CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761328Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Application Information

Application type	BLA
Application number(s)	761328
Priority or standard	Standard
Submit date(s)	9/26/2022
Received date(s)	9/26/2022
PDUFA goal date	9/26/2023
Division/office	Division of Antivirals (DAV)
Review completion date	7/13/2023
Established/proper name	Nirsevimab
(Proposed) proprietary name	Beyfortus
Pharmacologic class	Monoclonal antibody
Other product name(s)	MEDI8897
Applicant	AstraZeneca
Dosage form(s)/formulation(s)	Solution for intramuscular (IM) injection
Dosing regimen	Neonates and infants entering first RSV season: single IM dose 50 mg if weighing <5 kg, single IM dose of 100 mg if weighing greater or equal to 5 kg Children entering their second RSV season: single IM dose of 200 mg
Applicant-proposed indication(s)/ population(s)	Prevention of RSV lower respiratory tract disease in Neonates and infants entering or during their first RSV season, Children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season
SNOMED CT code for proposed indication disease term(s)[1]	55735094 Respiratory Syncytial Virus Infection (disorder)
Regulatory action	Approval
Approved dosage (if applicable)	Solution for intramuscular (IM) injection
Approved indication(s)/ population(s) (if applicable)	Prevention of RSV lower respiratory tract disease in neonates and infants born during or entering their first RSV season, children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season
SNOMED CT code for approved indication disease term(s)[1]	55735094 Respiratory Syncytial Virus Infection (disorder)

[1] For internal tracking purposes only.
Abbreviations: DAV, Division of Antivirals; IM, intramuscular; PDUFA, Prescription Drug User Fee Act; RSV, respiratory syncytial virus; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

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Glossary

AAP	American Academy of Pediatrics
ADA	anti-drug antibody
ADCC	antibody-dependent cell-mediated cytotoxicity
ADCD	antibody-dependent complement deposition
ADNKA	antibody-dependent NK cell activation
ADNP	antibody-dependent neutrophil phagocytosis
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BLA	biologics license application
CDC	Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CHD	congenital heart disease
CI	confidence interval
CLD	chronic lung disease
C_{max}	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
DART	Developmental and Reproductive Toxicology
EC_{50}	half maximal effective concentration
E-R	exposure-response
EU	European Union
FDA	Food and Drug Administration
FMQ	Food and Drug Administration Medical Dictionary for Regulatory Activities query
GA	gestational age
GLP	good laboratory practice
HAE	human airway epithelial
HIV	human immunodeficiency virus
ICH	International Council for Harmonisation
IM	intramuscular
IND	investigational new drug
ITT	intent-to-treat
K_D	binding affinity
LLOQ	lower limit of quantification
LRT	lower respiratory tract
LRTD	RSV LRT disease
LRTI	lower respiratory tract infection
MA	medically attended
MedDRA	Medical Dictionary for Regulatory Activities
NH	northern hemisphere
NIAID	National Institute of Allergy and Infectious Disease

NOAEL	no observed adverse effect level
OTC	over the counter
PCR	polymerase chain reaction
PFU	plaque-forming units
PI	Prescribing Information
PK	pharmacokinetic
PMC	postmarketing commitment
RRR	relative risk reduction
RSV	respiratory syncytial virus
RT	reverse transcriptase
RT-PCR	reverse transcriptase-polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
TEAE	treatment-emergent adverse event
TCR	tissue cross-reactivity
TM	triple mutation
ULN	upper limit of normal
YTE	three amino acid substitutions, M252Y/S254T/T256E

I. Executive Summary

1. Summary of Regulatory Action

This new biological license application (BLA) for nirsevimab-alip injection for intramuscular use, was submitted by Astra Zeneca AB and was reviewed by the FDA interdisciplinary review team. Nirsevimab-alip is henceforth referred to as nirsevimab in this review. The proposed proprietary name for nirsevimab, Beyfortus, has been conditionally granted. Nirsevimab is a respiratory syncytial virus (RSV) F protein-directed fusion inhibitor human immunoglobulin G1 kappa (IgG1κ) monoclonal antibody produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology. The intended indication for nirsevimab is prevention of Respiratory Syncytial Virus (RSV) lower respiratory tract disease (LRTD) in:

- Neonates and infants born during or entering their first RSV season.
- Children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season.

The Applicant submitted the original investigational new drug (IND) on May 11, 2014, for prevention of RSV disease in all infants. Fast track designation was granted on January 26, 2015, and Breakthrough Therapy Designation was granted on December 3, 2018. The BLA received a standard review and was presented at an Antimicrobial Drugs Advisory Committee Meeting on June 8, 2023, to discuss whether the available data support a favorable benefit-risk assessment for use of nirsevimab for the intended indication. A majority of the committee members agreed that the overall benefit-risk assessment is favorable for nirsevimab when used for the proposed indication.

No discipline (clinical, clinical virology, clinical pharmacology, pharmacometrics, pharmacology/toxicology, statistics, chemistry, and regulatory) identified any issues that preclude approval. I, the signatory authority for this application, concur with the approval recommendation. Please refer to the Approval Letter for further details.

To support the proposed indication, the Applicant conducted three pivotal clinical trials: two safety and efficacy trials in preterm and term neonates and infants (≥29 weeks gestational age, GA) and a trial in infants and neonates at highest risk for severe RSV LRT disease; i.e., those <29 weeks GA, those with chronic lung disease (CLD) of prematurity, and those with hemodynamically significant congenital heart disease (CHD). The primary efficacy endpoint used for Trials 03 and 04 was the incidence of medically attended RSV lower respiratory tract infection (MA RSV LRTI) caused by reverse transcriptase-polymerase chain reaction (RT-PCR)-confirmed RSV, characterized predominantly as bronchiolitis or pneumonia through 150 days after dosing. MA includes all healthcare provider visits such as physician office, urgent care, emergency room visits and hospitalizations. Signs of LRTI involvement included rhonchi, rales, crackles, or wheezing; and at least one sign of worsening clinical severity, including at least one of the following: increased respiratory rate, hypoxemia, acute hypoxic or ventilatory failure, new onset apnea, nasal flaring, retractions, grunting, or dehydration due to respiratory distress.

Incidence of RSV LRTI with hospitalization was a prespecified secondary endpoint. RSV hospitalization was defined as hospitalization for LRTI with a positive RSV test.

Trial 03 was a randomized, placebo-controlled trial that evaluated nirsevimab for prevention of MA RSV LRTI in preterm neonates and infants ≥29 weeks to <35 weeks GA; while Trial 04 was a randomized, placebo-controlled trial that evaluated nirsevimab for prevention of MA RSV LRTI in preterm and term infants ≥35 weeks GA. Trial 05, a randomized, palivizumab-controlled trial, evaluated the safety, pharmacokinetics and effectiveness of nirsevimab in palivizumab eligible neonates and infants in their first RSV season (premature infants and infants with CLD and CHD) and in children who remain vulnerable to RSV LRT disease in their second RSV season (i.e., children up to 24 months of age with CLD of prematurity and with hemodynamically significant congenital heart disease).

The proposed dosage for nirsevimab is 50 mg administered once by intramuscular (IM) injection in infants born during or entering their first RSV season and weighing <5 kg, and 100 mg administered once by IM injection in infants weighing ≥5 kg. For all children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season, the proposed dosage of nirsevimab is a single 200 mg IM dose. For infants and children undergoing cardiac surgery with cardiopulmonary bypass, an additional dose of nirsevimab is recommended as soon as the child is stable after surgery to ensure adequate postoperative nirsevimab serum levels.

The available efficacy data from the clinical trials demonstrate that nirsevimab is effective for its intended use. In Trial 03, in preterm infants (≥29 weeks to <35 weeks GA), the incidence of MA RSV LRTI was 25/969 (2.6%) in those who received nirsevimab and 46/484 (9.5%) in those who received placebo, for an estimated relative risk reduction (RRR) of 70.1% (95% confidence interval (CI):52.3% to 81.2%) with a p-value of <0.0001 in favor of nirsevimab. The secondary endpoint, hospitalization due to RSV, was also met in Trial 03, with RSV hospitalization reported in 8/969 (0.8%) subjects who received nirsevimab and 20/484 (4.1%) subjects who received placebo for an estimated RRR of 78.4% (95% CI: 51.9%, 90.3%) with a p-value=0.0002 favoring nirsevimab. In Trial 04, in term and late preterm infants born at ≥35 weeks gestation, the incidence of MA RSV LRTI was 12/994 (1.2%) in those who received nirsevimab and 25/496 (5.0%) in those who received placebo, for an estimated RRR of 74.9% (95% CI: 50.6% to 87.3%) with a p-value of <0.0001 in the primary analysis. For the secondary endpoint, hospitalization due to RSV, 6/994 (0.6%) subjects who received nirsevimab and 8/496 (1.6%) subjects who received placebo had an RSV hospitalization for an estimated RRR of 60.2% (95% CI: -14.6%, 86.2%) with a p-value=0.09.

In Trial 05, efficacy, measured as MA RSV LRTI through Day 150, was an exploratory endpoint; the incidence of MA RSV LRTI among subjects enrolled during their first RSV season was similar between nirsevimab and palivizumab treatment arms– 4/614 (0.6%) and 3/304 (1.0%), respectively. No subjects experienced MA RSV LRTI in either arm during their second RSV season. Efficacy in Trial 05 was established through extrapolation, based on similar nirsevimab exposures observed in subjects enrolled in Trial 05 (during their first or second RSV season) compared to nirsevimab exposures observed in subjects enrolled in Trial 03 (in those who received the recommended nirsevimab dose) and in Trial 04.

The available safety data from the clinical trials demonstrate that nirsevimab is safe for its intended use. The key safety concerns with nirsevimab are those observed with use of any monoclonal antibody, namely serious hypersensitivity reactions, such as anaphylaxis, and serious skin reactions. Severe or serious hypersensitivity reactions, such as anaphylaxis, and serious skin reactions were not reported in the nirsevimab clinical trials. Although a numerical imbalance in the incidence of death is noted in the nirsevimab clinical trials (12 deaths among subjects who received nirsevimab vs. 4 deaths in subjects who received the control), the overall incidence of deaths was similar between the two arms. No organ-specific toxicity was identified that could have contributed to or resulted in deaths. The review team concluded that the numerical imbalance in the incidence of death was not related to the use of nirsevimab. I concur that the risks identified in review of the clinical trial data can be mitigated through labeling and further evaluated through a pharmacovigilance strategy. In addition, the Agency is collaborating with the Centers for Disease Control and Prevention (CDC) to further monitor postmarketing safety events, as nirsevimab may potentially be used in a broader pediatric population than that in which palivizumab is currently used.

Based upon review of all available efficacy and safety data, the benefits of nirsevimab outweigh the risks when used for the prevention of RSV LRT disease in neonates and infants born during or entering their first RSV season, and in children up to 24 months of age who remain vulnerable to severe RSV disease in their second RSV season. The availability of nirsevimab will provide a new, effective, and convenient option for the prevention of RSV LRT disease in infants, including neonates, and in high-risk children up to 24 months of age. For detailed information supporting the basis for the benefit-risk assessment, please refer to the details in this Integrated Assessment document.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	**Respiratory Syncytial Virus (RSV)** • RSV is an enveloped RNA virus that causes respiratory tract infection. • RSV occurs in annual outbreaks each fall and winter in the majority of the United States. Most children and adults with symptomatic RSV infection have self-limited disease with signs and symptoms limited to the upper respiratory tract. However, RSV can present as a lower respiratory tract (LRT) disease, particularly in very young children and the elderly. Because of annual RSV outbreaks, almost all children have been infected with RSV by 2 years of age. • RSV is the most common cause of LRT infection (LRTI) in infants and young children both in the U.S. and worldwide. Approximately 20% to 30% of infants with RSV develop LRT disease with their first RSV infection. RSV LRT infection (LRTI) usually presents as bronchiolitis and/or pneumonia. The Centers for Disease Control and Prevention (CDC) estimates that RSV infection results in 2.1 million outpatient visits yearly among children younger than 5 years of age. Based on CDC's New Vaccine Surveillance Network (NVSN) analysis, it is estimated that RSV infections in pediatric patients < 24 months of age results in 472,000 visits to Emergency Departments each year (Lively et al. 2019). • RSV LRTI is the most common of hospitalization for infants in the United States. Approximately 1% to 3% all children in the U.S. will be hospitalized due to severe RSV disease. In children younger than 5 years of age, RSV infection results in 58,000 to 80,000 hospitalizations each year in	RSV virus is one of the most common causes of viral respiratory tract infection. While most experience mild upper respiratory tract infection, certain populations are at risk of lower respiratory tract disease, including pneumonia and bronchioloitis. RSV can lead to severe or serious diease in infants, including healthy term and preterm infants. Extreme preterms (e.g., <29 weeks of GA) and infants/children with certain underlying medical conditions are at greatest risk for severe or serious disease, including death.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	the U.S. The majority of children hospitalized with RSV infection improve with supportive care and are discharged in 2 to 3 days. • According to CDC, all infants (children ≤12 months of age), particularly those 6 months of age or younger, are at increased risk of hospitalization. While some studies have shown that the highest risk for severe RSV infection in otherwise healthy infants is for infants in the second month of life, the risk of hospitalization continues through at least six months of age. In a study by Hall et al. 2009, 58% of children hospitalized with RSV LRTI were 0 to <6 months of age, and 17% were from 6 to <12 months of age. Hospitalizations due to RSV LRTI were not reported in children older than 5 years of age. • Severe RSV disease and hospitalization are more common in certain pediatric populations. According to the CDC, the children at greatest risk for severe illness include premature infants in the first year of life, children younger than 2 year of years of age with chronic lung disease (CLD) of prematurity or hemodynamically unstable congenital heart disease (CHD), immunocompromised children, and children with neuromuscular disorders that have difficulty swallowing or handling secretions. The risk of severe RSV LRTI in infants born prematurely increases with decreasing gestational age (GA). Although the increased risk of severe RSV LRTI has been reported for all premature infants born at <35 weeks GA, the American Academy of Pediatrics (AAP) determined that the majority of studies show that the greatest risk for severe RSV LRTI is in infants born before 29 weeks GA. • According to the CDC, RSV infection leads to 100 to 300 deaths in children younger than 5 years of age in the U.S. each year. The Global Burden of Diseases, Injuries, and Risk Factors reported that there were more than 41,000 deaths due to RSV worldwide in 2016 in children <5 years of age. A recent meta-analysis reported 101,400 RSV-associated deaths globaly in children <5 years of age in 2019.	

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current treatment options	**Palivizumab** Palivizumab (Synagis®) is the only drug product approved by FDA for the prevention of serious lower respiratory tract disease caused by RSV. Palivizumab is a monoclonal antibody directed against a conserved epitope on the RSV fusion protein. Palivizumab is indicated for the prevention of serious RSV LRT disease in pediatric patients: • With a history of premature birth (less than or equal to 35 weeks gestational age) and who are 6 months of age or younger at the beginning of RSV season; • With bronchopulmonary dysplasia (BPD) that required medical treatment within the previous 6 months and who are 24 months of age or younger at the beginning of RSV season; • With hemodynamically significant congenital heart disease (CHD) and who are 24 months of age or younger at the beginning of RSV season. Palivizumab is administered by intramuscular injection; the first dose is administered prior to the start of the RSV season and remaining four doses are administered monthly during the RSV season. The trials supporting the licensure of palivizumab assessed RSV-related hospitalization as the primary efficacy endpoint. One trial was conducted during a single RSV season in children ≤24 months of age with broncho-pulmonary dysplasia or premature birth (<35 weeks gestation). The second trial was conducted over 4 seasons in children ≤24 months of age with hemodynamically significant congental heart disease. **Vaccines** Although there are multiple maternal RSV vaccines being developed to prevent RSV in infants, at the time of this BLA review, none have been licensed by FDA.	There is an unmet need for drug products to prevent RSV LRT disease in otherwise healthy term and late preterm infants born during or entering their first RSV season. There is a need for additional drug products to prevent RSV LRT disease in infants and children who are at greatest risk for severe RSV disease, including those with extreme prematurity or with certain underlying medical conditions. In addition, products requiring a single dose administration may increase compliance, and potentially decrease the frequency of adverse reactions associated with multiple intramuscular injections.
Benefit	**Prevention of MA RSV LRTI** • The primary efficacy endpoint used for Trials 03 and 04 (decribed below) was the incidence of MA RSV LRTI	Reduction in the incidence of MA RSV LRTI is a primary efficacy endpoint that demonstrates meaningful clinical benefit. Prospective, population-based surveillance studies have described the burden of RSV in infants by documenting the

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	caused by RT-PCR-confirmed RSV, characterized predominantly as bronchiolitis or pneumonia through 150 days after dosing. MA includes all healthcare provider visits such as physician office, urgent care, emergency room visits and hospitalizations. Signs of LRTI involvement included rhonchi, rales, crackles, or wheezing; and at least one sign of worsening clinical severity including at least one of the following: increased respiratory rate, hypoxemia, acute hypoxic or ventilatory failure, new onset apnea, nasal flaring, retractions, grunting, or dehydration due to respiratory distress. Incidence of RSV LRTI with hospitalization was a prespecified secondary endpoint. RSV hospitalization was defined as hospitalization for LRTI with a positive RSV test. • The efficacy of nirsevimab in otherwise **healthy infants born at ≥29 to <35 weeks GA**, who are at risk of severe RSV LRTI, was studied in Trial D5290C00003 (Trial 03). Trial 03 was a phase 2b, randomized, double-blind, placebo-controlled, safety, efficacy, and PK trial. Infants were enrolled in their first year of life, prior to their first RSV season, and were randomized in a 2:1 ratio to receive a single, 50 mg IM dose of nirsevimab or placebo. The primary endpoint was the incidence of MA- RSV LRTI over the 5-month RSV season. The incidence of MA-RSV LRTI was 2.6% in the nirsevimab arm and 9.5% in the placebo arm for a relative risk reduction of 70% (95% confidence interaval of 52.3, 81.2) (p<0.0001). • The efficacy of nirsevimab in otherwise **healthy infants born at ≥35 weeks GA** was supported by the results of the phase 3 trial, Trial D5290C00004 (Trial 04 or MELODY). Trial 04 was a phase 3 randomized, double-blind, placebo-controlled, safety, efficacy, and PK trial. Infants were enrolled in their first year of life prior to their first RSV season and were randomized in a 2:1 ratio to receive a single weight-based IM dose of nirsevimab or placebo. The trial was divided into two cohorts: the Primary Cohort, for analysis of efficacy, and the Safety Cohort, which was enrolled to increase the size of the safety database. The	increased rates of both RSV hospitalization and RSV outpatient visits (Hall et al. 2009). Infants with RSV LRTIs are less likely to be hospitalized today than they were in the past, when palivizumab was studied. Therefore, MA RSV LRTI, which is inclusive of both outpatient visits and hosptialization, is a reasonable, and clinically meaningful endpoint for evaluating nirsevimab for the prevention of RSV LRT disease. The submitted clinical data provide substantial evidence of nirsevimab efficacy to support the proposed indication of the prevention of RSV LRT disease in infants born during or entering their first RSV season; and in children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season. The data indicate that nirsevimab adminstered as a single dose prior to or during RSV season is superior to placebo in preventing MA RSV LRTI (Trial 03 and 04). Nirsevimab was highly effective in the reducing the incidence of MA RSV LRT disease in both trials. The prevention of RSV hospitalization was a secondary endpoint in Trial 03 and Trial 04. Efficacy was demonstrated for the prevention of hospitalization due to RSV in Trial 03, and there was a trend toward efficacy in the primary efficacy analysis cohort in Trial 04. Though nirsevimab was also compared to palivizumab (Trial 05), the trial was not designed for inferential statistics. Based on similar nirsevimab exposures observed in otherwise healthy infants and in high-risk infants enrolled in Trial 05, the efficacy of nirsevimab can be extrapolated to support use of nirsevimab to prevent MA RSV LRT disease in high-risk infants and children.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	primary efficacy endpoint in Trial 04 was the same as the primary efficacy endpoint in Trial 03 –the incidence of medically-attended lower respiratory tract infection (MA-LRTI) due to RT-PCR-confirmed RSV over the duration of the 5-month RSV season. In the Primary Cohort (the primary efficacy analysis population), the incidence of MA RSV LRTI was 1.2% in the nirsevimab arm and 5.0% in the placebo arm for a relative risk reduction of 75% (95% confidence interval of 49.6, 87.1) (p<0.0001). – The efficacy endpoint for the Safety Cohort is considered exploratory because results of the Primary Cohort were known to the Applicant while the Safety Cohort was ongoing. Therefore, FDA assessed the efficacy of nisevimab in Trial 04 based on the results of the Primary Cohort only. • The efficacy of nirsevimab in pediatric **subjects at high (or greatest) risk of severe RSV LRTI** was extrapolated from the efficacy in infants in Trials 03 and 04 after similar exposures of nirsevimab were demonstrated in subjects in Trials 03 and 04 and in subjects in Trial D5290C00005 (Trial 05 or MEDLEY). Trial 05 was a phase 2/3, randomized, double-blind, active (palivizumab)-controlled, safety, PK, and effectiveness trial in pediatric subjects born at <35 weeks GA and pediatric subjects with chronic lung disease (CLD) of prematurity and hemodynamically significant congenital heart disease (CHD). All subjects were randomized to receive nirsevimab or palivizumab prior to or during their first RSV season. Subjects with CLD or CHD were rerandomized prior to their second RSV season and received either nirsevimab or palivizumab prior to during their second RSV season. Based on similar nirsevimab exposures observed in pediatric subjects in Trials 03 and 04 and in Trial 05, the efficacy of nirsevimab can be extrapolated from Trials 03 and 04 populations to all high-risk infants in their first RSV season and for children with CLD and CHD in their second RSV season. • The secondary efficacy endpoint, incidence of MA RSV LRTI, was descriptive for Trial 05. The incidence of RSV in	Postmarket virologic surveillance for resistant variants is planned, and substitutions of interest will continue to be identified and evaluated.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	Trial 05 was very low due to the COVID-19 pandemic, but was similar in the nirsevimab and palivizumab arms. **Prevention of Hospitalization Due to RSV LRTI** • The incidence of hospitalization due to RSV LRTI was assessed as a secondary efficacy endpoint in Trials 03 and 04. In Trial 03, the incidence of RSV hospitalization in all subjects, regardless of weight and dose, was 0.8% in the nirsevimab arm and 4.1% in the placebo arm for a relative risk reduction of 78% (95% CI of 51.9, 90.3) (p-value=0.0002). • In Trial 04 Primary Cohort, the incidence of RSV-associated hospitalization through 150 days postdose was 1.5% in the nirsevimab arm and 1.2% in the placebo arm for a relative risk reduction of 60.2% 95%CI (-14.6% to 86.2%), (p=0.09). **Uncertainties Regarding Resistant Variants** • The future efficacy of nirsevimab could be impacted by naturally occurring variants which harbor F protein polymorphisms associated with reduced susceptibility to nirsevimab, or by selection of resistant variants following breakthrough infection. In Trial 04 and Trial 05, no known resistance-associated substitutions were identified at ≥25% frequency at any sampling time points.	
Risk and risk management	**Safety Summary** • Nirsevimab demonstrated an overall favorable safety profile in the clinical trials. • The safety database included 3,751 pediatric patients who received any dose of nirsevimab. In total, 3,285 received nirsevimab at the proposed (to-be-marketed) dose; of these, 3,224 subjects were enrolled in the 3 main clinical trials –Trial 03, Trial 04 and Trial 05. • In all three of the main trials included in the BLA, the percentage of subjects with at least one treatment-emergent adverse event was similar in the nirsevimab and control arms. In Trial 03, at least one adverse event was	The size of the safety database for both otherwise healthy infants and high-risk infants was adequate. No major safety issues related specifically to the use of nirsevimab have been identified. In general, nirsevimab had a similar adverse event profile compared to placebo or palivizumab in the pivotal clinical trials. Additonal long-term safety data from children enrolled in Trial 04 followed throught their second season will be submitted postmarketing. In addition, as part of the postmarket committment, long-term safety data from infants enrolled in other ongoing trials (e.g. HARMONIE trial) will be submitted.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	reported in 86.2% of infants who received nirsevimab and 86.8% who received placebo. In Trial 04, adverse events were reported in 83.7% of subjects who received nirsevimab and 81.8% who received placebo. In the first RSV season of Trial 05, adverse events were reported in 72.3% of subjects who received nirsevimab and in 70.7% of subjects who received palivizumab. • The differences in the incidences of AEs between the nirsevimab and control arms were small. No AE was reported with a frequency difference of 5% or greater between nirsevimab and the control arms. – The most common treatment-emergent AEs reported across the arms the three trials was upper respiratory tract infection, pyrexia, and nasopharyngitis –consistent with common childhood conditions. – The overall incidence of serious adverse events was low. Serious adverse events reported in at least 1% of subjects of any of the 3 trials were either respiratory tract infections (pneumonia, bronchitis and bronchiolitis) or gastroenteritis. • There were no premature discontinuations due to an adverse event in Trial 03 or 04. One subject in Trial 05 discontinued the trial prematurely after developing a rash following a placebo injection. • There are uncertainties regarding long-term adverse outcomes not observed in clinical trials including antibody dependent enhancement of RSV disease, and/or shifting of severe RSV LRT disease to the child's second RSV season. **Deaths** In the safety database, there were 12 deaths (0.32%) in subjects who received nirsevimab and 4 deaths (0.22%) in subjects who received control interventions. However, none of the deaths reported in nirsevimab-treated subjects are likely to be associated with use of nirsevimab	Overall, no adverse events consistent with a severe or serious allergic reaction to nirsevimab were reported in clinical trials of nirsevimab. There were no reports of anaphylaxis or hypersensitivity reactions (except skin reactions) in any of the trials of nirsevimab included in the BLA. The incidence of hypersensitivity skin reactions was <2% in both nirsevimab and control arms. Hypersensitivity skin reactions occuring within 3 days of trial drug administration were Grade 1 in intensity and in general were not associated with systemic symptoms. As serious hypersensitivity reactions, including anaphylaxis, have been observed with other human immunoglobulin G1 monoclonal antibodies, this risk will be included as a Warning and Precaution in labeling and a focus of postmarket surveillance. While numerically more deaths were reported among subjects who received nirsevimab, the causes of death were varied, suggesting lack of organ specific toxicity. Additionally, the majority of deaths were either due to underlying medical conditions, were complicated by underlying conditions, or had clear alternative causes. None of the deaths appeared to be related to trial drug.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• Most had either a preexsiting medical condition, or an intercurrent medical conditions contributing to the cause of death. • Two subjects died of unknown causes, both likely due to SIDS; one of the infants likely had underlying medical condition. **Hypersensitivity Reactions, Including Skin Reactions** • Hypersensitivity reactions, including analyphalaxis, have been associated with monoclonal antibody use, including palivizumab. There were no adverse events of anaphylaxis or hypersensitivity reactions (excluding skin reactions) observed during the clinical trials with nirsevimab. • In Trials 03, 04, and 05, more subjects in the nirsevimab arms experienced hypersensitivity skin reactions compared to subjects in the control arms. However, the percentage of hypersensitivity skin reactions was <1% in each arm. The hypersensitivity reactions reported within 3 days of trial drug administration were all Grade 1 and self-limited. No systemic symptoms were reported in combination with the hypersensitivity skin reactions. • In Trials 03, 04, and 05, the incidence of rash within two weeks of trial drug administration was similar in the nirsevimab and control arms. No increase in frequency of any specific preferred term for rash was observed in subjects who received nirsevimab.	

2.2. Conclusions Regarding Benefit-Risk

RSV is the most common cause of lower respiratory tract infection (LRTI) in infants and young children both in the United States and worldwide. RSV lower respiratory tract disease is a serious and potentially life-threatening illness in infants and children. Approximately 20% to 30% of infants with RSV develop LRT disease with their first RSV infection. (Committee on Infectious Diseases 2021-2024) RSV LRTI usually presents as bronchiolitis and/or pneumonia. Approximately 1% to 3% of children <12 months of age in the United States are hospitalized each year due to RSV. (Committee on Infectious Diseases 2021-2024) According to the CDC, there are 100 to 300 deaths per year in children younger than 5 years of age in the United States. In one retrospective review of deaths from 1999 to 2018 in the United States, the mean mortality rate for RSV in infants <12 months of age was 96 per 100,000 (Hansen et al. 2022).

Currently, the only approved drug for prevention of RSV LRTI is palivizumab, a monoclonal antibody against the RSV F protein, which is administered IM monthly throughout the RSV season. Although palivizumab is approved for use in preterm infants <35 weeks GA and in those with bronchopulmonary dysplasia (now known as chronic lung disease of prematurity) and in those with hemodynamically significant congenital heart disease, the use of palivizumab is only recommended by the American Academy of Pediatrics (AAP) in infants born at <29 weeks GA, and those with certain underlying medical conditions. In addition, palivizumab requires monthly intramuscular injections during the RSV season. For these reasons, palivizumab is not widely used.

Nirsevimab, also a monoclonal antibody against RSV F protein, has clearly demonstrated clinical benefit in preventing MA RSV LRTI in otherwise healthy preterm and term infants in their first RSV season. The efficacy of nirsevimab in preventing MA RSV LRTI in extremely premature infants (i.e., <29 weeks GA) during their first RSV season is extrapolated from moderate preterm and preterm infants based on the similarity of RSV disease pathophysiology and nirsevimab mechanism of action, and after similar exposures of nirsevimab were demonstrated between the two populations. Similarly, the efficacy of nirsevimab in those with CLD of prematurity, hemodynamically significant CHD, or other underlying conditions which place children at high risk for severe RSV disease in their second RSV season is extrapolated from moderate preterm and term infants, based on similarity of disease pathophysiology and demonstration of similar nirsevimab exposures in moderate preterm and term infants and in those with CLD or CHD.

The safety database for nirsevimab is adequate for the proposed indication, dosing regimen, and population. Overall, nirsevimab has a favorable safety profile. Key safety concerns with nirsevimab are hypersensitivity reactions, including anaphylaxis and serious skin reactions, although these were not reported in clinical trials. The potential risk of hypersensitivity reactions is described appropriately in labeling. Furthermore, a robust postmarketing pharmacovigilance strategy is planned for continued safety monitoring. The goal of the pharmacovigilance strategy will be to identify new safety signal(s); monitor for increased or unusual numbers of reports of a serious adverse event; and monitor for increase in the severity of a serious adverse event.

The overall benefit-risk profile of nirsevimab is favorable to support an indication for prevention of RSV LRT disease in:

- Neonates and infants born during or entering their first RSV season;
- Children up to 24 months of age who remain vulnerable to severe RSV in their second RSV season.

Areas of uncertainty include the potential for nirsevimab to result in adverse long-term outcomes not seen in clinical trials, for example, antibody dependent enhancement of RSV disease or shifting of severe RSV LRT disease to children's second RSV season. Longer-term data are needed to fully assess whether these potential risks should be of concern with nirsevimab use. In addition, the efficacy of nirsevimab could be impacted by naturally occurring variants which harbor F protein polymorphisms associated with reduced susceptibility to nirsevimab, or by selection of resistant variants following breakthrough infection. While the available nonclinical and clinical trial data (including surveillance programs) are encouraging, because RSV is continually evolving, the possibility that variants with reduced susceptibility to nirsevimab may emerge and become prevalent in the future should be considered; continued surveillance programs will be important with the approval of nirsevimab.

Although not currently licensed, maternal RSV vaccines, for passive immunization of infants are in late stages of development, and whether use of nirsevimab in such infants who received passive immunization by maternal RSV vaccination will provide added benefit is unknown. Although safety is not expected to be a concern in this setting, as both maternal RSV vaccination and nirsevimab provide passive immunization to the infant, this also remains unknown.

In our decision to approve nirsevimab, we considered the available safety and efficacy data, the recommendation for approval by all review disciplines, and the Antimicrobial Drugs Advisory Committee's recommendation where the majority of the committee members agreed that the overall risk-benefit assessment is favorable for nirsevimab in the populations evaluated in these trials. The availability of nirsevimab will provide a new, effective, and convenient option for prevention of RSV LRT disease in a broad population of infants in their first RSV season, and in certain children up to 24 months of age in their second RSV season.

II. Interdisciplinary Assessment

3. Introduction

Background on RSV Epidemiology and Disease

Human respiratory syncytial virus (RSV) is an orthopneumovirus in the Family Pneumoviridae, with a negative sense, nonsegmented RNA genome and lipid envelope. RSV consists of two antigenic subtypes, RSV-A and RSV-B, which vary in relative prevalence across seasons, and are further subdivided into different clades. The RSV fusion (F) surface glycoprotein mediates fusion between viral and host cell membranes, an essential step in the viral entry process. Nirsevimab targets antigenic site Ø on the prefusion conformation of F protein which locks the F protein in the prefusion state, preventing the conformation change and virus-cell membrane fusion needed for cell entry.

RSV occurs in annual outbreaks each fall and winter in most of the United States. Most children and adults with symptomatic RSV infection have self-limited disease with signs and symptoms limited to the upper respiratory tract. However, RSV can present as a lower respiratory tract (LRT) disease, particularly in very young children and the elderly. Because of annual RSV outbreaks, almost all children have been infected with RSV by 2 years of age (Committee on Infectious Diseases 2021-2024; Centers for Disease Control and Prevention 2023a).

RSV is the most common cause of LRT disease in infants and young children both in the United States and worldwide. Approximately 20% to 30% of infants with RSV develop LRT disease with their first RSV infection. RSV LRT disease (LRTD) usually presents as bronchiolitis and/or pneumonia. The CDC estimates that RSV infection results in 2.1 million outpatient visits yearly among children younger than 5 years of age. Investigators have estimated that RSV infections in pediatric patients results in 472,000 visits to emergency departments each year in children <2 years of age. RSV LRTD is the most common of hospitalization for infants in the United States (Ektare et al. 2022). Approximately 1% to 3% of children in the United States are hospitalized in the first 12 months of life due to severe RSV disease (Committee on Infectious Diseases 2021-2024). In children younger than 5 years of age, RSV LRTD results in 58,000 to 80,000 hospitalizations each year in the United States (Centers for Disease Control and Prevention 2022). The majority of children hospitalized with RSV LRTD improve with supportive care and are discharged in 2 to 3 days (Committee on Infectious Diseases 2021-2024). However, there are 100 to 300 deaths due to RSV LRTD in children younger than 5 years of age annually in the United States (Centers for Disease Control and Prevention 2022).

According to CDC, all infants (children ≤12 months of age), particularly those 6 months of age or younger, are at increased risk of hospitalization (Centers for Disease Control and Prevention 2023a). While some studies have shown that the highest risk for severe RSV LRTD in otherwise healthy infants is for infants in the second month of life, the risk of hospitalization continues to at least 12 months of age (Hall et al. 2013). In a study by Hall et al. 2009, 58% of children hospitalized with RSV LRTD were 0 to <6 months of age, and 17% were from 6 to <12 months

of age. Hospitalizations due to RSV LRTD were not reported in children older than 5 years of age (Hall et al. 2009).

Severe RSV disease and hospitalization are more common in pediatric patients born prematurely and in those with certain underlying conditions (Centers for Disease Control and Prevention 2023a). According to the CDC, the children at greatest risk for severe illness include premature infants in the first year of life, children younger than 2 years of age with chronic lung disease (CLD) of prematurity or hemodynamically significant congenital heart disease (CHD), immunocompromised children, and children with neuromuscular disorders that have difficulty swallowing or handling secretions. The risk of severe RSV LRTD in infants born prematurely increases with decreasing GA. Although the increased risk of severe RSV LRTD has been reported for all premature infants born at <35 weeks of gestation, the American Academy of Pediatrics (AAP) determined that most studies supported an increased risk of severe RSV LRTD in infants born before 29 weeks of gestation (American Academy of Pediatrics 2022).

The hospitalization rates for RSV decrease after the first year of life; approximately 75% of hospitalizations for RSV occur in the first year of life (Hall et al. 2009). However, some comorbidities, such as chronic lung disease (CLD) of prematurity with continued requirement for medical intervention and hemodynamically significant congenital heart disease (CHD), place children at risk of severe RSV disease in the second year of life (American Academy of Pediatrics 2022).

Palivizumab (Synagis®) is the only drug approved by FDA for the prevention of RSV lower respiratory disease (AstraZeneca 1998). Palivizumab is indicated for the prevention of serious RSV lower respiratory tract disease in high-risk infants. This indication was supported by trials in premature infants born at <35 weeks of gestation, infants with chronic lung disease of prematurity, and infants with hemodynamically significant congenital heart disease. The efficacy of palivizumab was assessed in two trials in which the primary efficacy endpoint was the incidence of RSV-associated hospitalization. In the first trial there was a 55% relative reduction in RSV-associated hospitalizations and in the second there was a 45% relative reduction in RSV-associated hospitalizations.

Palivizumab, like nirsevimab, is a recombinant humanized monoclonal antibody directed against a conserved epitope on the RSV fusion (F) protein. Because palivizumab is not modified to extend its serum half-life, a monthly intramuscular injection is required. The first dose of palivizumab is administered prior to the start of the RSV season and remaining four doses are administered monthly during the RSV season.

Aerosolized ribavirin is the only drug or biologic product approved for the treatment of RSV disease (Bausch Health US 1986). However, use of aerosolized ribavirin is limited due to its teratogenic effects and administration challenges, including risk of environmental spread. Aerosolized ribavirin must be administered in a hospital setting and is generally administered using an oxygen tent.

While palivizumab is the only FDA approved drug for the prevention of RSV lower respiratory tract disease, multiple vaccines are under development to prevent RSV disease. These include maternal vaccines to prevent RSV disease in infants by passive transfer of maternal antibodies to

the infant, (b) (4)

Pertinent Regulatory History and Clinical Trial Design

In March 2014, MedImmune submitted the initial IND application for the development program for nirsevimab, which was referred to as MEDI8897. The IND application proposed to evaluate nirsevimab for the prevention of RSV lower respiratory tract disease in infants entering their first RSV season, and in children with chronic lung disease (CLD) of prematurity and hemodynamically significant congenital heart disease (CHD) entering their first and second RSV seasons. During the course of the development program, both Fast Track and Breakthrough designations were requested and granted by the Agency. In 2021, a change in corporate name occurred, from MedImmune, LLC to AstraZeneca Pharmaceuticals LP. Refer to Section 12 for further regulatory history discussion.

During the development program, selection of key efficacy endpoints for the phase 2 and 3 clinical trials were discussed with the Applicant. Several reasons led to the selection of MA RSV LRTI as the primary efficacy endpoint, with prevention of hospitalization due to RSV as a secondary endpoint.

First, conducting a placebo-controlled trial to demonstrate the superiority of nirsevimab against placebo in preventing hospitalization would not be acceptable in infants and children for whom palivizumab is indicated. Second, while a noninferiority trial comparing nirsevimab with palivizumab to prevent hospitalization could be considered, the sample size needed to conduct such a trial was considered prohibitively large, and unlikely to be conducted within a reasonable time frame. Additionally, a noninferiority trial design, comparing nirsevimab to palivizumab for prevention of MA RSV LRTI is not feasible because a noninferiority margin cannot be determined as no randomized trials with the endpoint of MA RSV LRTI are available to estimate the treatment effect of palivizumab versus placebo. Therefore, alternative endpoints were considered.

The Agency held a public workshop with key opinion leaders in RSV clinical care and research to discuss drug development for the treatment or prevention of RSV on May 2, 2016. The workshop included a discussion of optimal endpoints for use in prevention trials. Participants acknowledged difficulties with the RSV hospitalization endpoint due to the change in clinical practice patterns with fewer infants hospitalized and noted that reduction in RSV-associated, MA illness in outpatient settings would likely be a clinically meaningful endpoint for prophylaxis trials. After the conclusion of the workshop, and with further deliberations within the FDA, the Agency developed draft guidance for industry "Respiratory Syncytial Virus Infection: Developing Antiviral Drugs for Prophylaxis and Treatment Guidance for Industry" which was published in October 2017. The guidance recommends use of laboratory confirmed RSV LRTI as a primary endpoint in prevention trials (October 2017).

MA RSV LRTI was adapted as a reasonable and clinically meaningful endpoint. The population for whom such an endpoint could be considered is also broader than the palivizumab-eligible population; and conducting a placebo-controlled superiority trial is feasible in this non-palivizumab eligible population.

3.1. Review Issue List

3.1.1. Key Efficacy Review Issues

3.1.1.1. Efficacy of Nirsevimab in Prevention of MA RSV LRTI in Neonates and Infants Born During or Entering Their First RSV Season: Assessment by Chronological and Gestational Age

Based on epidemiologic data, among otherwise healthy infants, the greatest risk for severe RSV LRT disease or hospitalization includes those who are <29 weeks of gestation, and infants younger than 6 months of age. The pivotal trials, Trials 03 and 04 enrolled preterm and term infants born at ≥29 weeks of gestation; at the time of enrollment, most subjects were also less than 8 months of chronological age.

- The incidence of MA RSV LRTI was infrequent in infants older than 6 months of chronological age.
- The efficacy of nirsevimab in infants born at <29 weeks of gestation is established based on demonstration of similar nirsevimab exposures among preterm and term infants enrolled in Trial 03 and 04, and extremely preterm infants (<29 weeks of gestation) enrolled in Trial 05.

3.1.1.2. Efficacy of Nirsevimab in Preventing MA RSV LRTI in Children Up to 24 Months of Age Who Remain Vulnerable to Severe RSV Through Their Second RSV Season

Based on epidemiologic data, infants and children with certain underlying medical conditions, such as CLD of prematurity and hemodynamically significant CHD are also at greatest risk for severe RSV LRT disease or hospitalization.

- The efficacy of nirsevimab in children who remain vulnerable to severe RSV disease during their second RSV season was established based on extrapolation of efficacy from infants enrolled in Trials 03 and 04. Trial 05, which enrolled children up to 24 months of age who remain vulnerable to RSV disease, included incidence of MA RSV as a secondary efficacy endpoint. Of note, the efficacy analysis was based on descriptive statistics as the trial was not designed for interferential statistics to demonstrate outcome difference between nirsevimab and palivizumab.

3.1.1.3. Potential for Reduced Susceptibility Through Natural Variation/Polymorphisms

The clinical efficacy of nirsevimab against RSV could be impacted by naturally occurring variants which harbor F protein polymorphisms associated with reduced susceptibility to

nirsevimab, or by selection of resistant variants following breakthrough infection. The potential for reduced susceptibility to nirsevimab was evaluated in cell culture studies, ongoing U.S.-based and global surveillance programs, and analysis of variants seen in breakthrough infections in clinical trials of nirsevimab.

3.1.2. Key Safety Review Issues

3.1.2.1. Hypersensitivity Reactions, Including Anaphylaxis and Rash

Immune-mediated adverse reactions, ranging from anaphylaxis to hypersensitivity skin reactions, are well-known adverse reactions associated with the use of monoclonal antibodies (Pintea et al. 2021). These adverse reactions have been reported with palivizumab, a monoclonal antibody against the RSV fusion protein with a similar mechanism of action as nirsevimab. We analyzed all adverse events in the safety dataset to identify those which could be hypersensitivity reactions.

3.1.2.2. Imbalance in Deaths During Clinical Trials

There was an unexpected imbalance in the number of deaths observed in subjects who received nirsevimab compared to in subjects who received controls (placebo or palivizumab). Therefore, deaths in all trials were carefully reviewed to determine if any death might be related to nirsevimab.

3.1.2.3. Pharmacovigilance

If approved, nirsevimab may be widely used for the prevention of RSV lower respiratory tract disease in neonates and infants, and certain children with underlying medical conditions. The pharmacovigilance strategy is important for continued assessment and risk characterization once nirsevimab is licensed for marketing.

3.2. Approach to the Clinical Review

Table 3 provides an overview of the clinical trials important to the review of nirsevimab for the prevention of RSV in infants, and in children up to 24 months of age who remain at high risk of severe RSV disease in the second year of life.

The results of Trials D5290C00003 (referred to as Trial 03 in this review) and D5290C00004 (referred to as Trial 04 in this review and also named the MELODY trial) were the primary support for the safety, pharmacokinetics, and efficacy of nirsevimab in the pediatric patients. Both Trials 03 and 04 were randomized, double-blind, placebo-controlled trials of nirsevimab in infants who were born during RSV season or were entering their first RSV season. Infants born at a gestational age of ≥29 weeks to <35 weeks were entered in Trial 03, while infants born at ≥35 weeks of gestational age were enrolled in Trial 04. All infants in Trial 03 received either placebo or a single 50 mg IM dose of nirsevimab. In Trial 04, nirsevimab was dosed according to

body weight; infants weighing <5 kg received a single 50 mg IM dose while infants weighing ≥5 kg received a single 100 mg IM dose.

Trial D5290C00005 (referred to as Trial 05 in this review and also named the MEDLEY trial) was a phase 2/3, randomized, double-blind, active-controlled trial comparing nirsevimab and palivizumab in the prevention of RSV in certain high-risk infants and children. The trial enrolled infants born at <35 weeks of gestational age and infants with chronic lung disease (CLD) of prematurity and hemodynamically significant congenital heart disease (CHD). The trial enrolled infants born during RSV season or prior to their first RSV season. Subjects with CLD of prematurity and hemodynamically significant CHD are considered at high-risk of serious RSV disease during their first 2 years of life; therefore, these subjects also received either nirsevimab or palivizumab prior to or during their second RSV season. The primary objective of this trial was to assess safety in this high-risk population; pharmacokinetic parameters and efficacy were assessed as secondary endpoints. Demonstration of efficacy in the high-risk population was based on extrapolation of efficacy from infants in Trials 03 and 04, based on similar nirsevimab exposures in infants enrolled in Trial 03 and 04, and infants and children enrolled in Trial 05. Efficacy outcome in Trial 05 was assessed with descriptive statistics.

The interim results of Trial D5290C00008 (referred to as Trial 08 in this review and also named the MUSIC trial) were submitted to support the safety and pharmacokinetics of nirsevimab in high-risk pediatric patients. Trial 08 was a single arm, open-label, safety, and pharmacokinetic trial of nirsevimab in immunocompromised pediatric subjects in their first 2 years of life. Sixty of a planned 100 subjects were enrolled prior to database lock, and the results of these 60 subjects were described in the interim Clinical Study Report. This was an open-label, uncontrolled trial that was not designed to assess efficacy; therefore, efficacy data from this trial is not discussed in this review.

The Clinical Study Report for Trial D5290C00002 (referred to in this review as Trial 02) was submitted to support the safety of nirsevimab. Trial 02 was a phase 1b/2a, randomized, double-blind, placebo-controlled, dose escalation trial in infants born at ≥29 weeks to <35 weeks gestational age, before their first RSV season. Trial subjects were randomized in a 4:1 ratio to receive a single 10 mg, 25 mg, or 50 mg IM dose of nirsevimab or of placebo. The safety results, including datasets, were submitted to support the safety of nirsevimab. Although the effectiveness results were described in the Clinical Study Report, the trial was not powered to assess efficacy and most subjects received a dose lower than the to-be-marketed dose; therefore, the efficacy results are not discussed in this review.

The Clinical Study Report for Trial D5290C00001 (referred to as Trial 01 in this review) was included in the BLA submission. This trial is the first-in-human trial of nirsevimab and was a phase 1, randomized, double-blind, placebo-controlled, dose-escalation trial in healthy adult volunteers from 18 to <50 years of age. Subjects in Trial 01 received a single intravenous or intramuscular dose of nirsevimab. The intravenous doses of nirsevimab ranged from 300 mg to 3,000 mg; the intramuscular doses of nirsevimab were 100 mg and 300 mg. The safety results from this trial were used to support the safety of higher doses in subjects who received nirsevimab replacement doses after cardiovascular surgery. See Section 7.2. Safety from this trial was also included in the analysis of anaphylaxis and hypersensitivity adverse events. Other safety results were reviewed but are not included in this integrated review, because of the

different doses of nirsevimab administered than that proposed for marketing by the Applicant, and because of the differences in trial populations.

The final Clinical Study Report and datasets were submitted for Trial 03. Interim Clinical Study Reports and datasets were submitted for Trials 04 and 05; long-term safety follow-up for these two trials was ongoing at the time of submission of the BLA. The Clinical Study Reports and datasets for demographics and adverse events were submitted for Trials 01 and 02. An interim Clinical Study Report and datasets were submitted for Trial 08, which was only partially enrolled at the time that the BLA was submitted. The statistical reviewer reviewed the trial designs and efficacy results for Trials 03 and 04. The clinical reviewer reviewed the trial designs and safety results from Trials 01, 02, 03, 04, 05, and 08. The clinical reviewer worked with the clinical data scientists to identify and perform pertinent safety analyses for Trials 03, 04, and 05 and to present these analyses in this review. The virology reviewer reviewed data from all clinical trials with a particular focus on amino acid substitutions conferring resistance. The clinical pharmacology reviewers reviewed pharmacokinetic data from each of the trials.

Table 3. Clinical Studies/Trials Submitted in Support of Efficacy and/or Safety Determinations[1] for Nirsevimab

Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
D5290C00001 (Trial 01)	Phase 1, R, DB, PC, dose escalation trial in healthy adults 18 years to <50 years of age	Phase 1, R, DB, PC, dose-escalating, safety, and PK trial of nirsevimab compared to placebo Control type: nirsevimab vehicle Randomization: 4:1 Blinding: Double-blind	Drug: nirsevimab Dosage: 300 mg, 1,000 mg, or 3,000 IV and 100 or 300 mg IM Number treated: 102 Duration (quantity and units): single dose on Day 1 Choose time unit.	Primary: AEs, SAEs, and AESIs Secondary: PK	136	1 country, 1 center
D5290C00002 (Trial 02)	Infants entering first RSV season. Born at ≥29 to <35 weeks GA	Phase 1b/2a, R, DB, PC, dose-escalating, safety, and PK trial of nirsevimab compared to placebo Control: Saline placebo Randomization: 4:1 Blinding: Double-blind	Drug: nirsevimab Dosage: Single 10 mg, 25 mg, or 50 mg IM dose Number treated:89 Duration: Single IM dose on Day 1	Primary: AEs, SAEs, AESIs Secondary: PK	89	3 countries, 10 centers

Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
D5290C00003 (Trial 03)	Infants entering first RSV season; Born at ≥29 to <35 weeks GA	Phase 2, R, DB, PC, safety, PK and efficacy trial of nirsevimab for the prevention of RSV MA-LRTI during their first RSV seasons Control: Saline placebo Randomization: 2:1 Blinding: Double-blind	Drug: nirsevimab Dosage: Single 50 mg IM dose Number treated:966 Duration: Single IM dose on Day 1	Primary: Incidence of MA-LRTI due to RT-PCR-confirmed RSV over 5-month RSV season Secondary: Incidence of hospitalizations due to RT-PCR-confirmed RSV nirsevimab PK Adverse events, SAEs, AESIs and incidence of ADA	Total planned: 1,500; Total randomized:1,453	23 countries 164 centers
D5290C00004 MELODY (Trial 04)	Infants entering first RSV season; Born at ≥35 weeks GA	Phase 3, R, DB, PC, 2-cohort, safety, PK and efficacy trial of nirsevimab for the prevention of RSV MA-LRTI during their first RSV seasons Control: Saline placebo Randomization: 2:1 Blinding: Double-blind	Drug: nirsevimab Dosage: 50 mg IM for subjects <5 kg and 100 mg for subjects ≥5 kg Number treated:1998 Duration: Single IM dose on Day 1	Primary Cohort: Primary endpoint: Incidence of MA-LRTI due to RT-PCR-confirmed RSV over 5-month RSV season Secondary endpoint: Incidence of hospitalizations due to RT-PCR-confirmed RSV Safety Cohort: Primary endpoint: AEs, SAEs, AESIs	Total planned: 3,000; Total randomized: 3,012 Primary Cohort randomized: 1,490 Safety Cohort randomized: 1,522	31 countries 211 centers

Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
D5290C00005 MEDLEY (Trial 05)	Infants entering first RSV season; Born at <35 weeks GA; Children ≤24 of age with CLD or CHD, entering first and second RSV seasons	Phase 2/3, R, DB, palivizumab-controlled, 2-cohort, safety, PK and effectiveness trial of nirsevimab for the prevention of RSV-MA-LRTI in pediatric patients at high risk of severe RSV disease Control: palivizumab, administered monthly x 5 as recommended in the PI Randomization: 2:1 Blinding: Double-blind	Drug: nirsevimab Dosage: In all trial infants in first year of life:50 mg IM for subjects <5 kg and 100 mg for subjects ≥5 kg In subjects with CLD and CHD in second year of life: 200 mg IM Number treated:614 Duration: Single IM dose on Day 1	Primary: AEs, SAEs, AESIs Secondary: PK Incidence of MA-LRTI due to RT-PCR-confirmed RSV over 5-month RSV season	Total planned: 900; Total randomized: 925	25 countries 126 centers
D5290C00008 MUSIC (Trial 08)	Immuno-compromised pediatric patients ≤24 months of age	Phase 2, OL, single arm, safety, PK and effectiveness trial of nirsevimab in immunocompromised children	Drug: nirsevimab Dosage: In infants in first year of life:50 mg IM for subjects <5 kg and 100 mg for subjects ≥5 kg In subjects in second year of life: 200 mg IM Number treated:60 Duration: Single IM dose on Day 1	Primary: AEs, SAEs, AESIs Secondary: PK Incidence of MA-LRTI due to RT-PCR-confirmed RSV over 5-month RSV season	Total Planned: 100; Total Randomized: 60	6 countries 22 centers

Source: Reviewer.
[1] Includes all submitted clinical trials, even if not reviewed in-depth, except for phase 1 and pharmacokinetic studies.
[2] If no randomization, then replace with "Actual Enrolled."
Abbreviations: BID, twice daily; d, day; DB, double-blind; h, hour; LTE, long-term extension; MC, multicenter; mo, month(s); N, number of subjects; NCT, national clinical trial; OL, open-label; PC, placebo-controlled; PG, parallel group; R, randomized; wk, week(s); y, year(s)

4. Patient Experience Data

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical Outcome Assessment Data Submitted in the Application		
☐	Patient-reported outcome	
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other Patient Experience Data Submitted in the Application		
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☒	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

5.1. Nonclinical Assessment of Potential Effectiveness

The potential effectiveness of nirsevimab was assessed nonclinically in nirsevimab/fusion protein binding assays and cell culture models of RSV neutralization, and in animal models of RSV prevention. The effector properties of nirsevimab were also evaluated in cell culture and animal models. These studies are described in detail in Section 20.

Mechanism of Action

Nirsevimab is a recombinant human immunoglobulin G1 kappa (IgG1κ) monoclonal antibody (mAb) that binds the prefusion conformation of the respiratory syncytial virus (RSV) fusion (F) protein. The Fc region of the parental antibody, 1G7, was modified with three amino acid substitutions, M252Y/S254T/T256E ("YTE") to extend the serum half-life in humans.

Co-crystallography studies were conducted with the mAb precursor to nirsevimab, D25, which differs from nirsevimab by five amino acids in the complementarity-determining region, and M252Y/S254T/T256E ("YTE") modifications in the Fc region. Analysis of D25 with RSV F protein trimer, showed that it binds to a quaternary epitope within antigenic site Ø, at the membrane-distal apex of the RSV F glycoprotein (McLellan et al. 2013). This interaction locks the F protein in its prefusion state, blocking the irreversible conformational change to the more stable postfusion conformation, and preventing viral entry. These studies identified 25 amino acid positions spanning a discontinuous binding site on the RSV F protein as contact residues for nirsevimab Fab, residing in the F2 subunit (residues 62 to 29) or the F1 subunit (residues 196 to 212).

Binding Activity

The binding kinetics of 1G7 (nirsevimab without YTE modification) were determined against recombinant RSV A and RSV B prefusion proteins. The binding affinity (K_D value) of 1G7 to RSV A2 and RSV B9320 prefusion F protein was 0.12nM and 1.22nM, respectively.

Reduced binding affinity was seen for F proteins harboring resistance-associated substitutions, with N208Y and N67I/N208Y substitutions in RSV A having K_D values reduced approximately 5- and 56-fold, respectively, and K68N and N201S substitutions in RSV B having K_D values reduced by 12- and 29-fold, respectively. No binding activity was detected to the RSV B F proteins containing N208D, N208S and double substitutions K68N/N201S and K68N/N208S. In general, reduction in binding activity to RSV A and RSV B F protein harboring resistance-associated substitutions correlated with the loss of neutralization activity.

Cell Culture Neutralization Activity

The antiviral activity of nirsevimab/1G7 was determined by microneutralization assays in HEp-2 cells, using laboratory strains and clinical isolates of RSV A and RSV B.

The 1G7 mAb neutralized RSV A2 and B9320 isolates with mean half maximal effective concentration (EC_{50}) values of 13pM (1.9 ng/mL) and 11pM (1.6 ng/mL), respectively, which were approximately 180-fold and 150-fold more potent, respectively, than palivizumab against these laboratory strains.

Nirsevimab or 1G7 were tested against clinical isolates collected from 2003 to 2017 from global locations, including Australia, China, Israel, Italy, Netherlands, and USA. For the isolates evaluated, the median EC_{50} value for nirsevimab/1G7 against RSV A isolates (n=70) was 21pM (3.2 ng/mL), ranging from 3pM (0.48 ng/mL) to 100pM (15.0 ng/mL). The median EC_{50} value for RSV B isolates (n=49) was 19pM (2.9 ng/mL), ranging from 2pM (0.3 ng/mL) to 398pM (59.7 ng/mL). The range of EC_{50} values was larger for RSV B isolates because of a single outlier, for which 1G7 had an EC_{50} value of 398pM (59.7 ng/mL). This outlier harbored

polymorphisms within both the F_2 (K65Q) and F_1 (S211N) regions of the nirsevimab/1G7 epitope.

The activity of nirsevimab in human airway epithelial (HAE) cells has also been assessed, using RSV A2 expressing green fluorescent protein. Following preincubation for 1 hour prior to infection, nirsevimab and palivizumab completely blocked RSV A2-GFP entry at concentrations of 3.3nM (0.5 µg/mL) and 68nM (10 µg/mL), respectively.

Evaluation of Effector Function

The potential of nirsevimab for Fc effector activity was evaluated by assessing binding activity to different recombinant human Fcγ receptors. The contribution of effector activity was evaluated in cell culture and the cotton rat model of RSV infection by comparing nirsevimab with 1G7 (unmodified Fc region) and 1G7-TM (triple mutation: L234F/L235E/P331S substitutions in Fc region which reduce effector function (Oganesyan et al. 2008). In cell culture, there was no clear difference in neutralization activity between 1G7 and the 1G7-TM mAb with reduced effector function, with EC_{50} values for nirsevimab, 1G7 and 1G7-TM of 2.2 ng/mL, 2.0 ng/mL and 2.0 ng/mL, respectively.

SPR affinity data indicated that nirsevimab is able to bind different human Fcγ receptors, with K_D values for FcγRI, FcγRIIA, FcγRIIB, or FcγRIII A158V of 8.94×10^{-9}, 1.87×10^{-5}, 5.30×10^{-4}, and 1.67×10^{-5} M, respectively. However, in the absence of control data, it is not known whether the binding affinities are biologically relevant.

In cell culture assays for antibody-dependent cell-mediated phagocytosis (ADCP) and antibody-dependent complement deposition (ADCD), nirsevimab demonstrated significant activity compared with the negative control antibody R347, and similar activity as seen for palivizumab. In addition, antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent NK cell activation (ADNKA) and antibody-dependent cell-mediated cytotoxicity (ADCC) activities were seen for nirsevimab at similar levels as palivizumab, although were not significantly different from the negative control.

In a cotton rat model of RSV infection, weight-based doses of 2.0, 1.0, or 0.5 mg/kg of IG7, IG7-TM, and negative mAb R347 dosed at 2.0 mg/kg, were administered to 4- to 6-week-old female cotton rats (groups of six to eight animals) by intramuscular injection. Administration of 1G7 or 1G7-TM one day prior to challenge with RSV A2 caused a dose-dependent reduction of RSV replication in lungs and nasal turbinates. There was no significant difference in viral titer in the lungs or nasal turbinates between animals treated with 1G7 and those with 1G7-TM at each mAb dose administered. However, it is not clear whether 1G7 binds cotton rat Fcγ receptors with the same affinity as human Fcγ receptors.

Animal Models of RSV Infection

The cotton rat model of RSV infection was used to assess the ability of 1G7 to inhibit replication of RSV A2 and RSV B9320 in lung tissue. Eight groups of four animals each were dosed intramuscularly with 1G7, ranging from 0.125 to 3 mg/kg, one day prior to intranasal challenge with 1×10^6 plaque-forming units (PFU) of RSV A2. For comparison, palivizumab doses ranging from 0.25 to 8 mg/kg were evaluated in parallel experiments, also using eight groups of four animals each. When administered one day prior to infection, the 1G7 mAb reduced RSV A2 lung

titers with mean EC_{50} and EC_{90} values of 19nM (2.9 µg/mL) and 45nM (6.8 µg/mL), respectively, and reduced RSV B9320 lung titers with mean EC_{50} and EC_{90} values of 12nM (1.8 µg/mL) and 39nM (5.8 µg/mL), respectively. Based on a comparison of mean EC_{50} serum concentrations, 1G7 activity was approximately 6-fold and 4-fold greater than seen with palivizumab for RSV A and RSV B, respectively.

5.2. Clinical Pharmacology/Pharmacokinetics

Table 5. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information
	Pharmacologic Activity
Established pharmacologic class (EPC)	Nirsevimab is a respiratory syncytial virus (RSV) F protein-directed fusion inhibitor
Mechanism of action	Nirsevimab (MEDI8897) is a recombinant neutralizing human IgG1κ mAb binding to the prefusion conformation of the RSV F protein. Nirsevimab is modified with a triple amino acid substitution (YTE) in the Fc region to extend serum half-life. Nirsevimab binds to a highly conserved epitope in antigenic site Ø on the RSV prefusion protein. Nirsevimab inhibits the essential membrane fusion step in the viral entry process, neutralizing the virus and blocking cell-to-cell fusion
Active moieties	Nirsevimab
	General Information
Bioanalysis	Validated ELISA based methods were used for quantification of nirsevimab in human serum (see Section 14.3 for details). The methods at sites of MedImmune GxP Testing Lab and [redacted] (b) (4) were fully validated with a lower limit of quantification (LLOQ) of 0.5 µg/mL.
Drug exposure following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	Nirsevimab is for a single dose administration. After either a single 50 mg IM dose for infants weighing <5 kg or a single 100 mg IM dose for infants weighing ≥5 kg, nirsevimab serum concentrations in term infants are similar to those in preterm infants and in infants with CLD/CHD. In Trial 04, the observed mean ± SD serum concentrations of nirsevimab following a single 50 mg or 100 mg dose in healthy late preterm and term infants in RSV Season 1 are summarized below:

Table 6. Observed Nirsevimab Serum Concentrations (mean ±SD) Following a Single 50 mg or 100 mg Dose in Trial 04 Subjects in RSV Season 1

Nirsevmab Dose and Serum Concentrations	Day 8	Day 15	Day 31	Day 151	Day 361
A single IM 50 mg dose in body weight <5 kg group					
N	32	131	349	257	312
Concentration, µg/mL	101±19	92±29	74±19	20±7.8	2.2±1.4
A single IM 100 mg in body weight ≥5 kg group					
N	44	167	684	396	463
Concentration, µg/mL	175±21	119±45	117±28	31±14	3.8±2.5

Source: The reviewer modified from CSR Table 47.

Characteristic	Drug Information

In Trial 05, the observed mean ± SD serum concentrations of nirsevimab following a single 50 mg or 100 mg dose in preterm infants and infants with CLD/CHD in RSV Season 1 are summarized below:

Table 7. Observed Nirsevimab Serum Concentrations (Mean ±SD) Following a Single 50 mg or 100 mg Dose in Trial 05 Subjects in RSV Season 1

Nirsevmab Dose and Serum Concentrations	Day 8	Day 15	Day 31	Day 151	Day 361
A single IM 50 mg dose in body weight <5 kg preterm cohort					
N	13	129	95	175	198
Concentration, µg/mL	127±22	97±23	83±23	22±8.1	2.5±1.5
A single IM 50 mg in body weight <5 kg CLD/CHD cohort					
N	5	50	46	77	87
Concentration, µg/mL	102±23	97±39	85±19	24±13	2.5±2.0
A single IM 100 mg dose in body weight ≥5 kg preterm cohort					
N	3	84	65	120	138
Concentration, µg/mL	181±35	142±27	109±33	35±10	4.4±3.6
A single IM 100 mg dose in body weight ≥5 kg CLD/CHD cohort					
N	3	42	55	94	91
Concentration, µg/mL	157±24	130±49	105±33	36±17	4.5±6.5

Source: The reviewer modified from CSR Table 30.

In Trial 05, the observed mean ± SD serum concentrations of nirsevimab following a single 200 mg dose in children with CLD/CHD in RSV Season 2 are summarized below:

Table 8. Observed Nirsevimab Serum Concentrations (Mean ±SD) Following a Single 50 mg or 100 mg Dose in Trial 05 Subjects in RSV Season 2

Nirsevmab Dose and Serum Concentrations	Day 8	Day 15	Day 31	Day 151	Day 361
A single IM 200 mg dose					
N	11	97	108	192	79
Concentration, µg/mL	260±49	180±64	154±72	52±25	6.7±4.9

Source: The reviewer modified from CSR Table 31.

Range of effective dose(s) or exposure	The recommended dosage of nirsevimab for neonates and infants born during or entering their first RSV season is based on body weight, a single intramuscular (IM) dose of 50 mg for infants <5 kg, or a single IM dose of 100 mg for infants ≥5 kg. The recommended dosage of nirsevimab for children less than 24 months of age who remain at increased risk for severe RSV in their second RSV season is a single IM dose of 200 mg.

Characteristic	Drug Information
Maximally tolerated dose or exposure Dose proportionality	An MTD was not determined. The highest evaluated dosage in pediatric subjects was 200 mg in children up to 24 months of age in Trials 05 and 08. Based on population PK analysis results, the PK (i.e., C_{max} and AUC) of nirsevimab is dose-proportional following a single IM administration of doses ranging from 25 mg to 200 mg in pediatric subjects.
Accumulation	Nirsevimab is administrated with a single dose. No drug accumulation has been assessed.
Bridge between to-be-marketed and clinical trial formulations	The final to be marketed formulation was used in pivotal clinical trials, therefore, formulation bridging is not needed.
	Absorption
Bioavailability	Bioavailability following IM administration is estimated to be 84%, based on population pharmacokinetics (popPK) analysis of adult data.
T_{max}	Based on Trial 02 in healthy preterm infants, observed mean T_{max} occurred 7 days postdose (the first postdose sampling time point). Based on population PK analysis results, the median time (range) to maximum concentration is 6 (1, 28) days.
Food effect (fed/fasted)	Nirsevimab is administered IM, so food effect was not assessed.
	Distribution
Volume of distribution	Central volume of distribution is 216 mL in a typical infant (White or Native Hawaiian/Pacific Islander, body weight of 5 kg, ADA negative, with a postmenstrual age of 11.1 months at the time of dose) in RSV Season 1, based on a popPK estimate. Volume of distribution increased with body weight and subjects in Season 2 of the Trial 05 had a total volume of 860 mL.
Plasma protein binding	Protein binding of nirsevimab has not been characterized.
	Elimination
Mass balance results	No mass balance studies were conducted for nirsevimab.
Clearance	Clearance is 3.42 mL/day in a typical infant (White or Native Hawaiian/Pacific Islander, body weight of 5 kg, ADA negative, with a postmenstrual age of 11.1 months at the time of dose) in RSV Season 1, based on popPK estimate. Clearance increased with body weight and subjects in Season 2 of the Trial 05 had a population clearance value of 7.46 mL/day.
Half-life	Terminal elimination half-life is 71.4 days in infants, based on popPK estimate.
Metabolic pathway(s)	As a mAb, nirsevimab is expected to be eliminated by intracellular catabolism and not primarily cleared via hepatic pathways.
Primary excretion pathways (% dose)	No specific excretion studies were conducted.

Characteristic	Drug Information
	Intrinsic Factors and Specific Populations
Body weight	The population pharmacokinetics (popPK) analysis identified body weight as a significant covariate influencing nirsevimab PK. With the same dosage regimen, the exposure of nirsevimab was lower in infants with higher body weight compared to infants with lower body weight.
Age	The postmenstrual age was identified to be a covariate as well, with a maturation t½ of 14.8 months. Nirsevimab clearance is 37% lower for a term infant at 12 months of age (postmenstrual age 21 months) compared to complete maturation (postmenstrual age 29 months).
Renal impairment	No dedicated trial of the effect of renal impairment on the PK of nirsevimab was conducted.
Hepatic impairment	No dedicated trial of the effect of hepatic impairment on the PK of nirsevimab was conducted.
	Drug Interaction Liability (Drug as Perpetrator)
Inhibition/induction of metabolism	No specific drug-drug interaction studies were conducted with nirsevimab.
Inhibition/induction of transporter systems	No specific drug-drug interaction studies were conducted with nirsevimab.
	Immunogenicity
Bioanalysis	Two versions of validated anti-drug antibody (ADA) assays are deemed to contain adequate sensitivity. However, the nirsevimab concentrations of most samples collected before or on Day 151 (Day 150 postdose) exceeded the drug tolerance limit of the ADA assays (see Section 14.4 for details).
Incidence	The ADA incidence in Trials 03, 04, and 05 were 3.3%, 7.0%, and 6.0%, respectively. The neutralizing antibody (NAb) incidence in Trials 03, 04, and 05 were 0%, 22.0%, and 6.0%, respectively. The anti-YTE incidence in Trials 3, 4, and 5 were 94.0%, 96.0%, and 97.0%, respectively (see Section 14.4 for details).
Clinical impact	In Trials 03, 04, and 05, nirsevimab-treated patients who developed anti-nirsevimab antibodies had a reduced nirsevimab serum concentrations at day 361 (50% to 60% lower compared to nirsevimab-treated patients who did not develop anti-nirsevimab-antibodies). Because of the low occurrence of ADA and MA RSV LRTI in clinical trials, the effect of these ADA on the safety and/or effectiveness of nirsevimab is unknown.

Abbreviations: ADA, anti-drug ant body; AUC, area under the concentration-time curve; CLD/CHD, chronic lung disease/congenital heart disease; C_{max}, maximum plasma concentration; ELISA, enzyme-linked immunosorbent assay; EPC, established pharmacologic class; Fc, fragment crystallizable; GxP, good practice; $IgG1_K$, immunoglobulin G1 kappa; IM, intramuscular; LLOQ, lower limit of quantification; mAb, monoclonal antibody; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; MTD, maximum tolerated dose; N, number of subjects; NAb, neutralizing ant body; PK, pharmacokinetic; popPK, population pharmacokinetic; (b) (4); RSV, respiratory syncytial virus; SD, standard deviation; T_{max}, time to maximum concentration; YTE, amino acid substitution

6. Efficacy (Evaluation of Benefit)

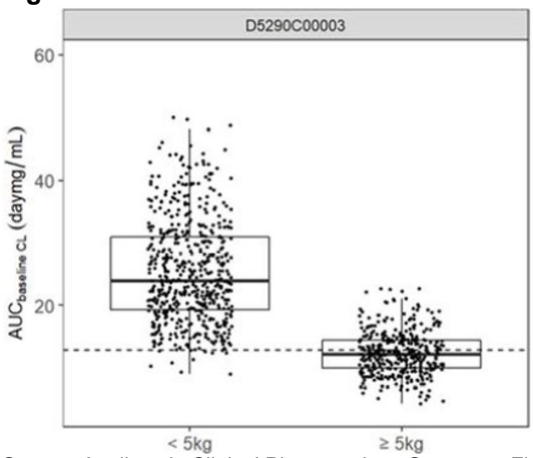
6.1. Assessment of Dose and Potential Effectiveness

6.1.1. Proposed Dosage Regimen in Healthy Term and Preterm Neonates and Infants During Their First RSV Season

The pivotal evidence of efficacy to support the proposed dosage regimen in healthy neonates and infants born during or entering their first RSV season was the clinical efficacy results of Trials 03 and 04. Exposure-response analyses were conducted to 1) identify the target exposure in infants and 2) support the proposed dosage regimen.

In Trial 03, a single, 50 mg IM dose was administered to all infants regardless of weight, based on PK simulations that indicated the 50 mg IM dose would maintain nirsevimab serum concentrations above 6.8 µg/mL (i.e., EC_{90}) over the 5-month RSV season. EC_{90} (concentration for 90% effectiveness) value was determined based on RSV challenge studies in cotton rats, a model that was used for dose selection of palivizumab. Most (97%) subjects in Trial 03 achieved nirsevimab serum concentrations on Day 150 postdose above the target of 6.8 µg/mL, EC_{90}. However, nirsevimab exposure (in terms of area under the concentration-time curve based on individual clearance at baseline, $AUC_{baselineCL}$) in infants weighing <5 kg was higher than the exposure in infants weighing ≥5 kg (Figure 1). $AUC_{baselineCL}$ was derived based on individual CL at baseline from the population PK model. In addition, the incidence of MA RSV LRTI was higher in infants weighing ≥5 kg compared to infants weighing <5 kg.

Figure 1. Box Plots of AUC Baseline CL Versus Weight Group, Trial 03 (D5290C0003)



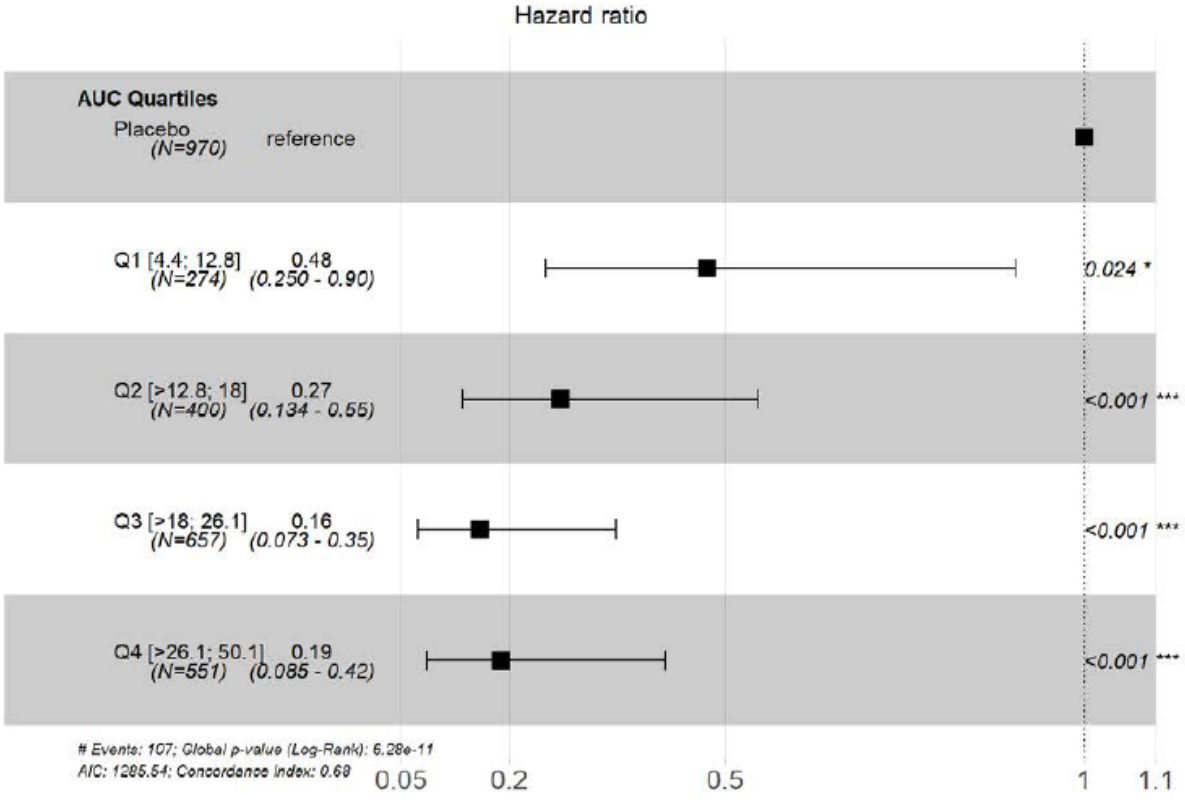
Source: Applicant's Clinical Pharmacology Summary, Figure 10, modified
Abbreviations: AUC, area under the concentration-time curve; CL, clearance

Additionally, the Applicant performed exposure-response (ER) analysis on data from Trial 03 to inform the dose for Trial 04. This analysis is not shown here as the analysis was later updated

with Trial 04 data, but the Trial 03 exposure-response results are consistent with the final analysis shown below. The PK, efficacy, and safety data from Trial 03 led to dose modification for Trial 04: a single, IM dose of 100 mg for infants weighing ≥5 kg; and a single, IM dose of 50 mg for infants weighing <5 kg.

To identify the target exposure in infants, the final ER analysis was performed based on pooled data from Trial 04 (Primary Cohort) and Trial 03 (all subjects, including subjects weighing ≥5 kg who received 50 mg IM dose). A Cox proportional hazards model was used to evaluate the influence of nirsevimab exposure (AUC quartiles) on the probability of having an MA RSV LRTI through 150 days postdose. The analysis indicated that the hazard ratio (HR) for subjects with exposures above the first quartile were significantly different from placebo (p<0.001) (Figure 2). The hazard ratio (HR) for the first quartile (Q1) was 0.48 (p =0.024), indicating lower efficacy in this exposure range. ER analysis demonstrated that a serum nirsevimab AUC above Q1 (12.8 day*mg/mL) was the target exposure to provide protection against MA RSV LRTI throughout the 5-month RSV season. In Trial 04, nearly all subjects received the proposed dosing regimen and 92.5% of subjects achieved exposure over target $AUC_{baselineCL}$.

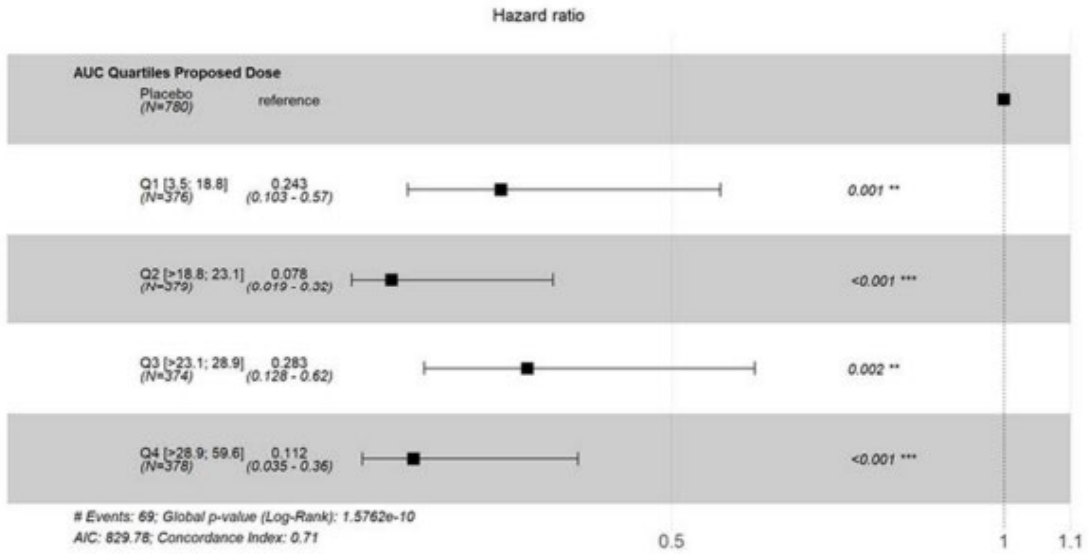
Figure 2. Applicant's Final Exposure-Response Model for MA RSV LRTI Through Day 151 in First RSV Season, Using All Data of Trials 03 & 04



Source: Applicant's Population PK Erratum
Hazard Ratio (95% CI). P-values based on the Wald test show testing of each quartile versus placebo; * = p-value<0.05, *** = p-value<0.001. Smaller values of the hazard ratio favor nirsevimab.
Abbreviations: AUC, area under the concentration-time curve; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; Q, quarter; RSV, respiratory syncytial virus

To further support the proposed dosing regimen, an additional ER analysis was performed based on the subset of data from Trials 03 and 04 –the subjects who received the proposed dose. The data included Trial 04 (Primary Cohort) and a subset of Trial 03 (i.e., only infants weighing <5 kg and received proposed dose of 50 mg). A Cox proportional hazards model was used to estimate the influence of $AUC_{baseline\ CL}$ on the probability of having a MA RSV LRTI through 150 days postdose. The analysis results indicated that the hazard ratios in all exposure bins were similar (<0.3), with no apparent ordering, demonstrating consistent efficacy over the exposure range after receiving the proposed dose (Figure 3).

Figure 3. Exposure-Response Based on Proposed Dose for MA RSV LRTI Through Day 151 Postdose Season 1, Hazard Ratio (95% CI)



Source: Applicant's Clinical Pharmacology Summary, Figure 12
Abbreviations: AUC, area under the concentration-time curve; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; Q, quarter

Based on nirsevimab serum exposure data, the proposed weight-based dosing of nirsevimab in neonates and infants entering their first RSV season (50 mg if less than 5 kg in body weight, or 100 mg if greater than or equal to 5 kg in body weight) is acceptable. Efficacy for this dosing regimen is on the plateau of the exposure response curve. The exposure response relationships were evaluated by the review team and found to be consistent with the Applicant's conclusions. Further details of the exposure response analysis are found in Section 14.5.

6.1.2. Proposed Dosage Regimen for High-Risk Neonates/Infants and Children During Their First or Second RSV Seasons

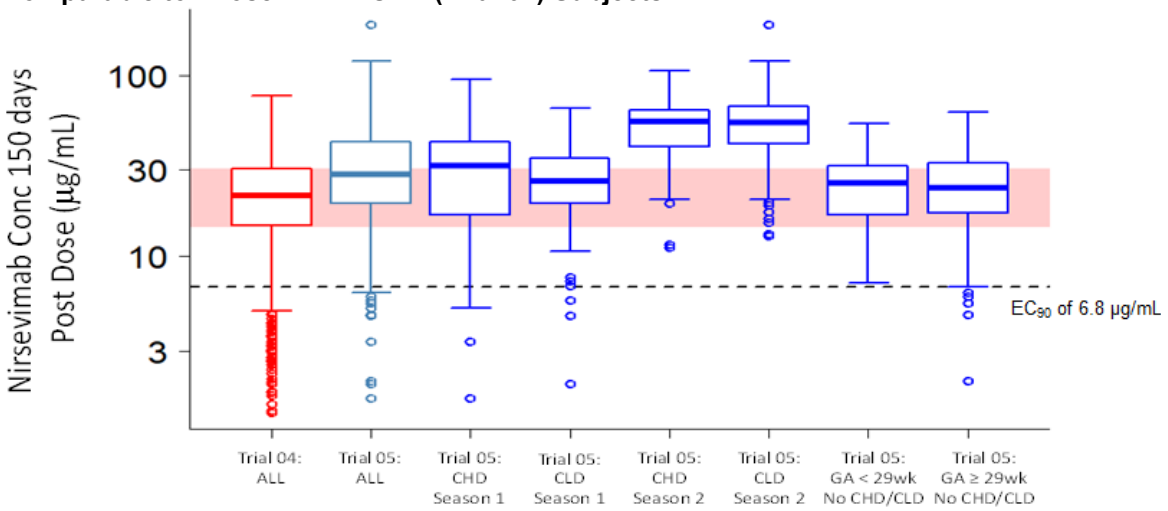
The doses for high-risk neonates/infants and children during their first or second RSV seasons were selected based on 1) similar nirsevimab serum exposure between healthy neonates/infants and high-risk infants/children who received the proposed doses; and 2) descriptive efficacy results in Trial 05. Please refer to Section 6.3.2 for details.

In Trial 05, in the first RSV season, nirsevimab was administered as a single 50 mg IM dose in infants weighing <5 kg, and as a single 100 mg IM dose in infants weighing ≥5 kg, the same doses administered in Trial 04. In the second RSV season, nirsevimab was administered as a 200 mg single IM dose to all children. A single 200 mg dose for the children entering the second RSV season was selected for evaluation to provide serum AUC above target exposure of 12.8 day*mg/mL based on expected body weight range.

Nirsevimab serum concentrations 150 days postdose (Day 151) and $AUC_{baselineCL}$ were chosen as the two PK parameters for efficacy extrapolation from Trials 03 and 04 to Trial 05 (refer to Section 6.3.2 for details). Day 151 nirsevimab serum concentration was selected based on the expected period of protection (i.e., 5 months) and duration of RSV season. $AUC_{baselineCL}$ was selected based on the ER analysis results with PK data from Trials 03 and 04. Please refer to Section 6.1.1 for details. The extrapolation of efficacy of nirsevimab from Trials 03 and 04 to Trial 05 was based on 1) the similar Day 151 serum nirsevimab concentrations in infants enrolled in Trials 03 and 04 and in infants enrolled in Trial 05 (Figure 4); and 2) percentage of subjects in Trial 05 with nirsevimab exposure above the target $AUC_{baselineCL}$ of 12.8 mg*day/mL (Table 9). The PK comparison was conducted for both the first and second RSV seasons in Trial 05.

As shown in Figure 4, the Day 151 serum concentrations in subjects enrolled in Trial 05 during their first season were comparable to the Day 151 serum concentrations observed among subjects enrolled in Trial 04. The Day 151 serum concentrations in subjects enrolled in Trial 05 during their second RSV season were higher than the concentrations observed in subjects enrolled in Trial 04. In both cases, because the Day 151 serum concentrations observed in Trial 05 were comparable or higher than those observed in Trial 04, it can be reasonably concluded that nirsevimab would be as effective in the extremely preterm infants or in infants with CHD/CLD.

Figure 4. Nirsevimab Day 151 Serum Concentrations in MEDLEY (Trial 05) Subjects Are Comparable to Those in MELODY (Trial 04) Subjects



Source: Reviewer's Analysis
The dashed line is EC90 value of 6.8 µg/mL determined based on RSV challenge studies in cotton rat model.
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; conc, concentration; EC, effective concentration; GA, gestational age

Table 9. Percent of Trial 05 Subjects With Nirsevimab Exposure Above Target $AUC_{baselineCL}$ of 12.8 mg*day/mL**

RSV Season	Extreme Preterm Infants <29 Weeks GA Without CLD or CHD	CLD	CHD
RSV Season 1	93.6% (44/47)	94.1% (128/136)	80.3% (53/66)
RSV Season 2	NA	97.7% (129/132)	100% (58/58)

Source: Applicant's Population Pharmacokinetic Report Erratum submitted on 5/18/2023, Table 13
**Target $AUC_{baselineCL}$ of 12.8 mg*day/mL is based on exposure-response analysis results from Trials 03 and 04.
Abbreviations: $AUC_{baselineCL}$, area under the concentration-time curve based on clearance at baseline; CHD, congenital heart disease; CLD, chronic lung disease; GA, gestational age; RSV, respiratory syncytial virus

Based on PK data and descriptive efficacy results in Trial 05, the proposed nirsevimab dosing is acceptable for high-risk neonates/infants during their first RSV season (a single dose of 50 mg if weighing <5 kg, or 100 mg if weighing ≥5 kg) and for high-risk children during their second RSV season (a single dose of 200 mg).

Extrapolation of efficacy using exposure comparison and descriptive efficacy results will be conducted when final data from Trial 08 in immunocompromised infants and children are available.

6.1.3. Proposed Dosage Regimen in Subjects Undergoing Cardiac Surgery With Cardiopulmonary Bypass

For individuals undergoing cardiac surgery with cardiopulmonary bypass, the Applicant proposed that an additional dose be administered as soon as the individual is stable after surgery, as summarized below.

- During the first RSV season: if surgery is within 90 days after receiving the first nirsevimab dose, the additional dose should be 50 mg or 100 mg IM according to body weight at the time of the additional dose. If more than 90 days have elapsed since the first dose, the additional dose should be 50 mg regardless of body weight.
- During the second RSV season: if surgery is within 90 days after receiving the first nirsevimab dose, the additional dose should be 200 mg IM, regardless of body weight. If more than 90 days have elapsed since the first dose, the additional dose should be 100 mg, regardless of body weight.

Based on the additional information provided by the Applicant, a total of 13 participants in Trial 05 underwent cardiac bypass surgery, 10 participants during their first RSV season and 3 participants during their second RSV season. Of these 13 subjects, 10 participants received an additional dose of nirsevimab. Subjects [redacted] (b) (6) during their first RSV season and subject [redacted] (b) (6) during the second RSV season did not receive an additional dose. It is noted that subject [redacted] (b) (6) received an additional dose that was not at the proposed dose level. Day 151 nirsevimab serum concentration was missing from three subjects, [redacted] (b) (6) during their first RSV season. Table 10 summarized the details for the 13 subjects who received cardiac surgery with cardiopulmonary bypass.

Table 10. Subject Level Data for Individuals Undergoing Cardiac Surgery With Cardiopulmonary Bypass

RSV Season/Subject ID	Baseline Body Weight, kg	Initial Dose Received, mg	Time Between First Dose and Additional Dose, Days	Body Weight at Time of Additional Dose, kg	Additional Dose Received, mg	Day 151 Conc., µg/mL	Conc Drop (Pre-Surgery Minus Post Surgery), µg/mL
First RSV season							
(b) (6)	3.4	50	131	6.4	50	Missing	8.3 (24.8-16.5)
	5.8	100	78	6.9	100	96.1	NA
	≥5	100	ND	ND	ND	Missing	NA
	7.5	100	68	8.2	100	47.2	NA
	3.8	50	86	5.7	100	114.8	19.0 (55.7-36.7)
	4.3	50	91	7	50	40.0	NA
	6.7	100	79	7.6	100	98.2	NA
	6.1	100	117	7.6	50	54.4	4.4 (10.5-6.1)
	5	100	91	7.1	100[$]	94.2	4.0 (33.6-29.5)
	<5	50	ND	ND	ND	Missing	NA
Second RSV season							
(b) (6)	11.9	200	23	12	100	42.8	NA
	≥5	200	ND	ND	ND	11.2	20.8 (31.7-11.0)
	9	200	113	10.4	100	104.3	39.3 (67.5-28.2)

Source: Response dated 1/13/2023 and response dated 4/13/2023
[$] The Subject (b) (6) did not receive the proposed additional dose.
Abbreviations: conc, concentration; NA, not applicable; ND, not dosed; RSV, respiratory syncytial virus

Pharmacokinetic Data

The Day 151 nirsevimab serum concentration from one subject who did not receive an additional dose was lower than the mean reference Day 151 concentration in Trial 04 and lower than concentrations from subjects who received an additional dose (see Table 11). In addition, Day 151 nirsevimab serum concentrations post additional dose were approximately 3-fold the reference Day 151 concentration in Trial 04 (see Table 11).

Table 11. Nirsevimab Day 151 Concentrations (Within ±14 Days Window) With an Additional Dose vs. Without an Additional Dose, in Subjects Who Underwent Cardiopulmonary Bypass Surgery, Compared With Reference Concentrations, Trial 04 (MELODY)

	Day 151 Nirsevimab Serum Concentration (µg/mL)		
	With Additional Dose	Without Additional Dose	Reference Concentrations from MELODY Study
Seasons 1 and 2			
Number of participants [a]	9	1	653
Arithmetic mean (SD)	76.88 (30.04)	11.19 (NC)	26.61 (12.97)

Source: Applicant's response dated 1/13/2023
Abbreviations: NC, not calculable; SD, arithmetic mean

Nirsevimab concentration reductions caused by cardiopulmonary bypass surgery were 29.3% within 90 days post-initial dose and 52.4% after 90 days post-initial dose (see Table 12). Three subjects ([redacted] (b) (6)) who underwent cardiac surgery with cardiopulmonary bypass within 90 days after initial dose had pre- and post-surgery nirsevimab serum concentration. Three subjects ([redacted] (b) (6)) who underwent cardiac surgery with cardiopulmonary bypass after 90 days after initial dose had pre- and post-surgery nirsevimab serum concentration.

Table 12. Nirsevimab Concentration Reductions Caused by Cardiopulmonary Bypass Surgery

	Nirsevimab Serum Concentration Drop Post Surgery (µg/mL)	Nirsevimab Serum Concentration Drop Post Surgery (%)
Within 90 days After Receiving the First Dose		
Number of participants [a]	3	3
Mean change	9.14	29.27
Median (min – max)	4.383 (4.05 - 19.00)	34.13 (12.05 - 41.62)
Post 90 days After Receiving the First Dose		
Number of participants [a]	3	3
Mean change	22.81	52.41
Median (min – max)	20.76 (8.34 - 39.32)	58.25 (33.57 - 65.40)

[a] Only participants with both pre-surgery and post-surgery samples available.
Source: Applicant's response dated 1/13/2023
Nirsevimab concentration drop (%) in each subject was calculated from 100x (pre-surgery concentration minus postsurgery concentration)/pre-surgery concentration.

In addition, nirsevimab serum concentrations at all timepoints post additional dose in subjects who received additional dose are within nirsevimab concentration ranges of Trials 03, 04, 05, and 08.

Safety in Subjects Who Received an Additional Dose of Nirsevimab After Cardiopulmonary Bypass

The Applicant reported no safety concerns observed in the 13 participants who underwent surgery with cardiopulmonary bypass and received an additional dose. Please see Section 7.3.3 for a clinical review of safety for subjects receiving an additional dose of nirsevimab.

In summary, based on the totality of the data, including the PK data, the proposal to administer an additional nirsevimab dose post-surgery as soon as a patient is stable is acceptable.

6.2. Clinical Studies/Trials Intended To Demonstrate Efficacy

6.2.1. Trials Reviewed for Efficacy

The clinical program comprises three randomized, double-blind trials. Two of those trials were designed for evaluation of efficacy (Trials 03 and 04) and the third trial was exploratory for efficacy (Trial 05). Trials 03 and 04 had a similar design, duration, and endpoints, but their trial populations were different. Trial 05 enrolled a different trial population and had different design features. A brief overview of trial designs and main features is presented below.

Trials 03 and 04 were double-blind efficacy trials, where subjects were randomized in a 2:1 ratio to receive nirsevimab or placebo and followed for one RSV season to assess efficacy. The primary efficacy endpoint for both trials was the incidence of MA RSV LRTI (hospitalized or

non-hospitalized) by Day 150 postdose (Day 151). All efficacy objectives and endpoints were the same across both trials.

The key differences between Trials 03 and 04 were the trial populations and dose selections. Specifically, Trial 03 was a phase 2b trial in very and moderately preterm infants (i.e., ≥29 to <35 weeks of gestation). All subjects randomized to the nirsevimab treatment arm received a 50 mg dose regardless of baseline body weight.

Trial 04 was a phase 3 trial in term and late preterm infants (i.e., ≥35 weeks of gestation). The dose selection for subjects randomized to the nirsevimab treatment arm was based on baseline body weight: subjects weighing less than 5 kg received a single, 50 mg IM dose, while subjects weighing ≥5 kg received a single, 100 mg dose of nirsevimab. Infants were for 360 days after dosing.

Both Trials 03 and 04 excluded subjects who were eligible to receive palivizumab (based on local standard of care).

Trial 05 was a PK and safety trial in palivizumab-eligible subjects. It was designed as a 2-season active-controlled trial in subjects considered to be at greatest risk for severe RSV disease (also referred to as 'high-risk' in this review). Subjects were randomized 2:1 to nirsevimab or palivizumab treatment arm. The enrolled population for the first RSV season comprised of preterm infants (including extremely preterm infants <29 weeks of gestation), and infants with CLD of prematurity or hemodynamically significant CHD; subjects with CLD/CHD continued into their second RSV season. Subjects were followed for 360 days after dosing in each RSV season.

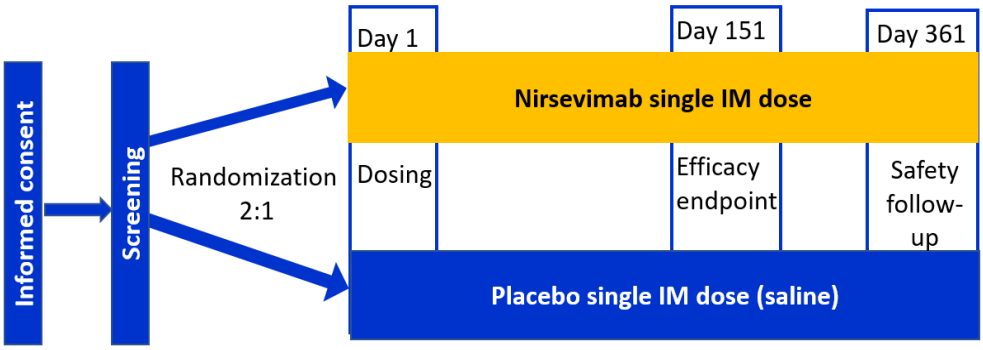
6.2.2. Trial 03

6.2.2.1. Design, Trial 03

Trial 03 was designed as a phase 2b, randomized, placebo-controlled trial to assess efficacy of nirsevimab in very and moderately preterm infants born ≥29 to <35 weeks of gestational age (GA) born during or entering their first RSV season.

Subjects were randomized 2:1 to receive nirsevimab or placebo. Randomization was stratified by the northern and southern hemispheres and by subjects' age at the time of randomization (i.e., ≤3months, >3 to ≤6 months, and >6 months). All infants were to be followed for 360 days after dosing (Figure 5).

Figure 5. Design, Trials 03 and 04



Source: FDA statistical reviewer
Abbreviations: IM, intramuscular

Primary Objective and Endpoint

The primary objective of this trial was to assess the efficacy of nirsevimab when administered as a single, 50 mg IM dose to healthy preterm infants born between 29 weeks 0 days and 34 weeks 6 days gestational age and entering their first RSV season for the reduction of MA LRTI due to RT-PCR confirmed RSV, compared to placebo.

The primary endpoint was the incidence of MA LRTI (with and without hospitalization) due to RT-PCR-confirmed RSV by day 150 postdose (Day 151) in infants entering their first RSV season.

Key Secondary Endpoint

Incidence of hospitalization due to RT-PCR-confirmed RSV over the duration of the 5-month RSV season (by Day 150 postdose).

An RSV hospitalization was defined as either:

1) a respiratory hospitalization with a positive RSV test within 2 days of hospitalization or
2) new onset of respiratory symptoms in an already hospitalized child, with an objective measure of worsening respiratory status and positive RSV test.

Prespecified Hypothesis Testing Order, Type I Error Control, and Interim Analysis

The primary and secondary efficacy hypotheses were tested in a prespecified hierarchical order. In this scenario, the secondary hypothesis was tested only if the treatment effect on the primary efficacy endpoint was demonstrated at the significance level of 2-sided alpha of 0.05.

Of note, the Statistical Analysis Plan (SAP) outlined a plan for an interim analysis, but an interim analysis was not conducted, according to the CSR.

6.2.2.2. Eligibility Criteria, Trial 03

Key Inclusion Criteria

- Healthy infants born between 29 weeks 0 days and 34 weeks 6 days gestational age.
- In all countries except European Union (EU): Infants who entered their first full RSV season at the time of screening. In EU: Infants who were ≤8 months of age and entered their first full RSV season at the time of screening.
- Written informed consent and any locally required authorization obtained from the subject's parent(s)/legal representative prior to performing any protocol-related procedures, including screening evaluations.

Exclusion Criteria

- Met American Academy of Pediatrics (AAP) or other local criteria to receive commercial palivizumab.
- Any fever (≥100.4°F [≥38.0°C], regardless of route) or lower respiratory illness within 7 days prior to randomization.
- Acute illness (defined as the presence of moderate or severe signs and symptoms) at the time of randomization.
- Active RSV infection (a child with signs/symptoms of respiratory infection must have had negative RSV testing) or known prior history of RSV infection.
- Any drug therapy (chronic or other) within 7 days prior to randomization or expected receipt during the study except for: 1) multivitamins and iron; 2) infrequent use of over-the-counter medications for the systemic treatment of common childhood symptoms (e.g., pain relievers, decongestants, or cough suppressants) that was permitted according to the judgment of the investigator.
- Any current or expected receipt of immunosuppressive agents, including steroids (except for the use of topical steroids according to the judgment of the investigator).
- History of receipt of blood transfusion or immunoglobulin products or expected receipt through the duration of the study.
- Receipt of any investigational drug.
- Known renal impairment.
- Known hepatic dysfunction, including known or suspected active or chronic hepatitis infection.
- History of chronic lung disease/bronchopulmonary dysplasia.
- Clinically significant congenital anomaly of the respiratory tract.
- Chronic seizure or evolving or unstable neurologic disorder.
- Congenital heart disease, except for children with uncomplicated congenital heart disease (e.g., patent ductus arteriosus, small septal defect).

- Prior history of a suspected or actual acute life-threatening event.
- Known immunodeficiency, including human immunodeficiency virus (HIV).
- Mother with HIV infection (unless the child had been proven to be not infected).
- Any known allergy, including to immunoglobulin products, or history of allergic reaction.
- Receipt of palivizumab or another RSV monoclonal antibody or any RSV vaccine, including maternal RSV vaccination.
- Receipt of any monoclonal or polyclonal antibody (for example, hepatitis B immune globulin, intravenous immunoglobulin).
- Any condition that, in the opinion of the investigator, would have interfered with evaluation of the investigational product or interpretation of subject safety or study results.
- Concurrent enrollment in another interventional study.
- Children of employees of the sponsor, clinical study site, or any other individuals involved with the conduct of the study, or immediate family members of such individuals.

6.2.2.3. Statistical Analysis Plan, Trial 03

All efficacy analyses were conducted using all randomized subjects following the Intent-to-Treat (ITT) principle. Subjects were included in their randomized treatment group, regardless of the treatment they received.

Analyses of Primary Endpoint

For the primary analysis, a Poisson regression model with robust variance was used to estimate the relative risk on the incidence of MA RSV LRTI between the nirsevimab and the placebo groups. The model contained the term of treatment group and age group at randomization (i.e., age ≤3 months, age >3 to ≤6 months, age >6 months) and dichotomous temperate (northern and southern) hemispheres as covariates. The relative risk reduction (RRR), defined as 1- Relative Risk (RR), and its corresponding 2-sided 95% CI, were estimated from the model.

Missing Data (To Evaluate the Impact of Missing Data)

Subjects who did not experience an event (MA RSV LRTI) and did not have their primary outcome status at Day 150 postdose were considered to be trial dropouts. The final status of those subjects was imputed based on the observed placebo RSV LRTI rate conditional on stratification factors using multiple imputation approach.

To further evaluate robustness of the primary analysis results, an additional sensitivity analysis was conducted. In this assessment all dropouts randomized to nirsevimab were considered as participants who experienced an event (MA RSV LRTI). The results were obtained using the same Poisson model with robust variance model as for the primary endpoint.

Analysis of Secondary Endpoint

Similar to the primary analysis, a Poisson regression model with robust variance was used to estimate the relative risk on the incidence of RSV LRTI hospitalization between nirsevimab and placebo. Of note, the model contained the treatment group as the only covariate in this model.

Missing data was imputed for subjects who did not have RSV LRTI and were not followed through 150 days postdose. The hospitalization status was imputed based on placebo rates using multiple imputations approach.

Exploratory Analyses

Exploratory analysis of very severe MA RSV LRTI cases was performed using the same approach as for the secondary endpoint (MA RSV LRTI with hospitalization). This analysis utilized a Poisson model with robust variance and treatment group as the only covariate. No missing data were imputed for this exploratory analysis.

Subgroup Analyses

The relative risk reduction was examined among subgroups: age, gestational age, sex, hemisphere, baseline weight, and race. The results were obtained using the same Poisson model with robust variance model as for the primary endpoint. Missing data were addressed using the same approach as for the primary endpoint.

6.2.2.4. Results of Analyses, Trial 03

This section summarizes patient disposition, demographics, baseline disease characteristics, and primary efficacy results based on data submitted by the Applicant.

Trial 03 had a relatively small percentage of screen failures, 5.6% of all screened subjects were not randomized (Table 13). Most of the screen failures (66 subjects of 87) did not meet inclusion/exclusion criteria, thirteen withdrew consent, and one subject was lost to follow-up.

Table 13. Patient Screening and Enrollment, Trial 03

Disposition	Trial A
Patients screened	1540
Screening failures	87 (5.6%)
Inclusion/exclusion criteria not met	66 (4.3%)
Lost to follow-up	1 (0.1%)
Consent withdrawn	13 (0.8%)
Other	7 (0.5%)
Patients randomized	1453 (94.4%)

Source: adsl.xpt; Tool: SAS

Overall, the baseline characteristics in each arm were balanced (Table 14). Of note, most (72%) of the subjects were White and 18% were Black or African American. About half of trial participants were male; baseline mean age and weight were approximately 3.3 months and 4.6 kg, respectively. The largest percentage of subjects was from the U.S. (20%) followed by South Africa (17%).

Table 14. Baseline Demographic Characteristics, Trial 03*

Characteristic	Nirsevimab 50 mg N=969	Placebo N=484	Total N=1453
Sex, n (%)			
Female	468 (48.3)	224 (46.3)	692 (47.6)
Male	501 (51.7)	260 (53.7)	761 (52.4)
Age at randomization, months			
N	969	484	1453
Mean (SD)	3.3 (2.22)	3.3 (2.31)	3.3 (2.25)
Median	2.9	2.8	2.8
IQR	1.5, 4.8	1.3, 4.9	1.4, 4.8
Min, max	0.1, 11.9	0.1, 11.3	0.1, 11.9
Age categories, n (%)			
Age ≤3 months	516 (53.3)	257 (53.1)	773 (53.2)
Age >3 to ≤6 months	320 (33.0)	153 (31.6)	473 (32.6)
Age >6 months	133 (13.7)	74 (15.3)	207 (14.2)
Race, n (%)			
American Indian or Alaska Native	0	1 (<1)	1 (<1)
Asian	5 (<1)	10 (2.1)	15 (1.0)
Black or African American	189 (19.5)	67 (13.8)	256 (17.6)
Multiple	12 (1.2)	5 (1.0)	17 (1.2)
Native Hawaiian or Other Pacific Islander	8 (<1)	3 (<1)	11 (<1)
Other	61 (6.3)	43 (8.9)	104 (7.2)
White	693 (71.5)	355 (73.3)	1048 (72.1)
Missing	1 (<1)	0	1 (<1)
Ethnicity, n (%)			
Hispanic or Latino	225 (23.2)	91 (18.8)	316 (21.7)
Not Hispanic or Latino	743 (76.7)	393 (81.2)	1136 (78.2)
Missing	1 (<1)	0	1 (<1)
Baseline weight, kg			
N	964	481	1445
Mean (SD)	4.6 (1.92)	4.5 (1.96)	4.6 (1.93)
Median	4.5	4.2	4.4
IQR	2.9, 6.0	2.7, 6.0	2.8, 6.0
Min, max	1.6, 11.1	1.2, 10.2	1.2, 11.1
Missing	5	3	8
Baseline weight category, n (%)			
Missing weight	5 (<1)	3 (<1)	8 (<1)
Weight ≤2.5 kg	186 (19.2)	96 (19.8)	282 (19.4)
Weight >2.5 to ≤5 kg	399 (41.2)	200 (41.3)	599 (41.2)
Weight >5 kg	379 (39.1)	185 (38.2)	564 (38.8)
Birth weight category, n (%)			
Weight ≤2.5 kg	905 (93.4)	454 (93.8)	1359 (93.5)
Weight >2.5 kg	64 (6.6)	30 (6.2)	94 (6.5)
Gestational age, n (%)			
29	37 (3.8)	20 (4.1)	57 (3.9)
30	53 (5.5)	30 (6.2)	83 (5.7)
31	103 (10.6)	51 (10.5)	154 (10.6)
32	170 (17.5)	84 (17.4)	254 (17.5)
33	234 (24.1)	106 (21.9)	340 (23.4)
34	365 (37.7)	193 (39.9)	558 (38.4)
35	7 (<1)	0	7 (<1)

Characteristic	Nirsevimab 50 mg N=969	Placebo N=484	Total N=1453
Region, n (%)			
Northern Hemisphere	659 (68.0)	329 (68.0)	988 (68.0)
Southern Hemisphere	310 (32.0)	155 (32.0)	465 (32.0)

Source: adsl.xpt; Tool: SAS
*Information about Country is located in (Section 16)
Abbreviations: IQR, interquartile range; ITT, intent-to-treat; N, number of subjects; n, number of subjects with specific characteristic; SD, standard deviation

Missing Data and Trial Discontinuations

Out of 1,453 subjects who were randomized, 35 (2.4%) discontinued and did not have their status outcome reported on Day 151 (primary endpoint). In addition, one subject from the placebo arm who experienced an event of MA RSV LRTI, died before Day 151. The main reason for trial discontinuation was 'withdrawal' by a parent or by a legal representative. Eighty-six (5.9%) subjects did not complete the trial (Day 361). The missing rates at the time of the primary endpoint analysis and the end of trial analysis were similar across treatment groups.

Table 15. Patient Disposition, Trial 03

Disposition	Nirsevimab 50 mg N=969	Placebo N=484
ITT population	969	484
Trial discontinuation reason (Day 151*), n (%)		
Death	2(0.2)	2(0.4)
Lost to follow-up	8(0.8)	3(0.6)
Withdrawal by parent/legal representative	13(1.3)	6(1.2)
Other	1(0.2)	1(0.2)
Total (Day 151)	24(2.5)	12(2.5)
Trial discontinuation reason (EOS, Day 361), n (%)		
Death	2 (0.2)	4 (0.8)
Lost to follow-up	26 (2.7)	11 (2.3)
Withdrawal by parent/legal representative	21 (2.2)	11 (2.3)
Other	7(0.7)	4(0.8)
Total (Day 361)	56(5.8)	30(6.2)

Source: asdl.xpt
*Primary endpoint; EOS end of the study, Day 361
Abbreviations: EOS, end of study; ITT, intent-to-treat; N, number of subjects; n, number of subjects with specific characteristic

Overall Outcome and Visualization of the Data

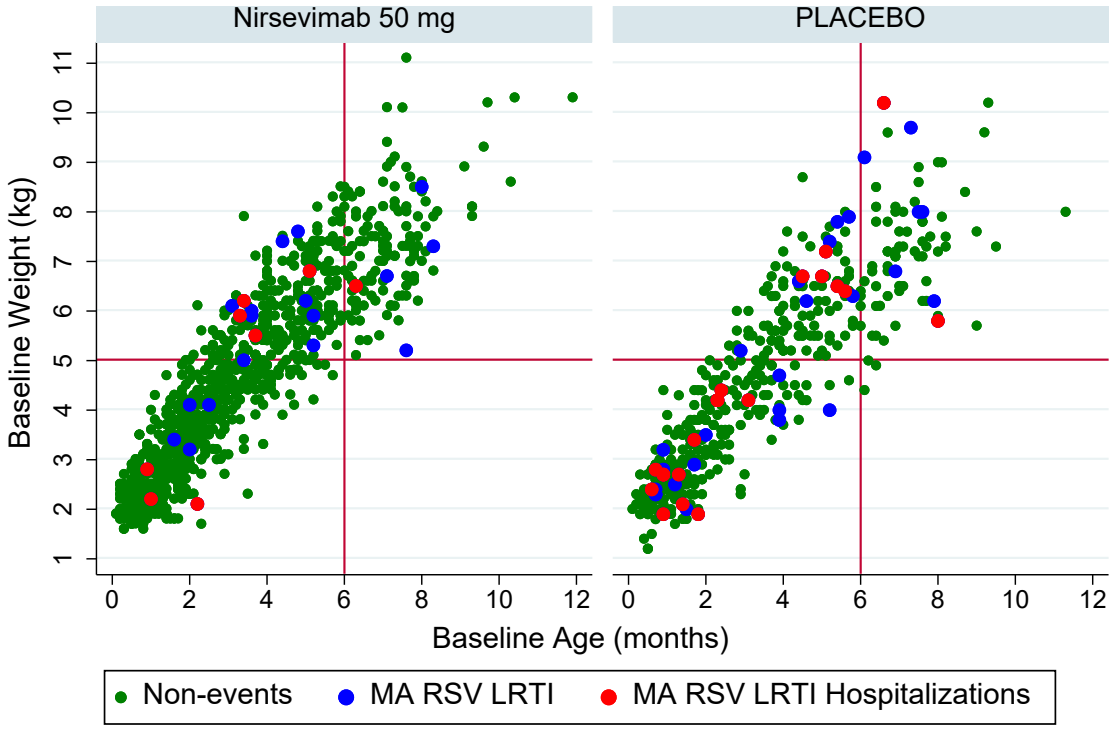
The overall outcome for the population enrolled in Trial 03 is visualized in the scatterplot figure below. The figure shows distribution of both MA RSV LRTI and hospitalization events and considers baseline characteristics such as age and weight. The primary and secondary endpoints, in addition to the subgroup analyses are further discussed in subsequent sections.

Because infants younger than 6 months of age are at greater risk for MA RSV LRTI, and because of dependence of the nirsevimab dose on body weight, examination of the relationship between baseline age, weight, and occurrence of MA RSV LRTI events is important. Overall, there were fewer MA RSV LRTI events (with and without hospitalization) in the group that received nirsevimab compared to the group that received placebo (Figure 6).

As previously mentioned, in Trial 03, all subjects randomized to the nirsevimab treatment group received a 50 mg dose, regardless of baseline body weight. Within the nirsevimab arm, there were fewer events of MA RSV LRTI in subjects weighing <5 kg compared to ≥5 kg. Because of this difference in event incidence, the Applicant reconsidered the adequacy of the dose for infants weighing ≥5 kg before the start of the phase 3 trial (Trial 04) and after the Trial 03 was completed. Subsequently, nirsevimab dose was optimized to 100 mg IM for subjects weighing ≥5 kg who enrolled in Trial 04.

As expected, only a few infants who were older than 6 months of age and weighing less than 5 kg (n=3) were enrolled in Trial 03. All of those subjects were in the placebo arm (Figure 6). Therefore, in this trial, the proposed weight-based dosing for nirsevimab (i.e., 50 mg IM for infants weighing <5 kg) was only examined in subjects younger than 6 months of age. Most MA RSV LRTI events in this trial, including hospitalization events, were reported in infants less than 6 months of age.

Figure 6. Relationship Between Baseline Weight, Baseline Age, and Outcome Status (Day 151), Trial 03



Source: FDA statistical reviewer
Legend: Relationship between baseline weight and baseline age for subjects who did not experience MA RSV LRTI (green circles), subjects who experienced MA RSV LRTI without hospitalization (blue circles), and those who experienced MA RSV LRTI and were hospitalized (red circles). Each circle represents age and weight at baseline so that each subject is represented only once.
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection events

Additional graphical exploration is presented in Section 16.

Primary Endpoint Results

The number of subjects who experienced MA RSV LRTI by Day 150 postdose in each of the treatment arms and the results of the analysis of the primary endpoint are presented in Table 16.

The estimated relative risk reduction (RRR) was 70.1% (95%CI: 52.3% to 81.2%) with p-value<0.0001 in favor of nirsevimab. Further exploration of MA RSV LRTI incidence in subgroups is presented later in this section.

Table 16. Incidence of MA RSV LRTI by Day 150 Postdose (Primary Endpoint)

	Trial 03	
Statistic	**Nirsevimab N=969**	**Placebo N=484**
Events (# of subjects, n (%))	25 (2.6)	46 (9.5)
Subjects requiring imputation*, n (%)	24 (2.5)	11 (2.3)
RRR (95% CI)§	70.1% (52.3% to 81.2%) p<0.0001	

Source: FDA statistical reviewer; adeff3.xpt; tool: SAS
* Subjects with missing outcomes on Day 151. The final status of those subjects was imputed based on the observed placebo rate conditional on stratification factors using multiple imputation approach.
§ Based on Poisson regression model with robust variance with of treatment group and age group at randomization (i.e., age ≤3 months, age >3 to ≤6 months, age >6 months) and dichotomous temperate (northern and southern) hemispheres as covariates
Abbreviations: CI, confidence interval; N, number of subjects; n, number of subjects with specific event; RRR, relative risk reduction

Sensitivity Analysis

A sensitivity analysis that repeated the primary analysis with an additional assumption that all subjects on nirsevimab who had a missing outcome (i.e., subjects requiring imputation) had their outcomes imputed as events yielded a relative risk reduction of 48.4% with 95%CI (24.2%, 64.9%) in favor of nirsevimab. This result suggests that the outcome of the primary analysis was robust.

RSV Subtypes

The RSV cases were evenly divided between RSV A and RSV B subtypes within each treatment group, a total of 35 RSV A and 36 RSV B (Table 17).

Table 17. RSV Virus Subtype by RT-PCR Test

RSV Subtype	Nirsevimab 50 mg N=969	Placebo N=484	Total N=1453
RSV A	11 (1.1)	24 (5.0)	35 (2.4)
RSV B	14 (1.4)	22 (4.5)	36 (2.5)

Source: FDA statistical reviewer; adeff3.xpt; tool: SAS
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus; RT-PCR, reverse transcriptase-polymerase chain reaction

Secondary Endpoint Results

The number of subjects who experienced MA RSV LRTI and were hospitalized by Day 150 postdose in each of the treatment arms and the results of the analysis of the secondary endpoint are presented in Table 18. The estimated relative risk reduction (RRR) was 78.4% (95%CI: 51.9%, 90.3%) with p-value=0.0002 in favor of nirsevimab.

Table 18. Incidence of RSV Hospitalization Events by Day 150 Postdose (Secondary Endpoint)

	Trial 03	
Statistic	**Nirsevimab N=969**	**Placebo N=484**
Events (# of subjects, n (%))	8 (0.8)	20 (4.1)
Subjects requiring imputation*, n (%)	24 (2.5)	11 (2.3)
RRR (95% CI)§	78.4% (51.9%, 90.3%) p-value=0.0002	

Source: FDA statistical reviewer; adeff3.xpt; tool: SAS
* Subjects with missing outcomes on day 151. The final status of those subjects was imputed based on the observed placebo rate conditional on stratification factors using multiple imputation approach.
§ Based on Poisson regression model with robust variance with treatment group as a covariate
Abbreviation: CI, confidence interval; N, number of subjects; n, number of subjects with specific event; RRR, relative risk reduction

Exploratory Analyses

Subgroup Analyses

The treatment effect based on the incidence of MA RSV LRTI (primary endpoint) were consistent across subgroups and with the overall treatment effect. The detailed subgroup results are presented in Section 16.

6.2.3. Trial 04

6.2.3.1. Design, Trial 04

Trial 04 was designed as a phase 3, randomized, placebo-controlled trial to assess efficacy of nirsevimab in term and late preterm infants born ≥35 weeks of gestation and entering their first RSV season.

Trials 03 and 04 had the same overall design (Figure 7). Similar to Trial 03, in Trial 04, subjects were randomized 2:1 to receive nirsevimab or placebo. Randomization was stratified by hemisphere (northern hemisphere (NH) and southern hemisphere (SH)) and subject age at randomization (i.e., ≤3.0 months, >3.0 to ≤6.0 months, >6.0 months).

Nirsevimab was administered as a single intramuscular dose (50 mg for infants weighing <5 kg or 100 mg for infants weighing ≥5 kg). The primary and secondary endpoints were evaluated on Day 151 and subjects were followed for safety through Day 361.

Impact of COVID-19 Pandemic on Trial Conduct

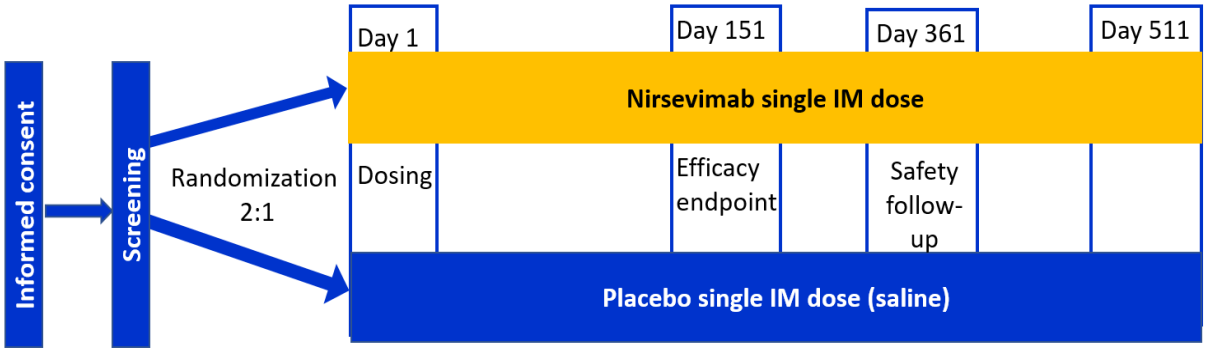
Because of the COVID-19 pandemic's impact on operational aspects of trial conduct, enrollment in the trial was paused. In this review, the cohort that was enrolled before the pause is referred to as the Primary Cohort. After a Type B meeting with the Agency (December 2, 2020), the trial design was amended, and it was agreed that the Primary Analysis would be based on the 1490 subjects randomized prior to the pause in enrollment (Primary Cohort). It was also agreed that the planned trial sample size would be completed by enrolling a complementary Safety Cohort.

New Sample Size and the Two Study Cohorts

The initial, planned sample size for this trial was 3000 subjects. Because of the interruption, the recruitment approach was changed.

As a result, the Primary Cohort consisted of 1490 subjects (994 on nirsevimab and 496 on placebo). The Safety Cohort consisted of 1522 subjects (1015 on nirsevimab and 507 on placebo). The overall trial design, randomization ratio, endpoints, and timing of those endpoints in both study cohorts were identical and followed the initial prespecified approach (Figure 7).

Figure 7. Design, Trial 04



Source: FDA statistical reviewer
Abbreviation: IM, intramuscular

Database Lock and Unblinding

According to the Applicant, the database lock for the primary analysis occurred when all participants in the Primary Cohort were followed through Day 361. Database lock for the Primary Cohort occurred on April 14, 2021. All but four subjects from the Safety Cohort were randomized after April 14, 2021. The enrollment to the Safety Cohort was complete on October 22, 2021.

Database lock for the Trial 04 All Subjects (Primary and Safety Cohorts together) analysis occurred when all participants in the Safety Cohort had been followed through Day 151.

Primary Objective and Endpoint

The primary objective of this trial was to assess the efficacy of nirsevimab in reducing MA LRTI due to RT-PCR confirmed RSV, compared to placebo.

The primary endpoint was the incidence of MA LRTI (with and without hospitalization events) due to RT-PCR-confirmed RSV by Day 150 postdose in infants entering their first RSV season.

Secondary Endpoint

The secondary endpoint was defined as the incidence of hospitalization events due to RT-PCR-confirmed RSV over the duration of the 5-month RSV season (by Day 150 postdose).

An RSV hospitalization was defined as either

1) a respiratory hospitalization with a positive RSV test within 2 days of hospitalization or
2) new onset of respiratory symptoms in an already hospitalized child, with an objective measure of worsening respiratory status and positive RSV test.

Prespecified Hypothesis Testing Order, Type I Error Control

The primary and secondary efficacy hypotheses were tested in a prespecified hierarchical order. In this scenario, the secondary hypotheses were supposed to be tested only if the treatment effect on the primary efficacy endpoint (using data from the Primary Cohort only) was demonstrated at the significance level of 2-sided alpha of 0.05.

For the secondary endpoints, the Applicant proposed to pool Trial 03 and Trial 04 together to ascertain efficacy of nirsevimab on hospitalization. Given that the populations of Trials 03 and Trial 04 were different (Trial 03 included very and moderately preterm infants born ≥29 to <35 week of GA, and Trial 04 included term/late preterm infants born ≥35 weeks 0 days of gestational age), hospitalization risks in those populations are expected to be different. Furthermore, the dosing regimen differed in the two trials. In Trial 04, baseline body weight was the determining factor in nirsevimab dose selection (100 mg for subjects weighing ≥5 kg; 50 mg for subjects weighing <5 kg); while in Trial 03, all participants received the 50 mg regardless of their baseline body weight. Because of these differences, pooling subjects from the two trials would be incongruous. The Agency alerted the Applicant about this issue during the pre-BLA meeting on July 26, 2022.

Therefore, no analysis based on pooled data from Trials 03 and 04 will be included or discussed in this review. The hospitalization endpoint based on the data from the Primary Cohort alone will be considered as the only prespecified secondary endpoint. All analyses based on the Primary and Safety Cohorts together were not planned to be adjusted for multiplicity and therefore will only be considered as exploratory analyses.

Mitigation of Discrepancies in Submitted Datasets

While examining the database of Trial 04, the Agency noticed that two subjects from the Primary Cohort were identified as 'nonevents' in the Primary Cohort dataset. At the same time, in the dataset that included All Subjects (Primary and Safety Cohorts together), the same two subjects were listed as 'lost to follow-up' with 'missing' event status. In response to the Agency's request for clarification, the Applicant confirmed that the two subjects in question were considered to be in the 'ongoing' status during the database lock. According to the Applicant, after the database lock (April 14, 2021), the Investigator recorded those subjects as 'lost to follow-up' prior to Day 151. Therefore, in the All Subjects (pooled cohort) dataset, those subjects were recorded as 'missing'.

For the purpose of this review, the results for the analysis of the Primary Cohort will be presented based on the All-Subjects dataset and therefore will include the observed outcomes for the two participants listed above as 'missing'. The analysis results based on the initial Primary Cohort dataset will be listed in the footnote.

6.2.3.2. Eligibility Criteria, Trial 04

Both the Primary and Safety Cohorts had the same eligibility criteria.

Key Inclusion Criteria

1) Healthy infants in their first year of life and born ≥35 weeks 0 days GA (infants with an underlying illness such as cystic fibrosis or Down syndrome with no other risk factors were eligible)
   a) In Japan, the inclusion criterion was healthy infants born ≥36 weeks 0 days GA and excluded infants with Down syndrome
2) Infants who were entering their first RSV season at the time of screening
3) Written informed consent and any locally required authorization was obtained from the subject's parent(s)/legal representative prior to performing any protocol-related procedures, including screening evaluations.

Exclusion Criteria

1) Met the national or other local criteria to receive commercial palivizumab
2) Any fever (≥100.4°F [≥38.0°C], regardless of route) or acute illness within 7 days prior to randomization
3) Any history of LRTI or active LRTI prior to, or at the time of, randomization
4) Known history of RSV infection or active RSV infection prior to, or at the time of, randomization
5) Any drug therapy (chronic or other) within 7 days prior to randomization or expected receipt during the study except for:
   a) multivitamins and iron
   b) infrequent use of over the counter (OTC) medications for the systemic treatment of common childhood symptoms (e.g., pain relievers) could be permitted according to the judgment of the investigator
6) Any current or expected receipt of immunosuppressive agents including steroids (except for the use of topical steroids according to the judgment of the investigator)
7) History of receipt of blood, blood products, or immunoglobulin products, or expected receipt through the duration of the study
8) Receipt of any investigational drug
9) Known renal impairment
10) Known hepatic dysfunction including known or suspected active or chronic hepatitis infection
11) History of CLD/bronchopulmonary dysplasia
12) Clinically significant congenital anomaly of the respiratory tract

13) Chronic seizure or evolving or unstable neurologic disorder
14) Congenital heart disease, except for children with uncomplicated CHD (e.g., patent ductus arteriosus, small septal defect)
15) Prior history of a suspected or actual acute life-threatening event
16) Known immunodeficiency, including HIV
17) Mother with HIV infection (unless the child was proven to be not infected)
18) Any known allergy, including to immunoglobulin products, or history of allergic reaction
19) Receipt of palivizumab or another RSV mAb or any RSV vaccine, including maternal RSV vaccination
    a) Exclusion criterion not applicable in Japan.
20) Receipt of any monoclonal or polyclonal antibody (for example, hepatitis B immunoglobulin, IV immunoglobulin)
21) Any condition that, in the opinion of the investigator, would interfere with evaluation of
22) the IP or interpretation of subject safety or study results
23) Concurrent enrolment in another interventional study
24) Children of employees of the Sponsor, clinical study site, or any other individuals involved with the conduct of the study, or immediate family members of such individuals.

6.2.3.3. Statistical Analysis Plan, Trial 04

All efficacy analyses were conducted using all randomized subjects following the ITT principle. Subjects were included in their randomized treatment group, regardless of the treatment they received.

The analyses of the primary and secondary endpoints were performed only using the data collected prior to the recruitment pause (Primary Cohort).

Analyses of Primary Endpoint

A Poisson regression model with robust variance was used to estimate the relative risk on the incidence of MA RSV LRTI between the nirsevimab and the placebo groups. The model contained the term of treatment group and age group at randomization (i.e., age ≤3 months, age >3 to ≤6 months, age >6 months) and dichotomous temperate (northern and southern) hemispheres as covariates. The relative risk reduction (RRR), defined as 1- Relative Risk (RR), and its corresponding 2-sided 95% CI were estimated from the model.

Missing Data (To Evaluate the Impact of Missing Data)

Subjects who did not experience an event (MA RSV LRTI) and did not have their primary outcome status at Day 150 postdose were considered to be trial dropouts. The final status of

those subjects was imputed based on the observed placebo RSV LRTI rate conditional on stratification factors using multiple imputation approach.

To further evaluate robustness of the primary analysis results, an additional sensitivity analysis was conducted. In this scenario all dropouts randomized to nirsevimab were considered as participants who experienced an event (MA RSV LRTI).

Analysis of Secondary Endpoint

Similar to the primary analysis, a Poisson regression model with robust variance was used to estimate the relative risk on the incidence of RSV LRTI hospitalizations between nirsevimab and placebo. Of note, the model contained the treatment group as the only covariate in this model.

Outcome results were imputed for subjects who did not have RSV LRTI and were not followed through 150 days postdose. The hospitalization status was imputed based on placebo rates using multiple imputations approach.

Exploratory Analyses

Appropriateness of Pooling of Primary and Safety Cohorts

To determine the appropriateness of pooling the cohorts together, baseline demographic characteristics and event rates were compared between Primary and Safety Cohorts.

Examining Endpoints Using Pooled Cohorts

The exploratory analyses of the primary and the secondary endpoints using the pooled cohort populations (Primary and Safety Cohorts together) were performed using the same statistical approaches as the prespecified primary and secondary endpoint analyses. In addition, all analysis models included a cohort indicator variable to adjust for potential differences between study cohorts. Missing data were imputed using the same approach as the prespecified primary and secondary endpoint analyses.

Examining Very Severe RSV Using Primary Cohort and Pooled Cohorts

Exploratory analysis of very severe MA RSV LRTI cases was performed using the Primary Cohort alone and using both Primary and Safety Cohorts pooled together. Similar to the analysis of the secondary endpoint (MA RSV LRTI with hospitalization), these analyses utilized a Poisson model with robust variance with treatment group as the only covariate in the model. No missing data imputations were performed for these exploratory analyses.

Subgroup Analyses

The relative risk reduction was examined among subgroups: age, gestational age, sex, hemisphere, baseline weight, and race. Results were obtained using the same Poisson model with robust variance model as for the primary endpoint. Missing data were addressed using the same approach as for the primary endpoint.

6.2.3.4. Results of Analyses, Trial 04

This section summarizes patient disposition, demographics, baseline disease characteristics, and efficacy results based on data submitted by the Applicant.

Screening and Enrollment

Primary Cohort

The proportion of subjects who were screening failures in the Primary Cohort was 8.4%. Most (6%) of the screen failures did not meet inclusion/exclusion criteria, 1.5% of the subjects withdrew consent and 0.4% were lost to follow-up (Table 19).

Safety Cohort

Among subjects in the Safety Cohort, screen failures were 10.1%. Most (5.4%) of the screening failures did not meet inclusion/exclusion criteria, 3.9% of subjects withdrew consent, and 0.3% were lost to follow-up.

Table 19. Patient Screening and Enrollment, Trial 04

Disposition	Primary Cohort	Safety Cohort
Patients screened	1626	1694
Screening failures	136 (8.4%)	171(10.1%)
Inclusion/exclusion criteria not met	98(6%)	92(5.4%)
Lost to follow-up	6 (0.4%)	5(0.3%)
Consent withdrawn	25 (1.5%)	67(3.9%)
Other	7 (0.4%)	7(0.4%)
Patients randomized	1490 (91.6%)	1522(89.9%)

Source: Primary Cohort asdl.xpt, Tool: SAS

Baseline Characteristics

Primary Cohort

Overall, the baseline characteristics in each arm were balanced. More than a half of all subjects was White (53%), 29% were Black or African American; and 10% of subjects were Hispanic or Latino. About half of trial participants were male (52%). The majority (98%) of subjects were younger than 9 months. Most were 3 months old or younger (58%, mean age 2.9 months) at baseline with a mean body weight of 5.5 kg. The majority (69%) of subjects were from the Northern Hemisphere. The largest percentage of subjects was from South Africa (31%) followed by the U.S. (29%) and Spain (8%). A detailed list of countries is presented in Section 16.

Safety Cohort

Demographics and baseline disease characteristics were well-balanced between treatment arms in the Safety Cohort. More than a half of all subjects was White (52%), only 1% of subjects were Black or African American, and 34% identified themselves as Other. Fifty-seven percent identified themselves as Hispanic or Latino. About half of trial participants were male (53%). Ninety-eight percent of subjects were younger than 9 months. Most (60%) of the subjects were 3

months old or younger (mean age 2.9 months), with a mean body weight of 5.6 kg at baseline. The majority of subjects were from the Northern Hemisphere (79%). The largest percentage of subjects was from Panama (33%), followed by Argentina (12%) and the U.S. (8%). A detailed list of countries is presented in Section 16.

Table 20. Baseline Demographics and Clinical Characteristics, Trial 04*

	Primary Cohort			Safety Cohort		
Characteristic	**Nirsevimab N=994**	**Placebo N=496**	**Total N=1490**	**Nirsevimab N=1015**	**Placebo N=507**	**Total N=1522**
Sex, n (%)						
Female	464 (46.7)	257 (51.8)	721 (48.4)	474 (46.7)	243 (47.9)	717 (47.1)
Male	530 (53.3)	239 (48.2)	769 (51.6)	541 (53.3)	264 (52.1)	805 (52.9)
Age at randomization, months						
N	994	496	1490	1015	507	1522
Mean (SD)	2.9 (2.21)	3.0 (2.25)	2.9 (2.22)	2.9 (2.23)	2.8 (2.29)	2.9 (2.25)
Median	2.6	2.6	2.6	2.5	2.4	2.5
IQR	1.0, 4.4	1.1, 4.6	1.1, 4.5	1.1, 4.3	1.1, 4.3	1.1, 4.3
Min, max	0.0, 11.1	0.0, 11.0	0.0, 11.1	0.0, 11.9	0.0, 14.0	0.0, 14.0
Age categories, n (%)						
Age ≤3.0 months	577 (58.0)	285 (57.5)	862 (57.9)	613 (60.4)	303 (59.8)	916 (60.2)
Age >3.0 to ≤6.0 months	317 (31.9)	162 (32.7)	479 (32.1)	319 (31.4)	161 (31.8)	480 (31.5)
Age >6.0 months	100 (10.1)	49 (9.9)	149 (10.0)	83 (8.2)	43 (8.5)	126 (8.3)
Age categories, n (%)						
Age <9.0 months	982 (98.8)	486 (98.0)	1468 (98.5)	996 (98.1)	497 (98.0)	1493 (98.1)
Age ≥9.0 months	12 (1.2)	10 (2.0)	22 (1.5)	19 (1.9)	10 (2.0)	29 (1.9)
Race, n (%)						
American Indian or Alaska Native	57 (5.7)	26 (5.2)	83 (5.6)	35 (3.4)	26 (5.1)	61 (4.0)
Asian	36 (3.6)	18 (3.6)	54 (3.6)	73 (7.2)	32 (6.3)	105 (6.9)
Black or African American	286 (28.8)	136 (27.4)	422 (28.3)	13 (1.3)	2 (<1)	15 (1.0)
Multiple	12 (1.2)	1 (<1)	13 (<1)	7 (<1)	7 (1.4)	14 (<1)
Native Hawaiian or Other Pacific Islander	6 (<1)	5 (1.0)	11 (<1)	9 (<1)	3 (<1)	12 (<1)
Other	70 (7.0)	38 (7.7)	108 (7.2)	350 (34.5)	168 (33.1)	518 (34.0)
White	524 (52.7)	272 (54.8)	796 (53.4)	528 (52.0)	269 (53.1)	797 (52.4)
Missing	3 (<1)	0	3 (<1)	0	0	0
Ethnic, n (%)						
Hispanic or Latino	100 (10.1)	51 (10.3)	151 (10.1)	578 (56.9)	284 (56.0)	862 (56.6)
Not Hispanic or Latino	890 (89.5)	443 (89.3)	1333 (89.5)	437 (43.1)	223 (44.0)	660 (43.4)
Missing	4 (<1)	2 (<1)	6 (<1)	0	0	0

	Primary Cohort			Safety Cohort		
Characteristic	**Nirsevimab N=994**	**Placebo N=496**	**Total N=1490**	**Nirsevimab N=1015**	**Placebo N=507**	**Total N=1522**
Baseline Weight, kg						
N	992	496	1488	1014	507	1521
Mean (SD)	5.5 (1.84)	5.6 (1.82)	5.5 (1.83)	5.6 (1.88)	5.6 (1.86)	5.6 (1.87)
Median	5.5	5.6	5.5	5.5	5.6	5.5
IQR	4.0, 6.8	4.1, 6.8	4.0, 6.8	4.2, 7.0	4.1, 7.0	4.1, 7.0
Min, max	1.8, 11.5	1.9, 11.0	1.8, 11.5	1.8, 11.5	2.0, 11.5	1.8, 11.5
Missing	2	0	2	1	0	1
Baseline weight category, n (%)						
Missing weight	2 (<1)	0	2 (<1)	1 (<1)	0	1 (<1)
Weight <5 kg	403 (40.5)	192 (38.7)	595 (39.9)	397 (39.1)	200 (39.4)	597 (39.2)
Weight ≥5 kg	589 (59.3)	304 (61.3)	893 (59.9)	617 (60.8)	307 (60.6)	924 (60.7)
Birth weight category, n (%)						
Missing weight	1 (<1)	0	1 (<1)	0	0	0
Weight ≤2.5 kg	145 (14.6)	88 (17.7)	233 (15.6)	127 (12.5)	52 (10.3)	179 (11.8)
Weight >2.5 kg	848 (85.3)	408 (82.3)	1256 (84.3)	888 (87.5)	455 (89.7)	1343 (88.2)
Gestational age, n (%)						
32	0	1 (<1)	1 (<1)	0	0	0
35	50 (5.0)	27 (5.4)	77 (5.2)	51 (5.0)	22 (4.3)	73 (4.8)
36	82 (8.2)	49 (9.9)	131 (8.8)	56 (5.5)	24 (4.7)	80 (5.3)
37	124 (12.5)	57 (11.5)	181 (12.1)	114 (11.2)	64 (12.6)	178 (11.7)
38	202 (20.3)	96 (19.4)	298 (20.0)	215 (21.2)	110 (21.7)	325 (21.4)
39	252 (25.4)	100 (20.2)	352 (23.6)	294 (29.0)	145 (28.6)	439 (28.8)
40	200 (20.1)	120 (24.2)	320 (21.5)	217 (21.4)	108 (21.3)	325 (21.4)
41	73 (7.3)	36 (7.3)	109 (7.3)	61 (6.0)	32 (6.3)	93 (6.1)
42	10 (1.0)	10 (2.0)	20 (1.3)	7 (<1)	2 (<1)	9 (<1)
Missing	1 (<1)	0	1 (<1)	0	0	0
Down syndrome, n (%)						
No	957 (96.3)	479 (96.6)	1436 (96.4)	947 (93.3)	477 (94.1)	1424 (93.6)
Yes	3 (<1)	0	3 (<1)	1 (<1)	0	1 (<1)
Missing	34 (3.4)	17 (3.4)	51 (3.4)	67 (6.6)	30 (5.9)	97 (6.4)
Cystic fibrosis, n (%)						
No	993 (99.9)	495 (99.8)	1488 (99.9)	1012 (99.7)	507 (100.0)	1519 (99.8)
Yes	0	1 (<1)	1 (<1)	3 (<1)	0	3 (<1)
Missing	1 (<1)	0	1 (<1)	0	0	0

	Primary Cohort			Safety Cohort		
Characteristic	**Nirsevimab N=994**	**Placebo N=496**	**Total N=1490**	**Nirsevimab N=1015**	**Placebo N=507**	**Total N=1522**
Region, (%)						
Northern Hemisphere	686 (69.0)	342 (69.0)	1028 (69.0)	804 (79.2)	393 (77.5)	1197 (78.6)
Southern Hemisphere	308 (31.0)	154 (31.0)	462 (31.0)	211 (20.8)	114 (22.5)	325 (21.4)

Source: adsl.xpt Software: SAS
* Information about Country is located in Section 16
Abbreviations: IQR, interquartile range, ITT, Intent-to-treat, N, number of subjects; n, number of subjects with specific characteristic; SD, standard deviation

Missing Data and Trial Discontinuations

Primary Cohort

Of the 1,490 subjects who were randomized, 23 (1.5%) discontinued the trial and did not have their status outcome on Day 151 (primary endpoint). Most discontinuations were due to 'withdrawal' by a parent or by a legal representative. Sixty-one subjects (4.1%) did not complete the trial (Day 361). The missing rate for both the primary endpoint and the end of trial were similar across the treatment groups.

Safety Cohort

Of the 1,522 subjects who were randomized, 26 (1.7%) discontinued and did not have their status outcome on day 151 (primary endpoint). Most of the discontinuations were withdrawal by a parent or by a legal representative. Forty-two subjects (4.1%) did not complete the trial (day 361). The missing rate for both the primary endpoint and the end of trial were similar across the treatment groups.

Table 21. Patient Disposition, Trial 04

	Primary Cohort		Safety Cohort	
Disposition	**Nirsevimab N=994**	**Placebo N=496**	**Nirsevimab N=1015**	**Placebo N=507**
ITT population	994	496	1015	507
Discontinued (Day 151), n (%)	17(1.7)	7(1.4)	15(1.5)	11(2.2)
Death	2(0.2)	0(0.0)	0(0.0)	0(0.0)
Lost to follow-up	2(0.2)*	2(0.4)*	2(0.2)	1(0.2)
Other	3(0.3)	2(0.4)	1(0.1)	1(0.2)
Withdrawal by parent/guardian	10(1.0)	3(0.6)	12(1.2)	9(1.8)
Discontinued trial (Day 361, EOS), n (%)	40(4.0)	21(4.2)	27(2.7)	15(3)
COVID-19 pandemic	3 (0.3)	1 (0.2)	0(0.0)	0(0.0)
Death	4 (0.4)	0(0.0)	1(0.1)	0(0.0)
Lost to follow-up	9 (0.9)	3 (0.6)	7(0.7)	2(0.4)
Due to AE	1 (0.1)	0(0.0)	0(0.0)	0(0.0)
Other	3 (0.3)	3 (0.6)	2(0.2)	1(0.2)
Withdrawal by parent/guardian	20 (2.0)	14 (2.8)	17(1.7)	12(2.4)

Source: asdl.xpt Software: SAS
*includes a subjects identified as lost to follow-up after the database lock was completed (one subject in each treatment group)
Abbreviations: AE, adverse events; COVID-19, coronavirus disease 2019; EOS, end of study; ITT, intent-to-treat; N, number of subjects; n, number of subjects with specific disposition

Overall Outcome and Visualization of the Data (Primary Cohort)

The overall outcome for the population enrolled in Trial 04 is visualized in the scatterplot figure below. The figure shows distribution of both MA RSV LRTI and hospitalization events and considers baseline characteristics such as age and weight. This section illustrates the association between those two baseline factors and the RSV efficacy outcomes. The primary and secondary endpoints, in addition to the subgroup analyses are further discussed in subsequent sections.

Because infants younger than 6 months of age are at higher risk for MA RSV LRTI, and because of dependence of the nirsevimab dose on body weight, the examination of the relationship

between baseline age, weight, and occurrence of MA RSV LRTI events is important. See the scatter plot (Figure 8).

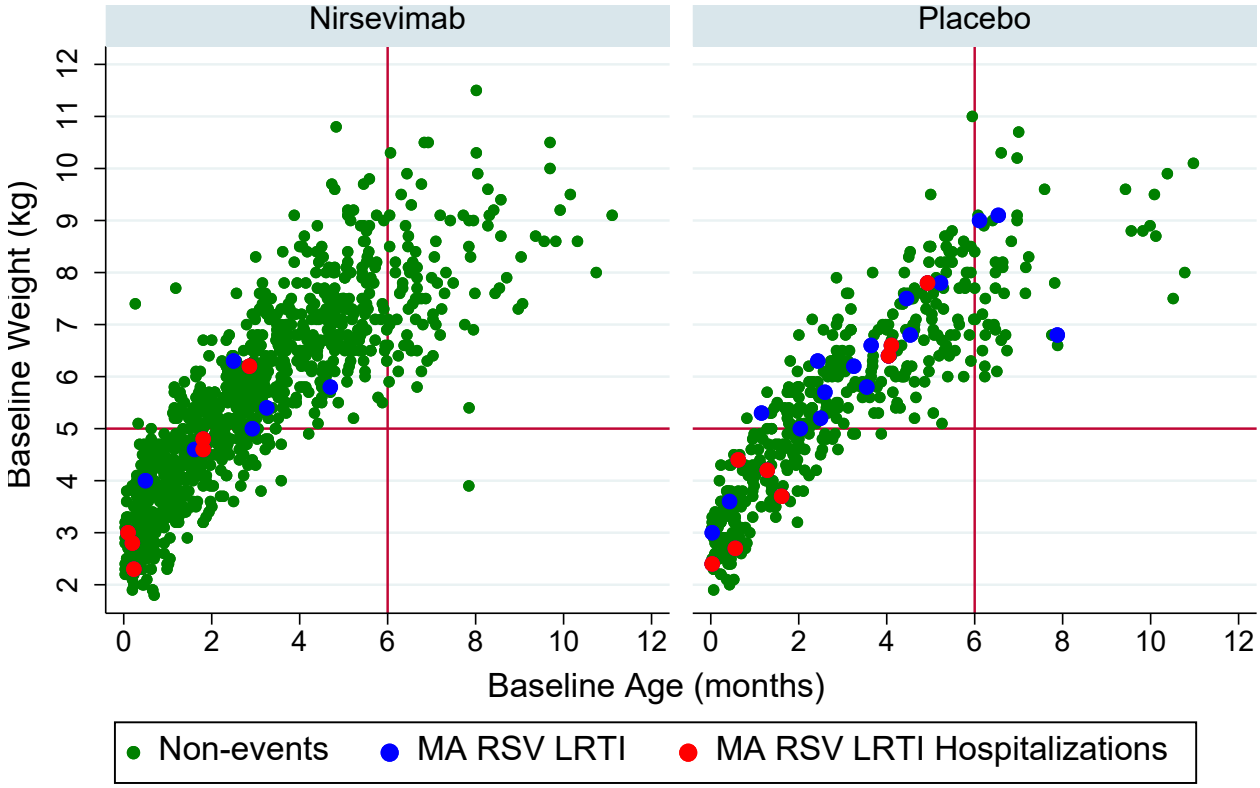
Baseline body weight (<5 kg versus ≥5 kg) essentially followed chronological age because, as generally expected, most infants weighing less than 5 kg are younger than 4 months of age. Only one subject older than 6 months weighed less than 5 kg at baseline. Thus, all other subjects older than 6 months who were randomized to nirsevimab received a 100-mg dose.

Overall, more subjects in the placebo group experienced MA RSV LRTI events (with or without hospitalization) than nirsevimab-treated subjects. The events mostly occurred in infants who were 6 months of age or younger at the time of enrollment. Events of MA RSV LRTI were less common in subjects older than 6 months of age in both treatment groups. With respect to the hospitalization endpoint, there were no hospitalization events in either treatment group among infants who were older than 6 months of age at the time of randomization. Because of the low number of MA RSV LRTI events in subjects who were 6 months of age or older at the time of randomization, there is limited data from which to draw definitive conclusions about the efficacy of nirsevimab in this chronological age subgroup.

As shown in Figure 8, there were only a few subjects who were older than 8 months of age at the time of enrollment (25 on nirsevimab and 10 on placebo). None of those subjects experienced MA RSV LRTI during the first 150 days postdose in the trial.

In addition, no nirsevimab-treated subjects weighing 7 kg or more at baseline experienced MA RSV LRTI, while subjects weighing 9 kg or less on placebo experienced events in all weight categories. Baseline body weight (<5 kg versus ≥5 kg) essentially followed chronological age because, as generally expected, most infants weighing less than 5 kg are younger than 4 months of age. Only one subject older than 6 months weighed less than 5 kg at baseline. Thus, all other subjects older than 6 months who were randomized to nirsevimab received a 100-mg dose. None of the nirsevimab-treated subjects older than 6 months experienced an MA RSV LRTI event. There were 3 MA RSV LRTI events among subjects on placebo who were older than 6 months at baseline.

Figure 8. Primary Cohort: Relationship Between Baseline Weight, Baseline Age, and Outcome Status (Day 151), Trial 04



Source: FDA statistical reviewer
Legend: Relationship between baseline weight and baseline age for subjects who did not experience MA RSV LRTI (green circles), subjects who experienced MA RSV LRTI without hospitalization (blue circles), and those who experienced MA RSV LRTI and were hospitalized (red circles). Each circle represents age and weight at baseline so that each subject is represented only once.
Abbreviation: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

Additional graphical exploration is presented in Section 16.

Refer to Section 16 for analyses and results for the pooled (Primary and Safety) cohort.

Primary Endpoint Results (for the Primary Cohort)

The number of subjects who experienced MA RSV LRTI by Day 151 in each of the treatment arms and the results of the analysis of the primary endpoint are presented in Table 22. The estimated relative risk reduction (RRR) was 74.9% (95%CI: 50.6% to 87.3%) with p-value<0.0001 in favor of nirsevimab. Further exploration of MA RSV LRTI incidence in subgroups is presented in this section.

Table 22. Primary Cohort: Incidence of MA RSV LRTI by Day 150 Postdose (Primary Endpoint), Trial 04

	Trial 04 Primary Cohort	
Statistic	**Nirsevimab N=994**	**Placebo N=496**
Events (# of subjects, n (%))	12(1.2)	25(5.0)
Subjects requiring imputation* n (%)	16(1.6)	7(1.4)
RRR (95% CI)§	74.9% (50.6% to 87.3%) p<0.0001	

Source: adeff3.xpt; tool: SAS
* Subjects with missing outcomes on day 151. The final status of those subjects was imputed based on the observed placebo rate conditional on stratification factors using multiple imputation approach. Includes subjects identified as lost to follow-up after the database lock was completed (one subject in each treatment group)
§ RRR based on initial Primary Cohort (assuming 2 lost to follow-up subjects as nonevents) 74.0%, 95%CI (49.6% to 87.1%)
Abbreviation: CI, confidence interval; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; n, number of subjects with specific incident; RRR, relative risk reduction

Sensitivity Analysis

A sensitivity analysis that repeated the primary analysis with an additional assumption that all subjects on nirsevimab who had a missing outcome (i.e., subjects requiring imputation) had their outcomes imputed as events, yielded a relative risk reduction of 44.8% with 95%CI (6.7%, 67.3%) in favor of nirsevimab. This result suggests that the outcome of the primary analysis was robust.

Secondary Endpoint Results (for the Primary Cohort)

The number of subjects who experienced MA RSV LRTI and were hospitalized by Day 150 postdose in each of the treatment arms and the results of the analysis of the primary endpoint are presented in Table 23. The estimated relative risk reduction (RRR) was 60.2% (95%CI: -14.6%, 86.2%) with p-value=0.09. Of note, few subjects in both treatment and placebo arms were hospitalized due to RSV (eight subjects on nirsevimab and six subjects on placebo).

Table 23. Primary Cohort: Incidence of RSV Hospitalization Events by Day 150 Postdose (Secondary Endpoint), Trial 04

	Trial 04 Primary Cohort	
Statistic	**Nirsevimab N=994**	**Placebo N=496**
Events (# of subjects, n (%))	6(0.6)	8(1.6)
Subjects requiring imputation*, n (%)	16(1.6)	7(1.4)
RRR (95% CI)§	60.2% (-14.6% to 86.2%) p=0.09	

Source: FDA statistical reviewer; adeff3.xpt; tool: SAS
* Subjects with missing outcomes on day 151. The final status of those subjects was imputed based on the observed placebo rate conditional on stratification factors using multiple imputation approach. Includes subjects identified as lost to follow-up after the database lock was completed (one subject in each treatment group)
§ RRR based on initial Primary Cohort (assuming 2 lost to follow-up subjects as nonevents) 62.1% (95%CI: -9.1%, 86.9%)
Abbreviations: CI, confidence interval; N, number of subjects; n, number of subjects with specific event; RRR, relative risk reduction; RSV, respiratory syncytial virus

Exploratory Analyses (for the Primary Cohort)

Subgroup Analyses

The treatment effects based on the incidence of MA RSV LRTI (primary endpoint) were consistent across subgroups and with the overall treatment effect. There were no events in the subgroup of subjects older than 6 months among participants treated with nirsevimab. Similarly, there were no events in the Southern Hemisphere. Because of this, the outcomes in those subgroups could not be evaluated. The detailed subgroup results are presented in Section 16.

6.2.4. Trial 05

Trial 05 is a phase 2/3, randomized, double-blind, active-controlled, safety and PK trial of nirsevimab compared to palivizumab for the prevention of RSV in subjects with greatest risk for severe RSV disease (high-risk infants). The trial enrolled infants born at ≤35 weeks of gestation (including infants <29 weeks of gestation) and infants with CLD of prematurity and/or hemodynamically significant CHD, who were born or entering their first or second RSV season.

The primary objective of Trial 05 was to evaluate the safety and tolerability of nirsevimab compared to palivizumab when administered to preterm infants and to infants with CLD of prematurity or hemodynamically significant CHD entering their first or second RSV season.

The secondary objectives were to:

a) To evaluate serum concentrations of nirsevimab (also known as MEDI8897),
b) To evaluate anti-drug antibodies (ADA) responses to nirsevimab in serum, and
c) To assess the descriptive efficacy of nirsevimab in reducing MA LRTI and hospitalization due to RT-PCR-confirmed RSV, compared to palivizumab.

Trial 05 was not designed to demonstrate efficacy through inferential statistics in this high-risk population. Efficacy in this population was to be demonstrated by extrapolation of efficacy from Trial 03 and Trial 04 to the trial population of Trial 05, provided that the exposures are comparable between the populations enrolled in Trial 03 and 04, and Trial 05. Please see Section 6.3.3 for a discussion of extrapolation of efficacy.

6.2.4.1. Design, Trial 05

Trial 05 is a phase 2/3, randomized, double-blind, active-controlled, safety and PK trial comparing nirsevimab and palivizumab in infants at high risk of developing RSV LRTI. Infants who were eligible for palivizumab, as defined in the palivizumab package insert, were enrolled in two cohorts. One cohort (preterm cohort) included infants born at ≤35 weeks gestational age, while a second cohort included infants with CLD of prematurity or hemodynamically significant CHD. The preterm cohort participated during the first RSV season and the safety follow-up period of the trial; subjects in the CLD/CHD cohort participated in the first and second RSV seasons as well as in the safety follow-up periods for both seasons. Subjects were randomized in a 2:1 ratio to receive nirsevimab or palivizumab.

Subjects in both cohorts received trial drug during the first RSV season of the trial. Subjects randomized to nirsevimab received a single dose of nirsevimab followed by 4 once-monthly IM doses of placebo. Nirsevimab was administered according to body weight, with subjects weighing <5 kg at the time of dosing receiving a single 50 mg IM (0.5 mL) dose and those weighing ≥5 kg receiving a single 100 mg IM (1.0 mL) dose. Subjects in the palivizumab arm received 5 once-monthly doses of 15/mg palivizumab, as labeled.

Only subjects from the CLD/CHD cohort were enrolled and dosed during the second RSV season. Subjects with CLD/CHD who received nirsevimab during the first RSV season of the trial received a single dose of nirsevimab and 4 doses of placebo in the second RSV season. Subjects with CLD/CHD who received palivizumab in the first RSV season were rerandomized to receive either a single dose of nirsevimab plus 4 doses of placebo or to receive 5 monthly doses of palivizumab in the following second RSV season. Nirsevimab was administered as a single 200 mg IM dose. The protocol included instructions for redosing with trial drug in subjects who underwent cardiac surgery with cardiopulmonary bypass in either RSV season. (See discuss of replacement doses in Section 6.1.3)

Subjects in the preterm cohort were followed through one year after the first dose for the first RSV season. Subjects in the CLD/CHD cohort were followed through one year after administration of nirsevimab for the second RSV season.

The definition of MA RSV LRTI was the same as used in Trials 03 and 04, except in infants with underlying lung disease. In infants with underlying lung disease the definition of LRTI was at least one new or worsened physical examination findings of rhonchi, rales, crackles, or wheezing PLUS at least one of the following:

- Increase in respiratory rate by ≥20% at rest and that rate is greater than age-based criteria provided in the LRTI definition above,
- Hypoxemia (O2 saturation <95% in room air, or O2 saturation drop of 5 percentage points from baseline in subjects with baseline O2 saturation <95% on room air, or acute documented need for supplemental oxygen, or increased oxygen required compared to baseline), OR
- Clinical signs of severe respiratory disease as described above with the addition of prescription of new or increased from baseline dose of medications including bronchodilators, steroids, diuretics, or cardiac medications.

Safety assessments were performed through Day 365 for both RSV seasons.

Blood samples for PK and for anti-drug antibody were collected at screening or on Day 1 Predose; on Days 31, 151, and 361; and at the time of hospitalization for subjects hospitalized with a respiratory infection through Day 361 in each season.

Statistical Analysis

Trial 05 was a double-blind, active (palivizumab)-controlled trial. Nirsevimab was administered once during each RSV season, and palivizumab was administered up to five times (once a month) during the RSV season. Subjects who were in the nirsevimab arm received normal saline IM to maintain blinding for palivizumab in months 2 to 5.

Subjects were stratified by hemisphere and by subject age (≤3 months, >3 months to ≤6 months, or >6 months). All subjects were randomized in a 2:1 ratio to receive a either nirsevimab or palivizumab.

The trial was originally designed to enroll 1,500 subjects. However, because of the global COVID-19 pandemic, enrollment was disrupted, and the Agency agreed to a small trial size of approximately 600 subjects. A sample size of 600 subjects provided a 95% probability of observing at least one adverse event if the true event rate was 0.5%.

The primary endpoint was safety and tolerability of nirsevimab as assessed by the occurrence of treatment-emergent adverse events (AE), serious adverse events, AEs of special interest, and NOCDs.

6.2.4.2. Eligibility Criteria, Trial 05

Infants were enrolled prior to entering their first RSV season.

In the preterm cohort subjects must have been in their first year of life and must have been eligible for palivizumab according to national or local guidelines. Infants in this cohort must have been born at ≤35 weeks of gestational age. These infants may have had the following types of CHD: uncomplicated small atrial or ventricular septal defects, patent ductus arteriosus (PDA), aortic stenosis, pulmonic stenosis or coarcation of the aorta if these defects occur alone.

In the CLD/CHD cohort:

- Subjects with CLD must have been in their first year of life and have a diagnosis of CLD of prematurity requiring medical intervention (i.e., supplemental oxygen, bronchodilators, or diuretics) within 6 months prior to randomization.
- Subjects with CHD must have been in their first year of life and have documented hemodynamically significant CHD (must be unoperated or partially corrected). Infants with hemodynamically significant acyanotic cardiac lesions must have had either pulmonary hypertension or the need for daily medication to manage CHD.

Pertinent exclusion criteria include the following:

- Previous receipt of palivizumab, other RSV monoclonal antibody, or any infant RSV vaccine including mother's receipt of a maternal RSV vaccination,
- Known history of RSV infection or active RSV infection prior to or at the time of randomization,
- Clinically significant anomaly of the respiratory tract,
- Anticipated cardiac surgery within 2 weeks of randomization,
- Any history of LRTI or active LRTI prior to or at the time of randomization,
- Any fever (≥100◦ F or ≥38◦ C, regardless or route) or acute illness within 7 days prior to randomization,

- Hospitalization at the time of randomization, unless discharge is expected within 7 days after randomization,
- Requirement for mechanical ventilation, ECMO, CPAP, or other mechanical respiratory or cardiac support at the time of randomization,
- Anticipated survival of <6 months after randomization,
- Known renal impairment or hepatic dysfunction including known or suspected hepatitis infection,
- Chronic seizure disorder or evolving unstable neurologic disorder,
- Prior history of a suspected or actual acute life-threatening event,
- HIV-infected mother unless the child has been proven to be uninfected,
- Receipt of immunosuppressive agents including steroids, except for the use of topical steroids according to the judgement of the investigator,
- History of receipt of blood, blood products, immunoglobulin products, or monoclonal / polyclonal antibodies, or
- Any known allergy or history of allergic reaction.

6.2.4.3. Results of Analyses, Trial 05

Results for Trial 05 are presented by RSV season.

First RSV Season

Demographic data and clinical characteristics at baseline are provided in Table 24. The results are provided for the entire trial population (N=918) as well as for the two study cohorts, preterm infants and infants with CLD/CHD. The majority of subjects were enrolled in the preterm cohort (N=612 or 66.7%); 306 subjects (33.3%) were enrolled in the CLD/CHD cohort. The mean age at baseline was 3.9 months in the nirsevimab arm and 3.8 months in the palivizumab arm; the chronological age range was 0.07 months to 11.14 months, and 0.07 months to 12.25 months, for the nirsevimab and palivizumab arms, respectively. The majority of subjects were 6 months of age or younger at baseline (78.5% in the nirsevimab arm and 79.3% in the palivizumab arm). The majority of subjects were White (79%) and non-Hispanic or Latino (85%) and were enrolled in the Northern Hemisphere (92%) including 116 subjects (13%) enrolled in the United States.

Baseline demographic and clinical characteristics were similar between the nirsevimab arm and palivizumab arm for the overall population, the preterm cohort, and the CLD/CHD cohort. Subjects in the CLD/CHD cohort were slightly older than in the preterm cohort. The mean age in the preterm cohort was 3.5 months in both the nirsevimab arm and palivizumab arm, while the mean age was 4.9 months in the nirsevimab arm and 4.5 months in the palivizumab arm in the CLD/CHD cohort. There was a higher percentage of Black or African American subjects in the premature cohort (12%) compared to the CLD/CHD cohort (5%). Prematurity is more common in Blacks and African Americans than in other races (March of Dimes 2022). More subjects in the CLD/CHD cohort (21%) than in the preterm cohort (8%) were enrolled in the U.S.; this may

have been related to the availability of specialized care centers in the U.S., such as pediatric cardiac surgery units.

In the CLD/CHD cohort, the majority of subjects had CLD (146 or 70% in the nirsevimab arm and 68 or 69% in the palivizumab arm). The percentages of subjects with CLD and CHD were similar in the two trial arms.

Many of the subjects in the CLD/CHD cohort were born prematurely: 69% of CLD/CHD subjects in the nirsevimab arm and 65% in the palivizumab arm were born at <35 weeks GA. Overall, in both the preterm and CLD/CHD cohorts together, 196 infants (21% of trial population) were born at <29 weeks of gestational age (21% in the nirsevimab arm and 22% in the palivizumab arm). This number is sufficient to assess PK and safety in extremely premature infants.

Overall, the baseline demographic and clinical characteristics were similar between the nirsevimab and palivizumab arms in both the overall population and two study cohorts. Therefore, the results should not have been affected by any differences in baseline characteristics.

Table 24. Baseline Demographic and Clinical Characteristics, Safety Population*, Trial 05 – Season 1

	Overall		Preterm		CLD/CHD	
Characteristic	**NIRS N=614**	**PALI N=304**	**NIRS N=406**	**PALI N=206**	**NIRS N=208**	**PALI N=98**
Sex, n (%)						
Female	296 (48.2)	131 (43.1)	201 (49.5)	92 (44.7)	95 (45.7)	39 (39.8)
Male	318 (51.8)	173 (56.9)	205 (50.5)	114 (55.3)	113 (54.3)	59 (60.2)
Age, months						
Mean (SD)	3.9 (2.6)	3.8 (2.5)	3.5 (2.4)	3.5 (2.4)	4.9 (2.7)	4.5 (2.4)
Median (min, max)	3.5 (0.1, 11.1)	3.5 (0.1, 12.3)	2.9 (0.1, 11.1)	2.8 (0.1, 10.3)	4.8 (0.2, 11.1)	4.3 (0.3, 12.3)
Age group, months, n (%)						
≤3.0 months	273 (44.5)	140 (46.1)	214 (52.7)	111 (53.9)	59 (28.4)	29 (29.6)
>3.0 to ≤6.0 months	209 (34.0)	101 (33.2)	125 (30.8)	59 (28.6)	84 (40.4)	42 (42.9)
>6.0 months	132 (21.5)	63 (20.7)	67 (16.5)	36 (17.5)	65 (31.2)	27 (27.6)
Race, n (%)						
American Indian or Alaska Native	11 (1.8)	5 (1.6)	11 (2.7)	5 (2.4)	0	0
Asian	36 (5.9)	14 (4.6)	26 (6.4)	9 (4.4)	10 (4.8)	5 (5.1)
Black or African American	59 (9.6)	29 (9.5)	49 (12.1)	24 (11.7)	10 (4.8)	5 (5.1)
Multiple	6 (1.0)	4 (1.3)	3 (0.7)	2 (1.0)	3 (1.4)	2 (2.0)
Native Hawaiian or Other Pacific Islander	4 (0.7)	1 (0.3)	3 (0.7)	1 (0.5)	1 (0.5)	0
Other	17 (2.8)	6 (2.0)	10 (2.5)	6 (2.9)	7 (3.4)	0
White	481 (78.3)	244 (80.3)	304 (74.9)	158 (76.7)	177 (85.1)	86 (87.8)
Missing	0	1 (0.3)	0	1 (0.5)	0	0
Ethnicity, n (%)						
Hispanic or Latino	99 (16.1)	41 (13.5)	77 (19.0)	35 (17.0)	22 (10.6)	6 (6.1)
Not Hispanic or Latino	515 (83.9)	262 (86.2)	329 (81.0)	170 (82.5)	186 (89.4)	92 (93.9)
Missing	0	1 (0.3)	0	1 (0.5)	0	0
Country of participation, n (%)						
Bulgaria	75 (12.2)	42 (13.8)	41 (10.1)	27 (13.1)	34 (16.3)	15 (15.3)
Spain	89 (14.5)	43 (14.1)	80 (19.7)	41 (19.9)	9 (4.3)	2 (2.0)
Latvia	28 (4.6)	10 (3.3)	6 (1.5)	2 (1.0)	22 (10.6)	8 (8.2)
Russia	69 (11.2)	33 (10.9)	31 (7.6)	13 (6.3)	38 (18.3)	20 (20.4)
United States	81 (13.2)	35 (11.5)	33 (8.1)	18 (8.7)	48 (23.1)	17 (17.3)
South Africa	47 (7.7)	26 (8.6)	45 (11.1)	23 (11.2)	2 (1.0)	3 (3.1)
Others	225 (36.6)	115 (37.8)	170 (41.9)	82 (39.8)	55 (26.4)	33 (33.7)
Region of participation, n (%)						
Northern Hemisphere	567 (92.3)	278 (91.4)	361 (88.9)	183 (88.8)	206 (99.0)	95 (96.9)
Southern Hemisphere	47 (7.7)	26 (8.6)	45 (11.1)	23 (11.2)	2 (1.0)	3 (3.1)

Characteristic	Overall		Preterm		CLD/CHD	
	NIRS N=614	PALI N=304	NIRS N=406	PALI N=206	NIRS N=208	PALI N=98
Is in the United States, n (%)						
United States	81 (13.2)	35 (11.5)	33 (8.1)	18 (8.7)	48 (23.1)	17 (17.3)
Non-United States	533 (86.8)	269 (88.5)	373 (91.9)	188 (91.3)	160 (76.9)	81 (82.7)

Source: adsl.xpt; Software: SAS
[1] Difference is shown between [treatment arms] [e.g., difference is shown between (drug name dosage X) vs. placebo].
Safety Population is all subjects who received any study drug, analyzed according to treatment received.
Abbreviations: CHD, congenital heart disease; CI, confidence interval; CLD, chronic lung disease; N, number of subjects in treatment arm/group; n, number of subjects with given characteristic; NIRS, nirsevimab; PALI, palivizumab; SD, standard deviation

A total of 960 infants were screened for trial participation; only 35 infants (3.6%) were screen failures (Table 25). The most common reasons for screen failure were not meeting entry criteria (N=22 or 63% of screen failures) and consent withdrawal (N=9 or 26%). A total of 925 subjects were randomized.

Table 25. Patient Screening and Enrollment, Trial 05

Disposition	Trial 05
Patients screened	960
Screening failures	35
Patients enrolled/randomized	925

Source: ds.xpt and Clinical Study Report; Software: R

Subject Disposition

As shown in Table 26, of the 925 subjects who were enrolled and randomized, 918 subjects received a dose of trial drug: 614 in the nirsevimab arm and 304 in the palivizumab arm. Ninety percent of subjects in the nirsevimab arm and 88% in the palivizumab arm completed treatment. Sixty subjects in the nirsevimab arm (9.7%) and 31 (10%) in the palivizumab arm discontinued treatment prematurely during the first 150 days postdose. For subjects in the nirsevimab arm, these subjects were discontinuing placebo injections, while subjects in the palivizumab arm discontinued monthly doses of palivizumab. The most common reasons for premature discontinuation of treatment were withdrawal by parent/guardian (5% in nirsevimab arm and 5.5% in the palivizumab arm) and COVID-19 (1.9% in the nirsevimab arm and 1.6% in the palivizumab arm). The percentage of subjects who discontinued the trial prematurely (after Day 150 but before Day 361) was 11.9% in the nirsevimab arm and 14.9% in the palivizumab arm. The most common reasons for premature trial discontinuation were withdrawal by parent/guardian (7% in the nirsevimab arm and 9.1% in the palivizumab arm) and loss to follow-up (2.8% in the nirsevimab arm and 2.3% in the palivizumab arm). More subjects in the palivizumab arm of the CLD/CHD cohort discontinued the trial prematurely (18.8%) compared to subjects in the nirsevimab (13.9%); this was mainly due to an increase in withdrawal of consent (12.9% in the palivizumab arm compared to 8.6% in the nirsevimab arm); the reason for this is unclear. Otherwise, the percentages of subjects discontinuing treatment prematurely or discontinuing the trial prematurely was similar between the two arms.

Only one subject discontinued the trial prematurely due to an adverse event. The subject was a 5-month-old white male in the nirsevimab arm who was withdrawn from the trial by the investigator due to a Grade 1 maculopapular rash on Day 93. The rash in this subject occurred after administration of placebo. Deaths that occurred in Trial 05 are discussed in Section 7.7.2 of this review.

Table 26. Subject Disposition, Trial 05 – Season 1

	Overall		Preterm		CLD/CHD	
Disposition Event	NIRS N=614 n (%)	PALI N=304 n (%)	NIRS N=406 n (%)	PALI N=206 n (%)	NIRS N=208 n (%)	PALI N=98 n (%)
Patients randomized	616	309	407	208	209	101
As-Treated population	614	304	406	206	208	98

	Overall		Preterm		CLD/CHD	
Disposition Event	NIRS N=614 n (%)	PALI N=304 n (%)	NIRS N=406 n (%)	PALI N=206 n (%)	NIRS N=208 n (%)	PALI N=98 n (%)
Discontinued treatment	60 (9.7)	31 (10)	35 (8.6)	15 (7.2)	25 (12)	16 (15.8)
Adverse event	1 (0.2)	0	1 (0.2)	0	0	0
Covid-19	12 (1.9)	5 (1.6)	10 (2.5)	4 (1.9)	2 (1)	1 (1)
Death	5 (0.8)	1 (0.3)	2 (0.5)	0	3 (1.4)	1 (1)
Lost to follow-up	8 (1.3)	2 (0.6)	3 (0.7)	0	5 (2.4)	2 (2)
Other	3 (0.5)	6 (1.9)	3 (0.7)	3 (1.4)	0	3 (3)
Withdrawal by parent/guardian	31 (5)	17 (5.5)	16 (3.9)	8 (3.8)	15 (7.2)	9 (8.9)
Discontinued trial	73 (11.9)	46 (14.9)	44 (10.8)	27 (13)	29 (13.9)	19 (18.8)
Covid-19	2 (0.3)	3 (1)	1 (0.2)	3 (1.4)	1 (0.5)	0
Death	5 (0.8)	1 (0.3)	2 (0.5)	0	3 (1.4)	1 (1)
Lost to follow-up	17 (2.8)	7 (2.3)	12 (2.9)	5 (2.4)	5 (2.4)	2 (2)
Other	6 (1)	7 (2.3)	4 (1)	4 (1.9)	2 (1)	3 (3)
Withdrawal by parent/guardian	43 (7)	28 (9.1)	25 (6.1)	15 (7.2)	18 (8.6)	13 (12.9)

Source: ds.xpt and adsl.xpt; Software: SAS
Duration is 5 months.
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; COVID-19, coronavirus disease 2019; N, number of subjects in treatment arm; n, number of subjects in specified population or group; NA, not applicable; NIRS, nirsevimab; PALI, palivizumab

Protocol Deviations

Major protocol deviations were reported in a similar percentage of subjects in the nirsevimab arm (18%) and in the palivizumab arm (17.8%) (Table 27). The Applicant divides the major protocol deviations as non-COVID and COVID-related. The first subject was enrolled in Trial 05 in July 2019 and the trial continued to enroll subjects into 2020 during the COVID-19 pandemic. COVID-related major protocol violations during the first RSV season were reported in 12.8% of subjects in the nirsevimab arm and in 13.3% of subjects in the palivizumab arm. Individual major protocol violations reported in more than 2% of subjects are provided in the following table. Fifty-three infants (8.6%) in the preterm cohort did not meet the definition for inclusion in preterm cohort, which was infants in first year of life, born at ≤35 weeks GA, and eligible to receive palivizumab in accordance with national or local guidelines. The overwhelming majority of these infants did not meet the national or local guidelines to receive palivizumab including infants in the U.S. who were not enrolled according to the American Academy of Pediatrics guidelines as instructed in the trial protocol. Although the percentage is similar in the two arms (9.3% in the nirsevimab arm and 7.2% in the palivizumab arm), this may be one reason that more subjects born at <29 weeks of gestational age were not enrolled. Missed doses were observed in both arms; a similar percentage of missed doses were observed in the non-COVID violations and in the COVID-related violations. When the missed doses are added, 10.4% of subjects in the nirsevimab arm missed a dose of trial drug/placebo and 9.1% of subjects in the palivizumab arm missed a dose of trial drug. Because nirsevimab was administered on Day 1 of the trial and subsequent doses of trial drug were placebo; missed doses did not affect subjects in the nirsevimab arm. However, subjects in the palivizumab missed active drug. This could have resulted in decreased efficacy of palivizumab. However, because of the decreased circulation of RSV with COVID-19, there were few events of MA RSV LRTI in either arm, and the impact on efficacy may have been minimal.

Table 27. Individual Major Protocol Violations Reported in >2% of Subjects, Trial 05 – Season 1 (ITT Population)

	Overall		Preterm		CLD/CHD	
Protocol Violation	**NIRS N=616**	**PALI N=309**	**NIRS N=407**	**PALI N=208**	**NIRS N=209**	**PALI N=101**
Definition of preterm infant	38 (6.2%)	15 (4.9%)	38 (9.3%)	15 (7.2%)	N/A	N/A
Missed dose*	37 (6.0%)	15 (4.9%)	23 (5.7%)	11 (5.3%)	14 (6.7%)	4 (4.0%)
Missed dose due to COVID-19	27 (4.4%)	13 (4.2%)	19 (4.7%)	11 (5.3%)	8 (3.8%)	2 (2.0%)

Source: BLA 761328, CSR, Trial 05, Table 14, pages 78-81
ITT population was defined as all randomized subjects, analyzed according to randomized treatment assignment.
*Missed doses in the nirsevimab arm were missed doses of the placebo. Nirsevimab was administered as the first dose and placebo was administered for the remaining four doses to match palivizumab dosing.
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; COVID-19, coronavirus disease 2019; N, number of subjects in treatment arm; n, number of subjects in specified population or group; N/A, not applicable; NIRS, nirsevimab; PALI, palivizumab

Efficacy Results (Descriptive)

MA RSV LRTI was reported in 4/616 (0.6%) subjects (95% CI: (0.18, 1.65) who received nirsevimab and in 3/309 (1.0%) subjects who received palivizumab (95% CI: (0.20, 2.81) through Day 150 postdose for the first RSV season. Three events of MA RSV LRTI occurred in the preterm cohort, 2 in the nirsevimab arm and one in the palivizumab arm (0.5% in each arm). One infant born at <29 weeks gestational age in each nirsevimab and palivizumab arms experienced MA RSV LRTI event. There were two events of MA RSV LRTI in each trial arm of the CLD/CHD cohort (1.0% in the nirsevimab arm and 2.0% in the palivizumab arm).

RSV hospitalization through Day 150 were reported in 2 (0.3%) subjects in the nirsevimab arm and in 2 (0.6%) subjects in the palivizumab arm. All four RSV hospitalizations were reported in subjects in the CLD/CHD cohort.

Overall, there were few events of MA RSV LRTI –including RSV hospitalization –reported in Trial 05; numerically and proportionally, the incidence of MA RSV LRTI and RSV hospitalization was similar between the nirsevimab and palivizumab arm. Efficacy in this population was established by extrapolation of efficacy from Trial 03 and Trial 04 to the Trial 05 population by demonstration of comparable nirsevimab exposures across the populations enrolled in the three trials. See Section 6.3.3.

Second RSV Season

Subjects in the CLD/CHD cohort were to continue in the trial through their second RSV season. Of the subjects who participated in the first season, 180 (87%) in the nirsevimab arm and 82 (84%) in the palivizumab arm continued into the second RSV season. The 180 subjects who received nirsevimab in the first season also received nirsevimab prior to the second RSV. The 82 subjects who received palivizumab in the first RSV season were randomized to receive nirsevimab (N=40) or palivizumab (N=42) in the second RSV season. The baseline demographics and clinical characteristics of subjects in the second RSV season are shown in Table 28. The majority of subjects in both arms were male (56.4% in the nirsevimab arms and 64.3% in the palivizumab arm) and White (85% in the nirsevimab arms and 90.5% in the palivizumab arm), and non-Hispanic or Latino (90.5% in the nirsevimab arms and 95.2% in the

palivizumab arm). As in the first RSV season, most subjects were enrolled in the Northern Hemisphere (98.6% in the nirsevimab arms and 97.6% in the palivizumab arm). More subjects in the nirsevimab arms (20%) than in the palivizumab arm (9.5%) were enrolled in the U.S. Because the palivizumab arm in the second RSV season is considerably smaller than the nirsevimab arms, it is difficult to reach any definite conclusions regarding differences between the two trial arms. However, overall, the baseline demographic and clinical characteristics were similar between the nirsevimab arms and the palivizumab arms.

Table 28. Baseline Demographic and Clinical Characteristics, Safety Population, Trial 05 – Season 2

Characteristic	NIRS/NIRS N=180	PALI/NIRS N=40	PALI/PALI N=42	All NIRS N=220
Sex, n (%)				
Female	81 (45.0)	15 (37.5)	15 (35.7)	96 (43.6)
Male	99 (55.0)	25 (62.5)	27 (64.3)	124 (56.4)
Age, months				
Mean (SD)	4.9 (2.7)	4.7 (2.5)	4.2 (2.1)	4.9 (2.7)
Median (min, max)	4.8 (0.2, 11.1)	4.6 (0.7, 10.5)	4 (0.7, 8)	4.8 (0.2, 11.1)
Age group, months, n (%)				
≤3.0 months	49 (27.2)	10 (25.0)	14 (33.3)	59 (26.8)
>3.0 to ≤6.0 months	76 (42.2)	19 (47.5)	19 (45.2)	95 (43.2)
>6.0 months	55 (30.6)	11 (27.5)	9 (21.4)	66 (30.0)
Race, n (%)				
Asian	10 (5.6)	3 (7.5)	2 (4.8)	13 (5.9)
Black or African American	9 (5.0)	2 (5.0)	1 (2.4)	11 (5.0)
Multiple	3 (1.7)	0	1 (2.4)	3 (1.4)
Native Hawaiian or Other Pacific Islander	1 (0.6)	0	0	1 (0.5)
Other	5 (2.8)	0	0	5 (2.3)
White	152 (84.4)	35 (87.5)	38 (90.5)	187 (85.0)
Ethnicity, n (%)				
Hispanic or Latino	19 (10.6)	2 (5.0)	2 (4.8)	21 (9.5)
Not Hispanic or Latino	161 (89.4)	38 (95.0)	40 (95.2)	199 (90.5)
Country of participation, n (%)				
Bulgaria	28 (15.6)	8 (20.0)	6 (14.3)	36 (16.4)
Estonia	8 (4.4)	4 (10.0)	5 (11.9)	12 (5.5)
Latvia	20 (11.1)	1 (2.5)	5 (11.9)	21 (9.5)
Poland	8 (4.4)	4 (10.0)	1 (2.4)	12 (5.5)
Russia	36 (20.0)	9 (22.5)	8 (19.0)	45 (20.5)
United States	38 (21.1)	6 (15.0)	4 (9.5)	44 (20.0)
Others	42 (23.3)	8 (20)	13 (31)	50 (22.7)
Region of participation, n (%)				
Northern Hemisphere	178 (98.9)	39 (97.5)	41 (97.6)	217 (98.6)
Southern Hemisphere	2 (1.1)	1 (2.5)	1 (2.4)	3 (1.4)
Is in United States, n (%)				
United States	38 (21.1)	6 (15.0)	4 (9.5)	44 (20.0)
Non-United States	142 (78.9)	34 (85.0)	38 (90.5)	176 (80.0)

Source: adsl.xpt; Software: R
Safety Population is all subjects who received any study drug, analyzed according to treatment received.
Abbreviations: N, number of subjects in treatment group; n, number of subjects with given characteristic; NIRS, nirsevimab; PALI, palivizumab; SD, standard deviation

Trial follow-up was available through Day 150 postdose for all subjects from the second RSV season. Subjects who discontinued the trial prior to Day 151 were considered to have

prematurely discontinued treatment. A total of 12 subjects discontinued treatment through Day 150: 9 (4.1%) in the nirsevimab arms and 3 (7.1%) in the palivizumab arm. See Table 29. The main reasons for discontinuing treatment before Day 150 were withdrawal by parent/guardian and other. Other reasons included subjects who moved, need for surgery, parent refusal, and trial personnel error. Although the interim CSR for the second RSV season includes safety data through Day 150, premature trial discontinuation data was provided for subjects who discontinued the trial after Day 150 of the second season. Subjects who discontinued the trial after Day 151 were considered to have prematurely discontinued from the trial. Seven subjects (3.2%) in the nirsevimab arms and 2 (4.8%) in the palivizumab arms discontinued the trial prematurely. The main reason for discontinuing the trial after Day 150 was withdrawal by parent/guardian. According to the Applicant, 96% of subjects in the nirsevimab arms and 95% in the palivizumab arm completed the trial through Day 150 postdose.

Table 29. Subject Disposition, Trial 05 - Season 2

Disposition Event	NIRS/NIRS N=180 n (%)	PALI/NIRS N=40 n (%)	PALI/PALI N=42 n (%)	All NIRS N=220 n (%)
Patients randomized	180	40	42	220
Safety population	180	40	42	220
Discontinued treatment	7 (3.9)	2 (5)	3 (7.1)	9 (4.1)
Lost to follow-up	1 (0.6)	0	0	1 (0.5)
Other	3 (1.7)	1 (2.5)	2 (4.8)	4 (1.8)
Withdrawal by parent/guardian	3 (1.7)	1 (2.5)	1 (2.4)	4 (1.8)
Discontinued trial	6 (3.3)	1 (2.5)	2 (4.8)	7 (3.2)
Lost to follow-up	1 (0.6)	0	1 (2.4)	1 (0.5)
Other	2 (1.1)	0	0	2 (0.9)
Withdrawal by parent/guardian	3 (1.7)	1 (2.5)	1 (2.4)	4 (1.8)

Source: ds.xpt and adsl.xpt; Software: R
Duration is 5 months.
Safety Population is all subjects who received any study drug, analyzed according to treatment received.
Abbreviations: N, number of subjects in treatment arm; n, number of subjects in specified population or group; NIRS, nirsevimab; PALI, palivizumab

Protocol Deviations

There were no major protocol violations reported in the palivizumab arm. Major protocol violations were reported in 14 subjects (6.4%) in the nirsevimab arms. The most commonly reported major protocol violation was missed dose. In the nirsevimab arm, the missed dose was a missed placebo dose, while in the palivizumab arm a missed dose would be a missed palivizumab dose. Missed dose was reported in 4 subjects (1.8%) in the nirsevimab arms and in none of the subjects in the palivizumab arm.

The entire second RSV season was conducted after the start of the COVID-19 pandemic. Thirteen subjects (5%) had a major protocol violation related to the COVID-19 pandemic, including 10 subjects (4.5%) in the nirsevimab arm and 3 (7.1%) in the palivizumab arm. The most commonly reported major protocol violation due to COVID-19 was a delayed visit (3.2% in the nirsevimab arms and 4.8% in the palivizumab arm). Only one subject in the nirsevimab arms and one subject in the palivizumab arm missed a trial visit. According to the Applicant, while some subjects discontinued trial drug prematurely and missed dose(s) of trial drug, no

subjects missed a dose of trial drug due to the COVID-19 pandemic. Overall, there was minimal disruption to the trial due to COVID-19.

Efficacy Results (Descriptive)

There were no events of MA RSV LRTI or of RSV hospitalization in the second year of Trial 05. This is likely due to the decreased circulation of RSV during the COVID-19 pandemic.

See Section 6.3.3 for a discussion of extrapolation of efficacy from Trial 03 and the Primary Cohort of Trial 04 to Trial 05.

6.3. Key Efficacy Review Issues

6.3.1. Efficacy of Nirsevimab in Prevention of MA RSV LRTI in Neonates and Infants Born During or Entering Their First RSV Season: Assessment by Chronological and Gestational Age

Issue

While the results of Trial 03 and 04 provide overall persuasive evidence of effectiveness for nirsevimab, the benefit of nirsevimab in preventing MA RSV LRTI disease in first year of life is unclear when assessed by baseline chronological age (i.e., birth to 12 months of age) and gestational age (<29 weeks of gestation through ≥38 weeks of gestation).

Background

The Applicant proposed an indication for the prevention of MA RSV LRTI disease in neonates and infants born during or entering their first RSV season.

Infants born during or entering their first RSV season were enrolled in Trial 03 and Trial 04. Infants up to 12 months of age were enrolled in both trials. Infants born at ≥29 weeks to <35 weeks of gestation were enrolled in Trial 03; infants born at ≥35 weeks of gestation were enrolled in Trial 04. Infants born at <35 weeks of gestation (including <29 weeks of gestation) were enrolled in Trial 05, which is discussed in the following section.

Severe RSV disease is more common with younger infants, in premature infants, and in infants with underlying comorbidities, such as CLD of prematurity and hemodynamically significant CHD. In a prospective, surveillance study conducted at three New Vaccine Surveillance Network (NVSN) sites during the 2002 to 2004 RSV seasons, information on outpatient visits for RSV was collected (Lively et al. 2019). The highest rates of emergency department visits were in infants who were 4 months of age (116 per 1,000 children) and the highest rates for pediatric office visits were at 5 months of age (289.2 per 1,000 children). In a review of both the literature and a national claims database, Paramore et al. 2010 reported the range for rates of outpatient visits in term infants as 128.8 to 171.3 visits per 1,000 children.

The timing for nirsevimab administration will also be influenced by whether the infant lives in a tropical/subtropical or temperate area of the United States. In tropical/subtropical climates, RSV circulates throughout the year, and infants could receive nirsevimab shortly after birth. In temperate climates, RSV occurs as seasonal outbreaks that typically last for 5 months.

Infants in temperate climates who are born immediately before or during the RSV season (~November through March) would receive nirsevimab shortly after birth. Infants born outside of the RSV season (April through October) would have an age range of 1 to 7 months before they enter their RSV season. As a result, nearly all infants in both temperate and tropical/subtropical climates would have experienced their first RSV season (and potentially exposed to RSV) by 7 months of age. Infants 8 months of age and above would have received nirsevimab during their first RSV season or would already have likely been exposed to RSV.

Assessment

Entry criteria for Trials 03 and 04 limited enrollment to infants born during or entering their first RSV season. Trial 03 enrolled infants born at a GA of ≥29 weeks to <35 weeks (very and moderately preterm infants), while Trial 04 enrolled infants born at >35 weeks of gestation (term and late preterm infants). The mean chronological age of infants enrolled in Trial 03 was 3.3 months (range of 0.1 to 11.9 months) and median of 2.8 months, and the mean chronological age of infants enrolled in Trial 04 was 2.9 months (range of 0.0 to 11.0 months) and median of 2.6 months.

Gestational Age

The results of efficacy in preterm infants born at ≥29 weeks <35 weeks GA are demonstrated in Trial 03; in this trial, the incidence of MA RSV LRTI was 0.8% in the nirsevimab arm and 4.1% in the placebo arm for a relative risk reduction of 78.4% (see Section 6.2.2.4). Efficacy results for infants ≥35 weeks GA were assessed in Trial 04. Because Trial 04 enrolled both preterm (≥35 weeks to <37 weeks GA) and term (≥37 weeks) infants, the efficacy of these subgroups was assessed and is shown in Table 30. The incidence of MA RSV LRTI is lower in the nirsevimab arm than in the placebo arm for both GA subgroups. The 95% CI for the younger GA subgroup (≥35 weeks to <37 weeks) is wide and crosses 0 because of the smaller number of subjects in that subgroup. The incidence of MA RSV LRTI is similar in the two GA groups for subjects in the nirsevimab arm. Trial 05 included infants born at <29 weeks GA; but because the subgroup was too small with too few events, and efficacy was descriptive, no definitive conclusions can be drawn from Trial 05 (see Trial 05). The efficacy of nirsevimab in preterm infants <29 weeks of gestation is extrapolated from the results in Trial 03 and 04 because nirsevimab exposures were similar across the various gestational age groups.

Table 30. Incidence of MA RSV LRTI by Gestational Age in the Primary Cohort of Trial 04

Gestational Age at Birth	Nirsevimab Arm	Placebo Arm	RRR (95%CI)
≥35 weeks to <37 weeks	2/132 (1.5%)	5/76 (6.6%)	76.97% (-16.79, 96.9)
≥37 weeks	10/861 (1.2%)	20/419 (4.8%)	75.67% (48.4, 89.1)

Source BLA 761328, CSR Trial 04, Figure 5, page 113.
Abbreviations: CI, confidence interval; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; RRR, relative risk reduction

Chronological Age

The Applicant analyzed the efficacy of nirsevimab by age at randomization in both trials and used three chronological age cohorts for the analysis: 3 months of age and younger, older than 3 months of age to 6 months of age, and older than 6 months of age. The results of these analyses are shown in Table 31 for all subjects as well as by chronological age at randomization and GA at birth.

The incidence of MA RSV LRTI was numerically lower in the nirsevimab arm than in the placebo arm for each chronological age subgroup in each trial. The incidence of MA RSV LRTI was numerically higher in the nirsevimab arm in each age subgroup in Trial 03 compared to the nirsevimab arm in the corresponding age subgroup in Trial 04, which is likely to be related to GA. Efficacy was demonstrated in the overall population in both trials. The treatment effect was consistent across subgroups and was consistent with the overall treatment effect.

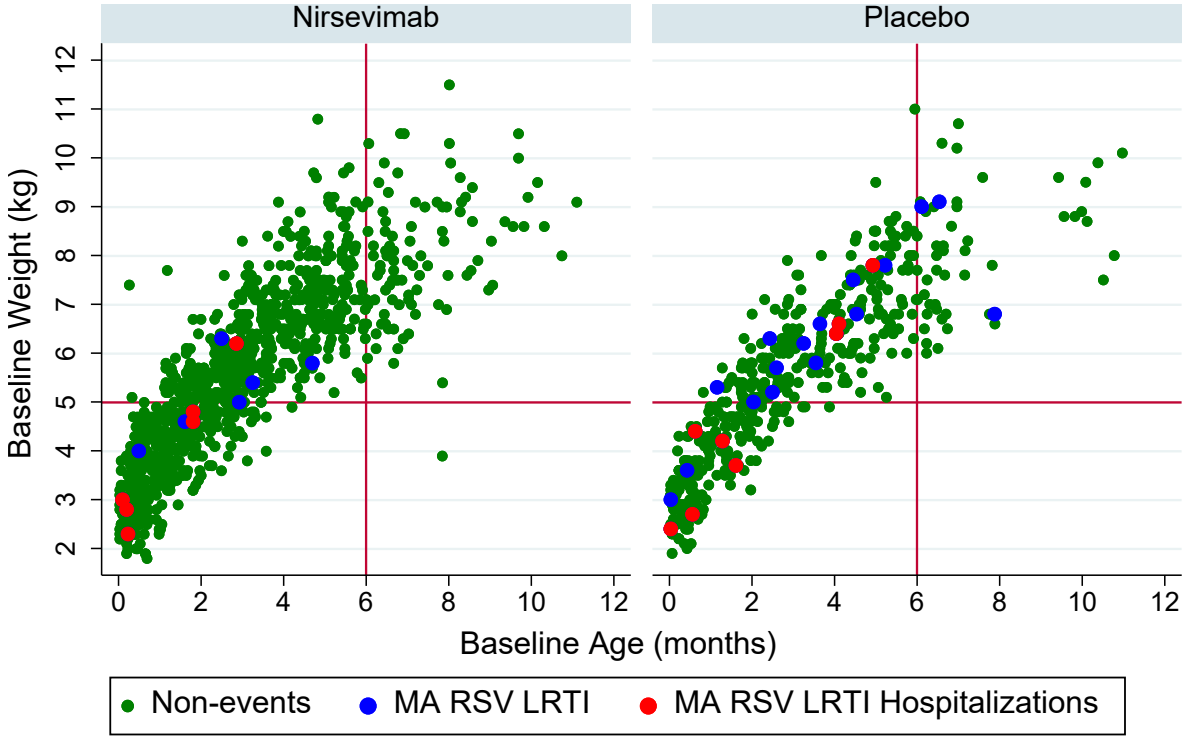
Table 31. Incidence of MA RSV LRTI in Trials 03 and 04 Through 150 Days Postdose by Chronological Age at Randomization and by Gestational Age (ITT Population)

	Trial 03 (≥29 Weeks to <35 Weeks GA)		Trial 04 (Primary Cohort) (>35 Weeks GA)	
Age	**Nirsevimab**	**Placebo**	**Nirsevimab**	**Placebo**
All subjects	25/969 (2.6%)	46/484 (9.5%)	12/994 (1.2%)	25/496 (5.0%)
≤3 months	7/516 (1.4%)	22/257 (8.6%)	10/577 (1.7%)	12/285 (4.2%)
>3 to ≤6 months	13/320 (4.0%)	16/153 (10.4%)	2/317 (0.6%)	10/162 (6.2%)
>6 months	5/133 (3.7%)	8/74 (10.8%)	0/100 (0%)	3/49 (6.1%)

Source: BLA 761328, CSR Trial 3: Table 15, page 42, Table 16, page 43 and CSR Trial 4: Table 24, page 109; Figure 5, page 111.
Abbreviations: CI, confidence interval; GA, gestational age; ITT, intent-to-treat; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

As shown in Figure 9, the numbers of subjects who were older than 6 months of age at the time of enrollment are considerably fewer compared to the younger age groups. This difference is likely due to the age at which infants typically enter their first RSV season. Efficacy results by chronological age for infants in Trial 04 are shown in Figure 6. The majority of subjects in Trial 04 were younger than 6 months of age, and there were very few subjects older than 8 months of age. In the nirsevimab arm, there were no cases of MA RSV LRTI (blue dots) in subjects older than 6 months of age, and only a few cases of MA RSV LRTI (blue dots) were reported in the placebo arm in this age group. There were no cases of RSV LRTI hospitalizations (red dots) in nirsevimab or placebo arms in subjects >8 months of age.

Figure 9. Primary Cohort: Relationship Between Baseline Weight, Baseline Age, and Outcome Status (Day 150) in Trial 04



Source: FDA statistical reviewer
Legend: Relationship between baseline weight and baseline age for subjects who did not experience MA RSV LRTI (green circles), subjects who experienced MA RSV LRTI without hospitalization (blue circles), and those who experienced MA RSV LRTI and were hospitalized (red circles). Each circle represents age and weight at baseline so that each subject is represented only once.
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

Conclusion

Efficacy was demonstrated in the overall population in both trials. The treatment effect was consistent across chronological age subgroups and was consistent with the overall treatment effect. However, few infants older than 8 months of age were enrolled. The prespecified analysis was for all infants enrolled; but in light of the limited enrollment of infants older than 8 months, and due to the small number of MA RSV LRTI events observed among these infants, the benefit of nirsevimab for this age group may need additional consideration. However, there may be times when administration of nirsevimab is appropriate for infants over 8 months of age, for example, infants who were lost to follow-up or who present late for health care, and when timing of RSV season is atypical.

Considering the overall persuasive evidence of effectiveness, the safety profile of nirsevimab (see Safety section), the recognition of real-life logistical challenges, and the risk of a future atypical RSV season, the review team supports approval of nirsevimab across the chronological age (up to age 12 months) and gestational age ranges for the prevention of RSV disease during their first RSV season.

6.3.2. Efficacy of Nirsevimab in Preventing MA RSV LRTI in Children Up to 24 Months of Age Who Remain Vulnerable to Severe RSV Through Their Second RSV Season

Issue

The clinical trial in the high-risk population (Trial 05) was not designed to perform efficacy analysis based on inferential statistics. The efficacy of nirsevimab for prevention of MA RSV LRTI in high-risk patients during RSV seasons 1 and 2 was extrapolated from efficacy in Trials 03 and 04 with demonstration of comparable serum nirsevimab exposures between the high-risk population (Trial 05 participants) and Trials 03 and 04 population; supportive descriptive efficacy data was provided with the demonstration of similar proportion of MA RV LRTI events in the nirsevimab and palivizumab treatment arms during the first RSV season. There were no MA RSV LRTI events in RSV Season 2.

Background

Trial 05 was a phase 2/3, double-blind, palivizumab-controlled trial in infants and children at high-risk of severe RSV disease. The trial enrolled infants in their first year of life who were born at <35 weeks of gestation, and who were eligible to receive palivizumab in accordance with national or local guidelines, as well as infants with CLD of prematurity, and infants with hemodynamically significant CHD. Infants who were born prematurely participated in the first year of the trial, while infants with CLD and CHD participated in RSV seasons 1 and 2. In the first RSV season, nirsevimab was administered as a single 50-mg IM dose in infants weighing <5 kg, and as a single 100-mg IM dose in infants weighing ≥5 kg. In the second RSV season, nirsevimab was administered as a 200-mg single IM dose to all children. A single 200-mg dose for the children entering the second RSV season is expected to provide serum AUC above target exposure of 12.8 day*mg/mL based on expected body weight range. Please refer to Trial 04 (above) for details of the target exposure. Trial 05 was designed to assess safety, as measured by the incidence and types of AEs, and serious adverse events (SAEs), and pharmacokinetics of nirsevimab; the efficacy analyses are descriptive. Please see Section 6.2.4.1 for further discussion of the trial design.

The Agency agreed with the Applicant that efficacy could be extrapolated from Trial 03 and Trial 04 to highest risk infants in Trial 05, and accepts that the efficacy of nirsevimab, as demonstrated in Trial 03 and the Primary Cohort of Trial 04, both adequate and well-controlled trials, can be leveraged to support the efficacy of nirsevimab in the high-risk population of Trial 05, provided that the observed nirsevimab exposures in Trial 05 were comparable to the exposures observed in Trial 04 (and 03). Therefore, the pharmacokinetic data were considered as bridging evidence to support the efficacy of nirsevimab for the population enrolled in Trial 05.

Extrapolation of efficacy was considered appropriate because:

1) The pathophysiology of RSV infection is sufficiently similar between infants with and without certain medical comorbidities.
2) The mechanism of action, and the viral protein target of nirsevimab is the same regardless of the host conditions.
3) The response to prevention is expected to be similar between infants with and without additional medical comorbidities.

The design of Trial 05 was discussed with FDA and agreed upon in 2017. A placebo-controlled trial would not be acceptable in infants and children for whom palivizumab is indicated. In addition, while a noninferiority trial comparing nirsevimab with palivizumab could be considered, the sample size needed to conduct such a trial was considered prohibitively large, and unlikely to be conducted within a reasonable time frame. Additionally, a noninferiority margin cannot be determined because no randomized trials with the endpoint of MA RSV LRTI are available to estimate the treatment effect of palivizumab versus placebo.

Assessment

A total of 925 infants were enrolled and randomized in Trial 05, including 615 preterm infants and 310 infants with CLD or CHD. Demographics for the first RSV season included the following:

- In the preterm cohort, 13% of infants were born at ≥22 weeks to <29 weeks GA, 81% were born at ≥29 to <35 weeks GA, and 6% were born at ≥35 weeks GA.
- The CLD/CHD cohort included 24% subjects with CLD and 11% subjects with CHD. Of infants in the CLD/CHD cohort, 40% were <29 weeks GA, 28% of infants were ≥29 weeks to <35 weeks GA; and 15% were ≥35 weeks GA.
- In all infants (premature infants, CLD, and CHD), 54% were male; 79% were White; 10% were Black; 5% were Asian, 2% were American Indian/Alaskan Native; and 57% weighed less than 5 kg.
- The median age was 3.5 months (range: 0.07 to 12.3 months); 45% were less than or equal to 3 months; 34% were greater than 3 months to less than or equal to 6 months; and 21% were greater than 6 months of age.

Of the 310 subjects with CLD or CHD who participated in the first RSV season of the trial, 262 (84.5%) subjects participated in Season 2.

The efficacy endpoint was the incidence of MA RSV LRTI through Day 150 postdose. In the first RSV season, the incidence of MA RSV LRTI through Day 150 postdose was 0.6% in the nirsevimab arm and 1.0% in the palivizumab arm.

- In the preterm cohort, two subjects had MA RSV LRTI in the nirsevimab arm and 1 in the palivizumab arm.
- In the CHD/CLD cohort, two subjects had MA RSV LRTI in each arm.

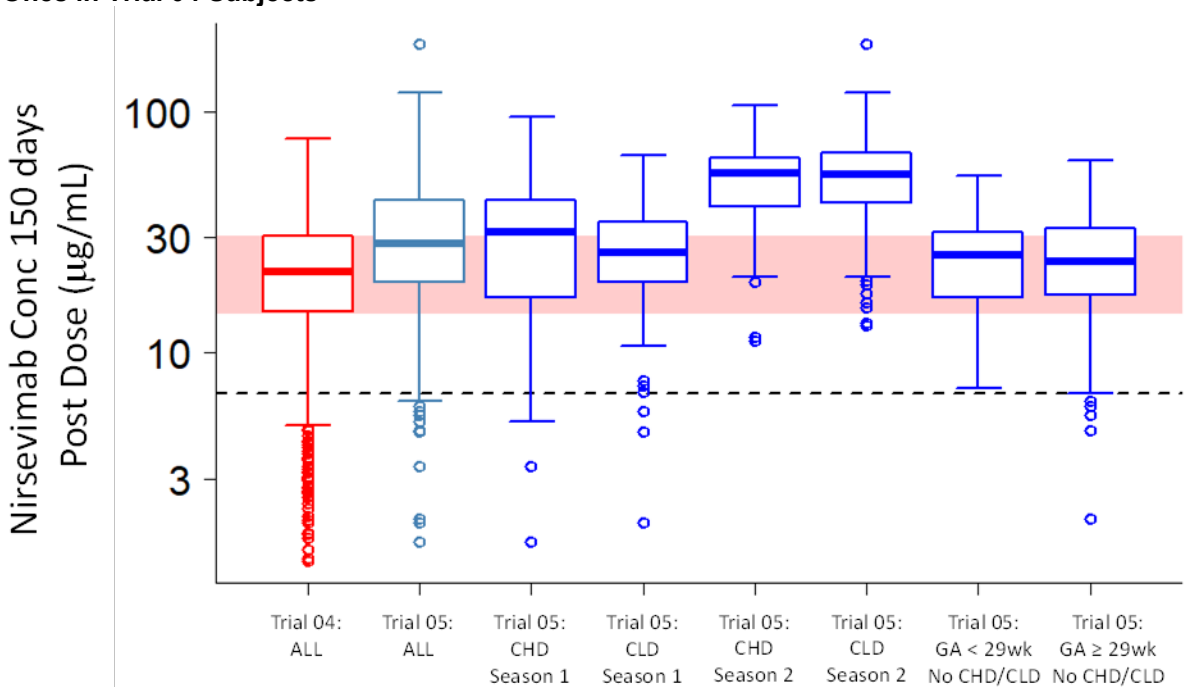
This trial enrolled subjects in 2019 and 2020, and the low numbers of RSV LRTI infections were likely related to the global COVID-19 pandemic. Despite a low incidence of RSV infections in the first RSV season, a similar number and percentage of cases of MA RSV LRTI were observed in the nirsevimab and palivizumab arms [4 (0.6%) and 3 (1.0%), respectively]. There were no cases of MA RSV LRTI through Day 150 postdose in Season 2.

Pharmacokinetic Data to Support Efficacy Extrapolation

Day 150 postdose nirsevimab serum concentration and AUCbaselineCL were chosen as the two PK parameters for efficacy extrapolation from Trials 03 and 04 to Trial 05. Day 150 nirsevimab serum concentration was selected based on the expected period of protection (i.e., 5 months) and duration of RSV season. AUCbaselineCL was selected based on the exposure-response analysis results with PK data from Trials 03 and 04. Please refer to Trial 04 above for details. The extrapolation of efficacy of nirsevimab from Trials 03 and 04 to Trial 05 was based on (1) the similar Day 150 postdose serum nirsevimab concentrations in infants enrolled in Trials 03 and 04 and in infants enrolled in Trial 05; and (2) percentage of Trial 05 subjects with nirsevimab exposure above the target AUCbaselineCL of 12.8-mg*day/mL (Table 32). More than 95 percent of the subjects in Trial 04 had AUCbaselineCL exceeding 12.8-mg*day/milk The PK comparison was conducted in both RSV seasons in Trial 05.

As shown in Figure 10, the Day 150 postdose serum concentrations in subjects enrolled in Trial 05 during their first season were comparable to the Day 150 postdose serum concentrations observed among subjects enrolled in Trial 04. The Day 150 postdose serum concentrations in subjects enrolled in Trial 05 during their second RSV season were higher than the concentrations observed in subjects enrolled in Trial 04. In both cases, because the Day 150 postdose serum concentrations observed in Trial 05 were comparable or higher than those observed in Trial 04, it can be reasonably concluded that nirsevimab would be expected to be as effective in very preterm infants or in infants with CHD/CLD.

Figure 10. Nirsevimab Concentrations 150 Days Postdose in Trial 05 Subjects Compared to the Ones in Trial 04 Subjects



Source: Reviewer's Analysis
The dashed line is EC_{90} value of 6.8 µg/mL determined based on RSV challenge studies in cotton rat model.
Abbreviations: CHD, hemodynamically significant congenital heart disease; CLD, chronic lung disease of prematurity; EC_{90}, 90% effective concentration; GA, gestational age

Table 32. Percent of Trial 05 Subjects With Nirsevimab Exposure Above Target $AUC_{baselineCL}$ of 12.8-mg*day/mL*

RSV Season	Extreme Preterm Infants <29 Weeks GA Without CLD or CHD	CLD	CHD
RSV Season 1	93.6% (44/47)	94.1% (128/136)	80.3% (53/66)
RSV Season 2	NA	97.7% (129/132)	100% (58/58)

Source: Applicant's Population Pharmacokinetic Report Erratum submitted on 5/18/2023, Table 13
*Target $AUC_{baseline}CL$ of 12.8-mg*day/mL is based on exposure-response analysis results from Trials 03 and 04.
Abbreviations: $AUC_{baseline}CL$, area under the concentration time curve from baseline to clearance; CHD, hemodynamically significant congenital heart disease; CLD, chronic lung disease of prematurity; GA, gestational age; RSV, respiratory syncytial virus

Conclusion

Trial 05 was a randomized, double-blind, active-controlled trial comparing the safety and PK of nirsevimab and palivizumab in high-risk infants and children. The trial was not designed to demonstrate efficacy. The descriptive efficacy data from the first RSV season in Trial 05 demonstrated a similar proportion of MA RV LRTI events in the nirsevimab and palivizumab treatment arms. There were no MA RSV LRTI events in RSV Season 2. The efficacy of nirsevimab for prevention of MA RSV LRTI in high-risk patients during RSV seasons 1 and 2 was extrapolated from efficacy in Trials 03 and 04 with demonstration of comparable serum nirsevimab exposures between the high-risk population (Trial 05 participants) and Trials 03 and 04 population.

6.3.3. Potential for Reduced Susceptibility Through Natural Variation/Polymorphisms

Issue

The clinical efficacy of nirsevimab against RSV could be impacted by naturally occurring variants which harbor F protein polymorphisms associated with reduced susceptibility to nirsevimab. In addition, prophylaxis with nirsevimab could theoretically select for variants with amino acid substitutions which reduce susceptibility and result in a breakthrough infection. Breakthrough infections could potentially result in worse disease outcomes and transmission of resistant virus.

Assessment

The evaluation of whether nirsevimab is likely to retain activity against the range of RSV variants circulating in the past or present relied on supporting data from nonclinical studies (detailed in Section 20), and ongoing surveillance programs in the U.S. and globally (Section 18.1). Cell culture studies included resistance passage experiments to identify nirsevimab resistance-associated substitutions, and phenotypic testing of binding site polymorphisms identified in sequence databases.

Analysis of clinical trial RSV sequence data was conducted to determine whether particular variants may be associated with breakthrough infection or more severe disease in nirsevimab-treated subjects (Section 18.6).

Nonclinical Supporting Data

Nirsevimab or 1G7 were tested against clinical isolates collected from 2003 to 2017 from global locations, including Australia, China, Israel, Italy, Netherlands, and USA (Section 20.6). For the isolates evaluated, the median EC_{50} value for nirsevimab/1G7 against RSV A isolates (n=70) was 21pM (3.2 ng/mL), ranging from 3pM (0.48 ng/mL) to 100pM (15.0 ng/mL). The median EC_{50} value for RSV B isolates (n=49) was 19pM (2.9 ng/mL), ranging from 2pM (0.3 ng/mL) to 398pM (59.7 ng/mL).

The ability of nirsevimab to neutralize RSV variants harboring polymorphic substitutions in the nirsevimab binding region was assessed using recombinant RSV in microneutralization assays. For RSV A (n=1,525) and RSV B (n=860), a total of 16 polymorphisms in each subtype were identified from GenBank® and internal databases spanning the years 1956 to 2014. For RSV A, 14 variant viruses were available for phenotypic testing, and did not confer reduced susceptibility (<3-fold) to nirsevimab. For RSV B, 15 variants were available for phenotypic testing, and of these, K65Q (n=11 sequences), K65T (n=2), and Q202R (n=2) substitutions caused 4-fold reductions in susceptibility to nirsevimab, and higher fold-reductions were seen with K65Q/K68N (n=1; 1,239-fold), L203I (n=1; 3,005-fold), and K65Q/S211N (n=6; 36-fold) substitutions.

To determine the potential for selection of RSV resistance to nirsevimab, and to identify amino acid residues associated with resistance, cell culture passage experiments were conducted of RSV A and RSV B in the presence of nirsevimab (Section 20.7). RSV A2 and RSV B9320

viruses were serially passaged three times in HEp-2 cells in the presence of 1.7nM (250 ng/mL; approximately 100 x EC_{50} value) of nirsevimab. For RSV A, the acquisition of N67I and N208Y substitutions in the nirsevimab binding site of F protein resulted in a 475-fold reduction in susceptibility to nirsevimab. Using recombinant RSV, these substitutions individually did not confer reduced susceptibility to nirsevimab. For RSV B, single substitutions of N208D or N208S were observed following serial passage, and double substitutions of K68N+N201S or K68N+N208S. These substitutions occurred in the nirsevimab binding site and conferred reduced susceptibility to nirsevimab of 5,532-fold for K68N+N201S, 14,623-fold for N208S, and >250,000-fold for N208D and K68N+N208S. Assessment of K68N and N201S substitutions individually using recombinant RSV showed a reduction in susceptibility to nirsevimab of 4-fold and 65-fold, respectively.

RSV Surveillance Programs

To determine whether nirsevimab maintains activity from one season to the next it is necessary to monitor contemporary and circulating clinical variants through sequencing of the F protein and phenotyping of any novel substitutions within and potentially outside of the antibody binding sites. To this end, the Applicant is conducting surveillance programs within the U.S. and internationally, summarized in Table 33, and described in detail in Section 18.1. The objectives of these programs are to characterize RSV A and RSV B seasonal variants circulating in the northern and southern hemispheres and determine the prevalence of variants harboring nirsevimab or palivizumab resistance-associated substitutions.

Table 33. Summary of RSV Molecular Surveillance Studies Supporting Nirsevimab

Study	Study Design	Population	RSV (+) Sample Size[a]	Study Period
OUTSMART-RSV	PS, MC, PV, OB	Infants (<2 yoa), children (2-12 yoa), adolescents (12-21 yoa), adults (≥21 yoa)	Total planned: >6,500 (50 samples/site/year)[b]	2015 to 2022+
INFORM-RSV	PS, MC, PV, OB	Infants (<2 yoa) and young children (2-5 yoa)	Total planned: >4,000 (50-100 samples/site/year)[c]	2017 to 2022+
SEARCH-RSV	PS, MC, PV, OB	Infants (<2 yoa) and young children (2-5 yoa)	Total planned: >1,200 (50 samples/site/year)[d]	2020 to 2024+

Source: page 10, study report ID8897-sVAP-002 amend 1
[a] First 10–20 RSV-positive nasal samples/month/site x ~5 months/year (typical length of RSV season)
[b] OUTSMART-RSV (USA); 4 geographical regions (West, Midwest, Northeast, South) at ~25-27 sites/year
[c] INFORM-RSV (global); GBR, ESP, NLD, FIN, JPN, BRA, ZAF, AUS (2017-2022+); CAN, FRA, GER, ITA (2018-2022+); KOR, TWN, RUS, MEX, CHL (2019-2022+) at 1 site/country except CAN with 2 sites
[d] SEARCH-RSV (CHN); 6 geographical regions (Northwest, North, Northeast, Southwest, South Central, East) at 6 sites/year
Abbreviations: INFORM-RSV, International Network For Optimal Resistance Monitoring of RSV; MC, multicenter; OB, observational; OUTSMART-RSV, Outsmart United States Targeted Surveillance of Monoclonal Antibody Resistance and Testing of RSV; P, prospective; PV, passive; RSV, respiratory syncytial virus; RSV(+), RSV-positive; SEARCH-RSV, Surveillance, Epidemiology and Research of China Hospital-Associated RSV; yoa, years of age

From the OUTSMART-RSV and INFORM-RSV surveillance programs, and a South African pilot program, overall, from 2015 to 2021, there was a high level of conservation (>99%) of amino acids in full-length F protein and in the nirsevimab binding site, including all positions in the RSV A binding site, and 22/25 positions in the RSV B binding site (Table 34). Of the three RSV B positions with ≤99% conservation, S211 was 98.8% conserved, and I206 and Q209 were

only approximately 31% conserved because of the emergence and predominance of I206M+Q209R co-occurring substitutions. In 2021, concurrent I206M+K209R+S211N substitutions were seen at a frequency of 29%. However, I206M+K209R substitutions together or with S211I or S211N did not confer reduced susceptibility to nirsevimab (<5-fold). The I206M substitution tested on its own confers a 5-fold reduction in susceptibility to nirsevimab, but it has been rarely (0.64% prevalence from 2015 to 2021) without the concurrent K209R substitution. Also, it is not known whether a 5-fold reduction in susceptibility is clinically relevant.

Variants with ≥5-fold reduced susceptibility to nirsevimab were seen rarely (<1%) from 2015 to 2021 and included RSV A substitutions K68E (13-fold) and S275F (6-fold), and RSV B substitutions N201S (127-fold), N201T (>406-fold), and N201T+I206M+Q209R (>418-fold). The only substitution conferring reduced susceptibility to both nirsevimab (6-fold) and palivizumab (>356-fold) was S275F in RSV A, which was seen at low frequency (0.15%) in 2020.

Table 34. Conservation of the Nirsevimab Binding Site in RSV F Protein Sequences, 2015-2021

	RSV A (n=2,875)			RSV B (n=2,800)		
Amino Acid Position	**Amino Acid[a]**	**Conservation (%)**	**Substitution[b] (Frequency, %)**	**Amino Acid[c]**	**Conservation (%)**	**Substitution[b] (Frequency, %)**
62	S	99.97	G/S (0.03)	S	100.00	-
63	N	100.00	-	N	99.96	S (0.04)
64	I	99.97	V (0.03)	I	100.00	-
65	K	99.86	Q (0.03), R (0.10)	K	99.89	R (0.11)
66	E	100.00	-	E	99.96	D (0.04)
67	N	100.00	-	T	99.96	A (0.04)
68	K	99.34	E (0.07), **N (0.56),** R (0.03)	K	99.57	N (0.32), **Q (0.04)**, R (0.07)
69	C	100.00	-	C	100.00	-
196	K	100.00	-	K	100.00	-
197	N	99.79	D (0.03), H (0.03), K (0.14)	N	99.57	D (0.39), S (0.04)
198	Y	100.00	-	Y	100.00	-
199	I	99.97	M (0.03)	I	100.00	-
200	D	99.97	N (0.03)	N	100.00	-
201	K	100.00	-	N	99.75	S (0.21), **T (0.04)**
202	Q	100.00	-	Q	100.00	-
203	L	100.00	-	L	100.00	-
204	L	99.97	I (0.03)	L	100.00	-
205	P	100.00	-	P	100.00	-
206	I	99.79	T (0.17), V (0.03)	I	31.04	I/M (0.07), **M (68.89)**[d]
207	V	99.97	I (0.03)	V	100.00	-
208	N	100.00	-	N	100.00	-
209	K	100.00	-	Q	31.43	K (0.21), L (0.11), Q/R (0.07), R (68.18)

	RSV A (n=2,875)			RSV B (n=2,800)		
Amino Acid Position	Amino Acid[a]	Conservation (%)	Substitution[b] (Frequency, %)	Amino Acid[c]	Conservation (%)	Substitution[b] (Frequency, %)
210	Q	99.97	L (0.03)	Q	99.93	H (0.07)
211	S	100.00	-	S	98.82	I (0.04), N (1.14)
212	C	100.00	-	C	100.00	-

Source: pages 26-28, study report ID8897-0102
[a] Based on reference strain RSV A/13-005275 from 2013
[b] RSV F protein substitutions among 2015 – 2021 RSV isolates compared to reference strains
[c] Based on reference strain RSV B/13-001273 from 2013
[d] I206M substitution has been seen rarely (<1%) from 2015-2021 without concurrent Q209R substitution, and together they do not cause reduced susceptibility to nirsevimab (<5-fold)
Bold data: Substitutions conferring ≥5-fold reduction in susceptibility to nirsevimab
Abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F protein, fusion protein; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; n, number of subjects; P, proline; Q, glutamine; R, arginine; RSV, respiratory syncytial virus; S, serine; T, threonine; V, valine; Y, tyrosine

OUTSMART-RSV 2021 to 2022

Late in the review cycle, the Applicant submitted a study report for the OUTSMART-RSV surveillance study during the 2021 to 2022 season (ID8897O-2122). In the U.S., RSV B remained dominant (91.7%; n=509) over RSV A (8.3%; n=8.3%) across sampling regions (West, Midwest, South and Northeast). Amino acid positions in the nirsevimab binding site remained >99% conserved at all 25 positions in RSV A, and 22 of 25 positions in RSV B. RSV B substitutions I206M, Q209R, and S211N were seen concurrently in 95.7% of isolates, and together do not confer reduced susceptibility (<5-fold change) to nirsevimab. The only other binding site substitutions identified were single instances (i.e., 0.2% of samples) of K68N and N201S in RSV B, which have been seen rarely in previous seasons, and respectively confer 30- and 127-fold reductions in susceptibility to nirsevimab. Regarding the palivizumab binding site, all amino acids were >99% conserved at all 14 positions, although there was one instance (0.2%) of RSV B substitution L273I (phenotype to be determined).

Other polymorphisms seen in antigenic site Ø outside of the nirsevimab binding site included single instances (0.2% each) of V76A, N88D, V90I, and N216S substitutions (no phenotypic data). For antigenic site II, other polymorphisms outside of the palivizumab binding site included single instances (0.2% each) of S255R and S276N substitutions (no phenotypic data).

Polymorphisms which were detected in ≥10% of full-length F protein sequences (i.e., amino acids 1 to 574) included RSV A substitutions: T12I (15.2%), T13A (34.8%), L15F (13.0%), A103T (21.7%), and T122A (39.1%), and RSV B substitutions: F15L (100%), R42K (63.3%), A103V (99.6%), L172Q (99.8%), S173L (100%), S190N (97.8%), K191R (100%), I206M (100%), Q209R (98.6%), S211N (97.6%), and S389P (97.1%). With respect to concurrent substitutions, there were two RSV B sequence clusters seen in ≥10% of sequences: R42K+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P (54.6%; 278 of 509), and A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P (26.1%; 133 of 509).

Of the individual substitutions seen in ≥10% of sequences, RSV A substitution T122A, and RSV B substitutions F15L, A103V, L172Q, S173L, K191R, Q209R, and S211N do not confer reduced susceptibility (<5-fold) to nirsevimab or palivizumab; I206M substitution on its own confers a 5-fold reduction in susceptibility but has been seen rarely in the absence of Q209R, and

together, or with Q209R+S211N, these substitutions do not confer a loss of susceptibility to nirsevimab. Phenotypic analysis of I206M+S211N (seen in 1.4% of sequences) and the other substitutions, including the two RSV B sequence clusters, is ongoing.

Clinical Supporting Data

Sequencing analyses of RSV variants from breakthrough infections were conducted for all clinical trials of nirsevimab, including Trial 03, Trial 04, Trial 05, and Trial 08 (described in detail in Section 18.6). These analyses included all RT-PCR-confirmed RSV isolates from subjects with MA RSV LRTI (protocol or nonprotocol defined) or hospitalization due to RSV illness and assessed full-length F protein and the impact of substitutions on susceptibility to nirsevimab and palivizumab.

Trial 03

In the placebo and nirsevimab groups of Trial 03, there were 54/484 (11.2%) and 40/969 (4.1%) subjects, respectively, in the resistance analysis population, of whom 44/484 (9.1%) and 25/969 (2.6%), respectively, had an MA RSV LRTI through Day 150. There were similar numbers of subjects with RSV A and RSV B infections overall, and for those with MA RSV LRTI through Day 150 (22 of each subtype in the placebo group, 11 RSV A and 14 RSV B events in nirsevimab-treated subjects). Table 35 summarizes the F protein substitutions which were detected through Day 150 in the binding site and extracellular regions and had reduced susceptibility to nirsevimab or no phenotypic data.

Table 35. Substitutions[a] in Binding Site/Extracellular Regions of F Protein With Reduced Susceptibility (≥5-Fold Change) to Nirsevimab or No Phenotypic Data, Detected in RSV Isolates From Subjects With MA RSV LRTI Through Day 150 in Trial 03

	RSV A		RSV B	
Trial 03	**Placebo**	**Nirsevimab**	**Placebo**	**Nirsevimab**
Number of Subjects	22/484 (4.5%)	11/969 (1.1%)	22/484 (4.5%)	14/969 (1.4%)
Major (≥25% Frequency) Variants				
Binding Site (Number of Subjects; Fold Change)	None	None	None	I64T[c]+K68E[c]+I206M +Q209R (1/14; >447-fold); N208S[c] (1/14; >387-fold)
Extracellular Regions (Number of Subjects; Fold Change)	S99N[c] (1/22, 5-fold); G71S, K419E (1/22 each; ND)	K419E (1/11; ND)	None	None

Trial 03	RSV A		RSV B	
	Placebo	Nirsevimab	Placebo	Nirsevimab
Minor (<25% Frequency) Variants				
Binding Site (Number of Subjects; Fold Change)	None	None	None	N208K[b,c] (1/14; >351-fold)
Extracellular Regions (Number of Subjects; Fold Change)	R49K[b] (1/22; ND)	None	L171M[b], L381F[b], Y457H (1/22 each; ND)	None

Source: FDA analysis
[a] Concurrent substitutions are shown as such if they were evaluated together phenotypically
[b] Observed at < LLOQ of NGS assay (4% for RSV A and 5% for RSV B)
[c] Substitutions: loss of susceptibility (≥5-fold change) to nirsevimab when tested individually
Abbreviations: F protein, fusion protein; ND, not determined (no phenotypic data); RSV, respiratory syncytial virus; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

In Trial 03, there were no RSV A variants detected with F protein substitutions in the nirsevimab binding site. For RSV B, approximately one third of the major (i.e., >25% frequency) variants harbored I206M+Q209R in the nirsevimab binding site, which have become predominant since 2017 and together do not cause reduced susceptibility to nirsevimab. Two of 14 nirsevimab-treated subjects infected with RSV B MA LRTI through Day 150 harbored variants with resistance-associated substitutions in the binding site: I64T+K68E+I206M+Q209R and N208S. These variants were not seen in surveillance studies. The I64T, K68E and N208S substitutions tested individually caused loss of susceptibility to nirsevimab (fold changes: >496, >283, and >387, respectively). Both subjects were hospitalized and had been dosed below the proposed dose.

Outside of the nirsevimab binding site but within the extracellular regions of F protein (amino acids 24-109 and 137-524), in placebo and/or nirsevimab-treated subjects with RSV A MA LRTI through Day 150, S99N was identified as a major substitution with reduced susceptibility to nirsevimab (5.3-fold change) in one placebo subject. Other substitutions showed no loss of susceptibility (<5-fold change), including S25N, V76I, A102S, and S213R, or have not been phenotyped (G71S, K419E). For RSV B, major substitutions A103V, L172Q, S173L, K191R, S276N, K327R, and E463D, were seen in placebo and/or nirsevimab-treated subjects through Day 150 and did not confer reduced susceptibility to nirsevimab when tested individually.

Within the intracellular regions of F protein (amino acids 1-23, 110-136, and 525-574), in placebo and/or nirsevimab-treated subjects with RSV A MA LRTI through Day 150, major substitutions included T13A, I14V, A17V, C21S, F22I, A23T, V127A, N116Y, N120S, and K123E; no phenotypic data were reported for these substitutions. For RSV B, major substitutions included L4P, F12L, F15I, A16T, A16V, T118I, V127I, I527M, A543T, and K574N; there were no phenotypic data for these substitutions.

There were no RSV A or RSV B variants or individual substitutions in Trial 03 which were clearly associated with nirsevimab-treated subjects compared with placebo subjects, or in

hospitalized subjects compared with nonhospitalized subjects, although in general there were too few examples to draw firm conclusions.

Trial 04

In the placebo and nirsevimab groups of Trial 04, there were 88/1,003 (8.8%) and 60/2,009 (3.0%) subjects, respectively, in the resistance analysis population, of whom 54/1,003 (5.4%) and 23/2,009 (1.1%), respectively, had an MA RSV LRTI through Day 150. Like Trial 03, there were similar numbers of subjects with RSV A and RSV B infections overall, and for subjects with MA LRTI included 25 RSV A and 29 RSV B events in the placebo group, and 13 RSV A and 10 RSV B events in nirsevimab-treated subjects. Table 36 summarizes the F protein substitutions which were detected through Day 150 in the binding site and extracellular regions and had reduced susceptibility to nirsevimab or no phenotypic data.

Table 36. Substitutions in Binding Site/Extracellular Regions of F Protein With Reduced Susceptibility (≥5-Fold Change) to Nirsevimab or No Phenotypic Data, Detected in RSV Isolates From Subjects With MA RSV LRTI Through Day 150 in Trial 04

	RSV A		RSV B	
Trial 04	**Placebo**	**Nirsevimab**	**Placebo**	**Nirsevimab**
Number of Subjects	25/1003 (2.5%)	23/2009 (1.1%)	29/1003 (2.9%)	10/2009 (0.5%)
Major (≥25% Frequency) Variants[d]				
Binding Site (Number of Subjects; Fold Change)	None	None	None	L204S (1/10, ND)
Extracellular Regions (Number of Subjects; Fold Change)	T245N, V406I, D486N (1/25 each, ND)	S255N, K419E, D479N (1/23 each, ND)	T91N, V239I, K327E, V365I, P376S, S436P, T522A (1/29 each; ND); S190N, S389P (22/29 each; ND)	S190N, S389P (9/10 each, ND); K272R[b] (1/10; ND)

Trial 04	RSV A		RSV B	
	Placebo	Nirsevimab	Placebo	Nirsevimab
Minor (<25% Frequency) Variants[d]				
Binding Site (Number of Subjects; Fold Change)	None	None	None	I64T[a,d]+K68E[a,d] (1/10, >280-fold); K65E[c]+ N200Y (1/10, ND); N208I (1/10, ND)
Extracellular Regions (Number of Subjects; Fold Change)	I79M[c], A89V[c], N165S[c], N515H (1/23 each, ND)	I79M[c], N228S[c], V247L[c] (1/23 each, ND); N515H (2/23, ND)	D356N[c], I475M, Y477H (1/29 each; ND)	None

Source: FDA analysis
[a] Concurrent substitutions are shown as such if they were evaluated together phenotypically
[b] Substitution at amino acid position associated with palivizumab resistance
[c] Observed at < LLOQ of NGS assay (4% for RSV A and 5% for RSV B)
[d] Substitutions: loss of susceptibility (≥5-fold change) to nirsevimab when tested individually
Abbreviations: F protein, fusion protein; ND, not determined (no phenotypic data); RSV, respiratory syncytial virus; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

There were no major (i.e., >25% frequency) RSV A variants detected with F protein substitutions in the nirsevimab binding site through Day 511. One minor (i.e., ≥4% to <25% frequency) variant with D200N+K201N substitutions (no phenotypic data) was seen in a nirsevimab-treated subject with MA RSV LRTI from Day 360 to 511. Given the timing and because the variant was only seen in one subject, the significance of this event is not clear. Like Trial 03, for RSV B, most major variants harbored I206M+Q209R in the nirsevimab binding site, which have become predominant since 2017 and together do not cause reduced susceptibility to nirsevimab. Other variants which had substitutions in the nirsevimab binding site included ones harboring I206M+Q209R+S211N substitutions, seen in 22/29 placebo and 8/10 nirsevimab subjects with MA LRTI through Day 150, and one with L204S+I206M+Q209R+S211N substitutions, seen in one nirsevimab subject with MA LRTI through Day 150. The I206M+Q209R+S211N substitutions were seen at increased prevalence (28.6%) in 2021, at a time when Trial 04 was in progress, and together did not confer reduced susceptibility (<5-fold change) to nirsevimab or palivizumab. The L204S+I206M+Q209R+S211N substitutions have not been detected concurrently in surveillance studies and are being evaluated phenotypically. Additional substitutions in the nirsevimab binding sites seen at low frequency (<25%) through Day 150 only, included I64T+K68E, K65E+N200Y, and N208I, in one nirsevimab-treated subject each. Of these substitutions, I64T and K68E conferred reduced susceptibility to nirsevimab individually (>496-fold and >283-fold, respectively) and together (>280-fold). K65E, N200Y and N208I substitutions are being evaluated phenotypically.

Outside of the nirsevimab binding site but within the extracellular regions of F protein (amino acids 24-109 and 137-524), in placebo and/or nirsevimab-treated subjects with MA RSV LRTI through Day 150, major substitutions seen in RSV A variants either did not cause reduced susceptibility to nirsevimab (A103T, A107T, V144I, S276N, Q354R, I384T), or have not been tested phenotypically (T245N, S255N, V406I, K419E, D479N, D486N). For RSV B, none of the

substitutions with phenotypic data showed reduced susceptibility to nirsevimab when tested individually (A103S, L172Q, S173L, K191R S330T), and other substitutions in the extracellular domains have not been evaluated (T91N, S190N, V239I, K272R, K327E, V365I, P376S, S389P, S436P, T522A).

Within the intracellular regions of F protein (amino acids 1-23, 110-136, and 525-574), in placebo and/or nirsevimab-treated subjects with MA RSV LRTI through Day 150, major substitutions in RSV A variants seen mostly in one or two subjects included L6H, T8I, T12I, T13A, L15F, L20F, A23T, F114S, M115T, L119F, T122A, K123Q, V127A, and V127I, for which phenotypic data were only available for T122A (no change in susceptibility; observed in 3/23 placebo and 4/12 nirsevimab subjects with MA LRTI through Day 150). For RSV B, major substitutions, mostly seen in single placebo subjects, included I11L, F12I, F15L, A15V, N18S, L22P, A111V, N116S, and A529V, for which phenotypic data were only available for F15L (no change in susceptibility, observed in 26/29 placebo and 10/10 nirsevimab subjects).

There were no RSV A or RSV B variants which were clearly associated with nirsevimab-treatment or hospitalization. Most variants were identified in single subjects, in both nirsevimab and placebo groups, so limited conclusions can be drawn regarding individual substitutions. Overall, there were no individual substitutions or specific amino acid positions within or outside the nirsevimab binding sites which were significantly increased in frequency in nirsevimab-treated subjects compared with placebo, or in hospitalized subjects compared with nonhospitalized subjects.

Trial 05 and Trial 08

In the palivizumab and nirsevimab groups of Trial 04 (, there were 10/309 (3.2%) and 12/616 (1.9%) subjects, respectively, in the resistance analysis population, of whom 3/309 (1.0%) and 4/616 (0.6%), respectively, had an MA RSV LRTI through Day 150. In the palivizumab group, 1/3 subjects had RSV A, and 2/3 had an RSV B infection, and in the nirsevimab group 4/4 subjects had an RSV A infection. For the Trial 08 trial, there were only three subjects with NGS data in the resistance analysis population, of whom 2 were treated with nirsevimab in the first year of life, and one with nirsevimab in the second year of life. None of these three subjects had a protocol-defined MA LRTI. Table 37 summarizes the F protein substitutions in Trial 05 through Day 150 which were detected in the binding site and extracellular regions and had reduced susceptibility to nirsevimab or no phenotypic data.

Table 37. Substitutions[a] in Binding Site/Extracellular Regions of F Protein With Reduced Susceptibility (≥5-Fold Change) to Nirsevimab or No Phenotypic Data, Detected in RSV Isolates From Subjects With MA RSV LRTI Through Day 150, Season 1, in Trial 05

	RSV A		RSV B	
Trial 05	**Palivizumab**	**Nirsevimab**	**Palivizumab**	**Nirsevimab**
Number of Subjects	1/309 (0.3%)	4/616 (0.6%)	2/309 (0.6%)	0/616 (0.0%)
Major (≥25% Frequency) Variants				
Binding Site (Number of Subjects; Fold Change)	None	None	None	N/A
Extracellular Regions (Number of Subjects; Fold Change)	S275L[b] (1/1; ND)	None	None	N/A
Minor (<25% Frequency) Variants				
Binding Site (Number of Subjects; Fold Change)		None	None	N/A
Extracellular regions (#subjects; fold-change)	K272M[b], K272T[b] (mix in 1 subject; <5-fold each)	None	K272T[b,c] (1/2; <5-fold); E294V[c] (1/2, ND)	N/A

Source: FDA analysis
[a] Concurrent substitutions are shown as such if they were evaluated together phenotypically
[b] Palivizumab resistance-associated substitutions
[c] Observed at < LLOQ of NGS assay (4% for RSV A and 5% for RSV B)
Abbreviations: N/A, not applicable (no RSV B infections in nirsevimab arm); F protein, fusion protein; ND, not determined (no phenotypic data); RSV, respiratory syncytial virus; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

For Trial 05, there were no major (i.e., ≥25% frequency) variants through Day 360, Season 1, with substitutions in the nirsevimab binding sites. One subject treated with palivizumab was infected through Day 150 with a variant harboring S275L substitution, which is associated with palivizumab resistance (AstraZeneca 1998), and is being assessed for susceptibility to nirsevimab. A minor variant with a mix of K272M and K272T substitutions was also seen in one palivizumab-treated subject; both these substitutions showed loss of susceptibility to palivizumab (>179.9- and >213.9-fold change, respectively), but not to nirsevimab (<5-fold change). There were no RSV B infections in nirsevimab-treated subjects through Day 150. Two variants were seen in palivizumab-treated subjects at >25% frequency through Day 150, both of which harbored the I206M+Q209R double substitution. A K272T substitution in the palivizumab binding site was seen at low frequency (<lower limit of quantification (LLOQ) of NGS assay) in one palivizumab-treated subject.

Major RSV A variants in Trial 05 through Day 150, Season 1, included a few substitutions outside the nirsevimab and palivizumab binding sites: A103T (extracellular region, no loss of susceptibility to nirsevimab or palivizumab), E110G, M115T, T122A, and N126Y (intracellular regions, no phenotypic data except for T122A [no change]). For RSV B through Day 150, the two variants in palivizumab-treated subjects included major substitutions A103V, L172Q, L173L, and K191R (<5-fold change), in the extracellular regions, and L4P, S9I, F12L, F15L, and I525V in the intracellular regions, for which only F15L has been phenotyped (no change).

Given the small numbers of subjects with RSV A or RSV B infections in Trial 05 and Trial 08, conclusions cannot be drawn regarding association of variants or F protein substitutions with nirsevimab treatment or hospitalization.

Conclusions

In cell culture, nirsevimab had broad antiviral activity across laboratory strains and clinical isolates which were globally and temporally diverse. In surveillance programs and clinical trials of nirsevimab, there were few RSV isolates which were found to harbor resistance-associated substitutions. Analysis of breakthrough infections from clinical trials of nirsevimab did not identify F protein amino acid positions or individual substitutions for RSV A or RSV B which were clearly associated with increased frequency of infection or hospitalization in nirsevimab-treated subjects compared with control subjects.

In general, the RSV variants and F protein substitutions identified in clinical trials concurred with those seen in surveillance data. It is possible that in infected subjects treated with nirsevimab, resistant virus may have been selected, but not detected because it was only present in the lower respiratory tract, which was not sampled. Also, most samples were taken at a single time point, limiting the ability to detect treatment-emergent resistant virus. If there were resistant virus present as a small minority species, it may propagate as a mixture with wild-type virus, and/or be transmitted through cell-cell fusion, also limiting the ability to detect.

Given that RSV is continually evolving, it is possible that variants with reduced susceptibility to nirsevimab may emerge and become prevalent in the future, so it will be important for the Applicant to continue their surveillance programs while nirsevimab is being used clinically.

7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

Nirsevimab nonclinical safety studies included a good laboratory practice (GLP) 4-week intravenous/intramuscular repeat-dose toxicology study in cynomolgus monkeys with a 25-week recovery period, a GLP tissue cross-reactivity (TCR) study in normal adult human and select fetal, neonatal and juvenile human tissues. All pertinent studies and findings are summarized below. Full reviews for all studies are located in Section 13.1.

No adverse, drug-related findings were observed in the GLP 4-week toxicology study in cynomolgus monkeys up to the highest doses tested (no observed adverse effect level (NOAEL) =300 mg/kg IM or IV). Minor, nonadverse effects were observed in clinical pathology parameters (increased APTT, globulin and total protein) and the spleen (red pulp macrophage hypertrophy/hyperplasia) at 300 mg/kg IV and/or IM. Further, no injection site findings were observed following subcutaneous or intramuscular dosing. Lastly, no off-target binding was observed with nirsevimab in TCR studies in either normal adult human or select fetal, neonatal and juvenile human tissues. Genotoxicity, carcinogenicity and developmental and reproductive toxicology (DART) studies have not been conducted with nirsevimab based on International Council for Harmonisation (ICH) S6(R1) guidelines.

Overall, the nonclinical safety assessment for nirsevimab was considered acceptable to support licensing from a pharmacology/toxicology perspective. The exposure multiples at the NOAEL for intravenous and intramuscular administration in the GLP 4-week toxicology study in cynomolgus monkeys are presented in Table 38 and are acceptable.

Table 38. Mean Exposure and Safety Margins of Nirsevimab at the NOAEL in the 1-Month Repeat Dose IV/IM Toxicity Study in Cynomolgus Monkeys and in Human Infants and Adults

Cynomolgus monkey				Human					
300 mg/kg/week IV NOAEL		300 mg/week IM NOAEL		Infants (IM 50 mg <5kg, 100 mg ≥5kg)[a, b]		Children - 2nd Season (200 mg)[c, d]		Adults (3000 mg IV)[b]	
$C_{max,5}$ (µg/mL) [SD]	AUC_{1-31} (µg day/mL) [SD]	$C_{max,5}$ (µg/mL) [SD]	AUC_{1-31} (µg day/mL) [SD]	C_{max} (µg/mL) [SD]	AUC_{0-365} (µg day/mL) [SD]	C_{max} (µg/mL) [SD]	AUC_{0-365} (µg day/mL) [SD]	C_{max} (µg/mL) [SD]	AUC_{0-365} (µg day/mL) [SD]
13360 [2704]	208500 [43270]	4,982 [863.4]	92380 [9428]	120 [28.0]	12200 [3,550]	194 [42.2]	21500 [5,520]	1090 [163]	53900 [19,800]
Safety Margins (IV/IM)				111/42	17/8	69/26	10/4	12/5	4/2

$AUC_{(1-31)}$ = cumulative area under the curve from Day 1 to Day 31; $C_{max,5}$ = maximum observed concentration after fifth dose on Day 29; IM = intramuscular; IV = intravenous; SD = standard deviation

[a] Dose of 50 mg for infants weighing <5 kg and 100 mg for those weighing ≥5 kg entering their first RSV season

[b] Modeling and Simulation Report: Population PK and Exposure-Response Modeling of Nirsevimab in Term and Preterm Children, and Extrapolation to High-Risk Children, 2021

[c] Modeling and Simulation Report: Population PK Modeling of Nirsevimab in Term and Preterm Children, and Extrapolation to Higher-Risk Children, 2022

[d] For children at higher risk of severe RSV disease entering their second RSV season, the recommended dose is 200 mg given as 2 IM injections (2 x 100 mg).

Source: Sponsor table; Toxicology Written Summary; page 12

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

7.2.1. Drug Class Considerations

Nirsevimab is a recombinant human immunoglobulin G1 kappa monoclonal antibody directed against the fusion (F) protein of RSV protein. It is related to palivizumab, which is also a monoclonal antibody targeting the F protein of RSV. Palivizumab was approved by the FDA in 1998 for the prevention of serious RSV lower respiratory tract disease in high-risk infants, infants born ≤35 weeks gestational age and infants with bronchopulmonary dysplasia (chronic lung disease of prematurity) or hemodynamically unstable congenital heart disease. Unlike

palivizumab, nirsevimab has a triple amino acid substitution (YTE mutation) to extend the serum half-life. As a result, nirsevimab is administered as a single intramuscular dose prior to or during the RSV season, while palivizumab is administered as monthly IM doses during the RSV season (with a maximum of five doses in a single season).

Nirsevimab and palivizumab are both recombinant, humanized IgG1 monoclonal antibodies that target the F protein of RSV and block RSV entry into the cell. The safety obtained with use of palivizumab is relevant to nirsevimab because of the similarities between the two monoclonal antibodies. Palivizumab was approved by FDA in 1998; as a result, there is more than 20 years of experience with the use of palivizumab. The following safety findings are included in the palivizumab package insert:

- Anaphylaxis, anaphylactic shock, including fatal cases, and hypersensitivity reactions have been reported following both the initial exposure and to re-exposure to palivizumab. Clinical presentations have included urticaria, angioedema, dyspnea, cyanosis, hypotonia, hypotension, cyanosis, and unresponsiveness.
- In clinical trials of palivizumab, fever was reported more frequently with palivizumab (27%) than with the placebo control (25%). Rash was reported in 12% of subjects who received palivizumab and in 10% who received placebo. Of note, the differences between the palivizumab arm and placebo control arm were slight.
- Adverse reactions reported postmarketing are severe thrombocytopenia and injection site reactions.

In summary, extensive experience with palivizumab is a useful reference for what could be expected with wider use of nirsevimab.

7.2.2. Other Drug-Specific Factors

See Section 6.1.3 for the Clinical Pharmacology review of replacement dosing and PK.

Pediatric subjects with hemodynamically significant congenital heart disease were enrolled in Trial 05. Some trial subjects with CHD had cardiac surgery requiring cardiopulmonary bypass during the surgery. To maintain adequate nirsevimab exposure in the postoperative period, an additional (replacement) dose was administered in these subjects. The Applicant has proposed to include these additional postoperative dosing recommendations in the nirsevimab package insert.

In this setting, a key safety consideration is to evaluate the exposure-response (safety) relationship among subjects requiring additional doses and assess if there are any differences in adverse events reporting between these subjects and the nonsurgical subjects enrolled in Trials 03, 04 or 05.

Subjects who had cardiac surgery in their first year of life and within 90 days of their initial nirsevimab dose received an additional postoperative (replacement) nirsevimab dose based on their body weight at the time of the additional dose administration: 50 mg for subjects weighing <5 kg, and 100 mg for subjects weighing ≥5 kg. If more than 90 days had elapsed since the first

nirsevimab dose, a 50 mg additional nirsevimab dose was administered to all subjects, regardless of body weight.

If the subject had cardiac surgery during the second RSV season and within 90 days after receiving the first (Season 2) nirsevimab dose, an additional postoperative dose of 200 mg was administered, regardless of body weight. If more than 90 days had elapsed since the first (Season 2) nirsevimab dose, subjects received a nirsevimab replacement dose of 100 mg, regardless of body weight. The Applicant has also proposed to include these dosing recommendations in the nirsevimab package insert.

Thirteen subjects had cardiac surgery with cardiopulmonary bypass. Ten of these subjects received additional doses of nirsevimab. The nirsevimab serum concentrations on Day 151 in subjects who received an additional dose after cardiac surgery were 3- to 4-fold higher than the reference concentrations from Trial 04. In order to assess the safety of nirsevimab in these ten subjects, adverse events were assessed for 30 days after the additional doses were administered. Of these 10 subjects, three subjects had no AEs after the additional dose. Two subjects had AEs that were clearly related to their surgery, such as postoperative pain, electrolyte imbalances, pulmonary hypertension, and pressure sores. Five subjects had postoperative complications. Postoperative infections were reported in three subjects: one subject had pneumonia and a urinary tract infection, one reported an upper respiratory tract infection, and one had positive tests for coronavirus, enterovirus, and rhinovirus. The subject with multiple positive viral laboratory tests was also diagnosed with systemic inflammatory response syndrome (SIRS). SIRS was reported as an SAE that was judged as not trial product related and that resolved in 5 days. SIRS is a condition with inflammation throughout the body and can be associated with high or low body temperature, increased heart rate, increased respiratory rate or an abnormal white count. It can be caused by an infection, surgery, ischemia, and other conditions such as autoimmune disorders. SIRS may have been associated with surgery in this subject. When SIRS occurs with infection, it is typically called sepsis; this subject was not diagnosed with sepsis or with infections other than the three positive laboratory tests for viruses; therefore, it is possible that this SAE was related to surgery and not to an infection or to nirsevimab. Two subjects reported rashes. One subject had and AE of contact dermatitis reported 8 days after the additional dose, and another subject reported an AE of “rash” 30 days after their additional dose. Although the AE of contact dermatitis was judged by the investigator as unrelated to trial drug product, the contact dermatitis was temporally related to nirsevimab administration (e.g., within two weeks of administration). The AE of “rash” also may have been related to nirsevimab because of the long half-life of nirsevimab.

To further support the safety of nirsevimab when administered as an additional dose, the safety profile of nirsevimab was evaluated from other clinical trials where higher doses were administered.

A phase 1, dose-escalation study of nirsevimab was conducted in healthy adults aged ≥18 to <50 years of age. In this study, 18 subjects received a single intravenous dose of nirsevimab; six subjects received 300 mg IV, six received 1,000 mg IV, and six received 3,000 mg IV. The maximum plasma concentration (C_{max}) values (which represents the highest exposure potential) for the adult subjects ranged from 97 to 1,163 µg/mL, which were considerably higher than the C_{151} values for the pediatric subjects in Trial 05 who received additional nirsevimab (mean C_{151}

of 76.88). None of the adult subjects in Trial 01 reported Grade 3 or Grade 4 AEs, and there were no SAEs in the adult subjects who received IV nirsevimab. Of the 18 adult subjects, five (28%) reported AEs. AEs reported in more than one subject included nausea (n=2), headache (n=2), URTI (n=2), and contact dermatitis / dermatitis (n=3). There does not appear to be an increase in severity or in the number of adverse events reported at the higher C_{max} levels in adult subjects who received IV nirsevimab. However, there were two AEs of contact dermatitis and one of dermatitis, in adults who received IV nirsevimab.

Contact dermatitis was reported in one subject in Trial 05 who received an additional nirsevimab dose and had therefore had a high nirsevimab serum concentration. In addition, contact dermatitis was reported in two subjects and dermatitis in one subject in a phase 1 trial in adults who received IV nirsevimab and who also had high serum concentrations of nirsevimab. It is possible that rash may be more common in patients who receive a higher dose of nirsevimab, such as patients who receive an additional dose of nirsevimab after surgery. However, the number of subjects with higher serum nirsevimab concentrations and rash is small; therefore, it is difficult to reach a firm conclusion regarding this finding. In addition, rash in these subjects was not associated with any other signs or symptoms associated with hypersensitivity, and all rashes were self-limited and resolved.

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

Nirsevimab was approved by the European Commission in October 2022, by the UK Medicines and Healthcare products Regulatory Agency (MHRA) in November 2022, and by Health Canada in April 2032. Nirsevimab has not been launched in any countries or regions at this time, and therefore, no postmarketing safety data is available.

Nirsevimab has not been approved by any other regulatory authority.

7.3.1. Adverse Events Identified in Postmarket Experience

There are no postmarketing data available for review as nirsevimab was only recently approved for use by EMA and MHRA; however, nirsevimab is currently not in use at this time.

7.3.2. Expectations on Safety

Expectations of safety in the postmarketing experience are based on safety data from the phase 2 and 3 clinical trials of nirsevimab and the safety profile of palivizumab, which has been approved for use in the U.S. since 1998. See Section 7.5 in this review for a description of adverse events reported with palivizumab use.

Safety analyses and conclusions in this review are primarily based upon data from the submitted phase 2 and 3 trial populations. There were no safety concerns observed that necessitate issuing a Risk Evaluation and Mitigation Strategy for nirsevimab. Emergence of new events can be

managed by the planned pharmacovigilance activities. Please refer to Section 7.7.3 for pharmacovigilance discussion, and Section 24 for description of postmarketing requirements and commitments.

There are no specific safety concerns that are expected from unapproved uses of nirsevimab.

7.3.3. Additional Safety Issues From Other Disciplines

Not applicable.

7.4. FDA Approach to the Safety Review

Approach to Assessment of Clinical Trial Data

Data from two pivotal trials (Trials 03 and 04), two additional controlled trials (Trials 02 and 05), and an open-label, uncontrolled trial (Trial 08) were analyzed individually to support safety for nirsevimab in the prevention of MA RSV LRTI in infants entering or born during their first RSV season and in high-risk children entering their second RSV season. The results from Trials 03, 04, and 05 were considered pivotal for supporting the proposed indication. Safety data analysis for Trials 03, 04, and 05 were conducted by the Clinical Data Scientists (CDS). Safety analysis of Trials 02 and 08 were conducted by the clinical reviewer using JMP statistical software.

The results from Trials 03 and 04 were pooled, because both trials enrolled otherwise healthy infants born prior to or during their first RSV season. Infants in Trial 03 were born at ≥29 weeks to <35 weeks gestational age, while infants in Trial 04 were born at ≥35 weeks GA. Because all subjects were otherwise healthy without major underlying diseases, prematurity in and of itself should not impact the safety results of either trial. All infants who received nirsevimab in Trial 03 received a single 50 mg IM dose, regardless of weight. Subjects who received nirsevimab in Trial 04 received a weight-based dose, as recommended in the proposed package insert. Subjects weighing <5 kg received a single, 50 IM dose, and subjects weighing ≥5 kg received a single, 100 mg IM dose. Therefore, only subjects who received the recommended dose of nirsevimab in Trial 03 were pooled with the subjects in Trial 04.

Data from Trials 02, 05, and 08 were not pooled with other trial data except in the analyses of deaths. Trial 02 was a phase 1 /2 dose-escalation trial including infants born at ≥29 weeks to <35 weeks gestational age, who were randomized to receive nirsevimab doses of 10 mg, 25 mg, or 50 mg. Since most subjects in this trial were likely underdosed, it was not considered appropriate to pool these data with data from other trials. All subjects in Trial 02 had safety laboratory monitoring; laboratory results from Trial 02, as well as laboratory results from Trials 04, and 05 were reviewed. The trial populations in Trials 05 and 08 had underlying conditions that precluded pooling with other trials. Trial 05 enrolled premature infants as well as infants with CLD of prematurity and hemodynamically significant CHD. In addition, infants with CLD and CHD received a second dose of trial drug prior to their second RSV season and were followed through two RSV seasons. Immunocompromised children younger than 2 years of age were

enrolled in Trial 08. These subjects were enrolled in either their first or second year of life and were followed for one RSV season. The types and severity of underlying diseases in Trial 08 varied, which complicated the analysis of safety in this trial.

Adequacy of Applicant's Clinical Safety Assessments

Clinical safety assessments in all five trials included evaluations of adverse events, including adverse events of special interest, and of vital signs. Adverse events were assessed for 360 days after the first dose of trial drug was administered.

In Trial 04 and in both RSV seasons in Trial 05, subjects were seen at the trial site on Day 1, 8, 15, 31, 91, 121, 151, and 361. Trial drug was administered on Day 1 at the clinical trial site. Vital signs (temperature, blood pressure, heart rate, and respiratory rate) were obtained 60 minutes prior to dosing as well as 30 minutes and 60 minutes postdosing. Subjects in Trial 04 were followed with routine telephone calls from Day 362 until Day 511 to monitor for any MA RTIs.

Subjects in Trial 03 were seen at the trial site on Days 1, 31, 91, 151, and 361; subjects in Trial 02 had an additional trial visit on Day 271. Subjects in Trial 08 had trial visits on Days 1, 8, 31, 91, 151, and 361.

Subjects with CLD and CHD were eligible to continue in Trial 05 for a second RSV season. Subjects were followed for 360 days after nirsevimab administration in Season 2 for safety assessment. The safety database was closed after the last subject reached Day 150 postdose, and safety database for this BLA submission was only available through Day 150 postdose from the second RSV season. Safety laboratory assessments were evaluated only in Trial 02 and in the subset of subjects who were enrolled at Japanese trial sites in Trials 04 and 05. Subjects were followed for 511 days in Trial 04 and for 360 days postdose in all other trials.

No major data quality or integrity issues were identified that would preclude performing an adequate safety review for this BLA. There were no major issues identified with respect to recording, coding, and categorizing AEs. The Applicant's translations of verbatim terms to Medical Dictionary for Regulatory Activities (MedDRA) preferred terms for the events reported in Trials 03, 04, and 05 were reviewed, and found to be acceptable.

Adverse events in all trials were graded using Common Terminology Criteria for Adverse Events.

7.5. Adequacy of the Clinical Safety Database

Nirsevimab Safety Database From Clinical Trials

Overall, the safety database of 3,751 pediatric subjects who received a single dose of nirsevimab in Trials 02, 03, 04, 05 and 08 is adequate to assess the safety of nirsevimab for the proposed indication of prevention of MA RSV LRTI. A total of 3,285 subjects in the safety database received nirsevimab at the to-be-marked dose.

The safety database includes 1,567 healthy, term infants who received nirsevimab in Trials 04 and 05. Trial 05 enrolled a total of 614 preterm infants and infants with CLD and/or CHD;

subjects in the CLD/CHD cohort could be born at any gestational age, and many were born prematurely. In Trial 05, there were a total of 138 infants with CLD of prematurity, 61 infants with hemodynamically significant CHD, and 9 infants with both CLD and CHD. Of these infants in the CLD/CHD plus the preterm cohorts, 128 infants in were born at <29 weeks GA. The safety database also includes 60 immunocompromised children, who were enrolled in Trial 08, and 71 premature infants enrolled in Trial 02. These numbers do not reflect all of the subjects enrolled in the nirsevimab clinical trials.

During the clinical development of nirsevimab, the Agency requested a safety database of at least 3,000 subjects exposed to nirsevimab and 3,285 infants and children received the to-be-marked dose. This size allows estimations regarding frequency of rare adverse events. For example, anaphylaxis was not reported in the clinical development program, and from the statistical rule of three, it can be concluded with 95% confidence that fewer than 1 person in 1,000 (or 3/3,000) will experience anaphylaxis with nirsevimab. Therefore, the size of this safety database is reasonably sufficient to predict that nirsevimab-associated adverse reactions not observed in the clinical trials are unlikely to be common in the real-world setting.

In all five trials, nirsevimab was administered at the trial site on Day 1 and subjects were observed for at least 60 minutes postdose. Because nirsevimab is administered as a single dose (for an RSV season), all subjects received the entire course of nirsevimab.

Duration of exposure is not shown for Trials 02, 03, 04 or 08, in which subjects were randomized to a single dose of nirsevimab or placebo.

In Trial 05, subjects were randomized in a 2:1 ratio to receive either nirsevimab or palivizumab. Palivizumab is administered once monthly during RSV season for 5 total doses. Subjects in the nirsevimab arm received a single dose of nirsevimab, then received monthly doses of placebo to equal five total doses. Subjects were monitored for 60 minutes postdose for all nirsevimab, placebo, and palivizumab doses. The mean duration of treatment in the palivizumab arm was 16.6 weeks; 88% of subjects in the palivizumab arm received all 5 doses of palivizumab.

In summary, the size of the safety database for nirsevimab appears sufficient to identify anticipated safety issues. In addition, the extensive experience with palivizumab, a closely related product, provides some assurance regarding the safety of nirsevimab.

7.6. Safety Results

Populations

All subject treated with trial drug were included in the safety analyses. The primary safety results in this section are presented based on analyses from pooled data: Trial 03 (infants who received the proposed dose of nirsevimab) and Trial 04 (Primary and Safety Cohorts); this combined Trial 03 and 04 population is referred to as the 'Pooled Safety Population'. Safety results from Trial 05 are also presented in this section.

Key Safety Assessments

The key safety assessments presented here include serious adverse events, including death, adverse events leading to discontinuation, common treatment-emergent adverse events and adverse reactions, and adverse events of special interests (e.g., hypersensitivity reactions). Safety information from Trials 02 and 08 are included where appropriate, such as in the evaluations of death. Information on deaths and hypersensitivity reactions, including anaphylaxis, are presented in Section 7.7.

Please see Section 17 for a discussion of the safety results of the individual trials (Trials 02, 03, 04, 05 and 08). Laboratory results from all trials, including Trial 02, are also described in Section 17.

7.6.1. Safety Results From Clinical Trials

7.6.1.1. Overview of Treatment-Emergent Adverse Events

Nirsevimab demonstrated an overall favorable safety profile in the pivotal placebo-controlled clinical trials. The incidence of SAEs, AEs leading to discontinuation of trial drug, any treatment-emergent adverse event (TEAE), and severe AEs were similar or higher in the placebo group compared to the nirsevimab treatment arm. (See Table 39).

Treatment-emergent AE for Trial 03 was defined as events present at baseline that worsened in intensity after administration of investigational product (IP) or events absent at baseline that emerged after administration of IP, for the period extending to 360 days after the last dose of investigational product. For Trial 04, treatment-emergent adverse event was defined as events present at baseline that worsened in intensity after administration of IP or events absent at baseline that emerged after administration of IP, for the period extending through 360 days postdose.

Table 39. Overview of Treatment-Emergent Adverse Events, Pooled Safety Population[a]

Event	Nirsevimab N=2,570 n (%)	Placebo N=1,284 n (%)
SAE	195 (7.6%)	135 (10.5%)
SAEs with fatal outcome	6 (0.2%)	3 (0.2%)
Life-threatening SAEs	7 (0.3%)	5 (0.4%)
AE leading to permanent discontinuation of trial drug	0 (0%)	0 (0%)
Any AE[b]	2,158 (84%)	1,060 (82.6%)
Severe and worse	102 (4.0%)	81 (6.3%)
Moderate	524 (20.4%)	289 (22.5%)
Mild	1,532 (59.6%)	690 (53.7%)

Source: CDS: adae.xpt; Software: R.
[a] Pooled Safety Population: subjects from Trial 03 (infants who received the proposed dose of nirsevimab) and Trial 04 (Primary and Safety Cohorts) who received study drug, analyzed according to drug received.
[b] Severity as assessed by the investigator using NCI CTCAE.
Abbreviations: AE, adverse event; CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects with at least one event; SAE, serious adverse event

For Trial 05, treatment-emergent adverse events, defined as any AE that started on/after the Season 1 Day 1 dose (start of Season 1) and prior to the end of Season 1, Day 361 (end of Season 1) or the last day prior to Season 2 Dose 1 (start of Season 2).

Safety results were similar in the nirsevimab and palivizumab arms during the first RSV season in Trial 05. (Table 40). The percentage of subjects with SAEs was low in both treatment arms and was similar between arms in both the preterm and CLD/CHD cohorts. The percentages of subjects with adverse events and severe adverse events were also similar in the nirsevimab and palivizumab arms. There was one adverse event leading to premature treatment discontinuation in the nirsevimab arm. Nirsevimab was administered on Day 1 and saline was administered to subjects in the nirsevimab arm as a placebo match for palivizumab, so premature trial drug discontinuation in the nirsevimab arm was discontinuation of the saline placebo doses.

Table 40. Overview of Adverse Events, Trial 05 – RSV Season 1

	Overall		Preterm		CLD/CHD	
Event Category	**NIRS N=614 n (%)**	**PALI N=304 n (%)**	**NIRS N=406 n (%)**	**PALI N=206 n (%)**	**NIRS N=208 n (%)**	**PALI N=98 n (%)**
SAE	80 (13.0)	38 (12.5)	35 (8.6)	13 (6.3)	45 (21.6)	25 (25.5)
SAEs with fatal outcome	5 (0.8)	1 (0.3)	2 (0.5)	0	3 (1.4)	1 (1.0)
AE leading to permanent discontinuation of trial drug	1 (0.2)	0	1 (0.2)	0	0	0
Any AE[a]	444 (72.3)	215 (70.7)	287 (70.7)	141 (68.4)	157 (75.5)	74 (75.5)
Severe and worse	50 (8.1)	25 (8.2)	18 (4.4)	8 (3.9)	32 (15.4)	17 (17.3)
Moderate	113 (18.4)	65 (21.4)	61 (15.0)	37 (18.0)	52 (25.0)	28 (28.6)
Mild	281 (45.8)	125 (41.1)	208 (51.2)	96 (46.6)	73 (35.1)	29 (29.6)

Source: CDS: adae.xpt; Software: R
[a] Severity as assessed by the investigator.
Abbreviations: AE, adverse event; CHD, congenital heart disease; CI, confidence interval; CLD, chronic lung disease; N, number of subjects in treatment arm; n, number of subjects with at least one event; NIRS, nirsevimab; PALI, palivizumab; SAE, serious adverse event

In the second RSV in Trial 05, the palivizumab arm was considerably smaller (N=42) than the nirsevimab arm (N=220), making comparison of safety difficult. (Table 41) Serious adverse events were reported in 21 subjects (9.5%) who received nirsevimab in the second RSV season, but no SAEs were reported in subjects who received palivizumab. The most common SAEs reported in subjects who received nirsevimab were infections (N=17 or 7.7%). The type of infections varied and included both upper and lower respiratory tract infections and gastrointestinal SAEs. There were also more severe AEs in the nirsevimab arm. The reasons for these differences are unclear. The percentage of subjects with any adverse events is similar in the two treatment arms.

Table 41. Overview of Adverse Events, Trial 05 – Season 2

Event Category	PALI/PALI N=42 n (%)	All NIRS N=220 n (%)
SAE	0	21 (9.5)
SAEs with fatal outcome	0	0
AE leading to permanent discontinuation of trial drug	0	0
Any AE[a]	29 (69.0)	155 (70.5)
Severe and worse	1 (2.4)	18 (8.2)
Moderate	14 (33.3)	52 (23.6)
Mild	14 (33.3)	85 (38.6)

Source: CDS: adae.xpt; Software: R
[a] Severity as assessed by the investigator.
Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with at least one event; NIRS, nirsevimab; PALI, palivizumab; SAE, serious adverse event

7.6.1.2. Deaths: Trials 02, 03, 04, 05, and 08 (Pooled)

Please refer to section 7.7.2 for discussion of deaths in these trials.

7.6.1.3. Serious Treatment-Emergent Adverse Events: Trials 03 and 04 (Pooled) and Trial 05

Trials 03 and 04

A serious adverse event was defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect. Treatment-emergent SAEs were pooled for the subjects in Trial 03 who received the recommended dose and for all subjects in Trial 04. A total of 195 subjects (7.6%) in the nirsevimab arm reported at least one SAE compared to 135 (10.5%) in the placebo arm.

Individual serious adverse events that were reported in more than 0.5% of subjects in Trials 03 and 04 are included in Table 42. Bronchiolitis was the only SAE reported in more than 1% of subjects and was reported more frequently in subjects who received placebo than in those who received nirsevimab. Bronchiolitis is the most common cause of hospitalizations in infants and may be caused by viruses other than RSV (Justice and Le 2023). Therefore, it is not unexpected that bronchiolitis was the most common SAE. The percentage of subjects with other SAEs was similar in the nirsevimab and placebo arms.

Overall, the percentage of subjects with individual SAEs was low and was similar in the nirsevimab and placebo arms.

Table 42. Serious Treatment-Emergent Adverse Events Reported in >0.5% of Subjects, Pooled Safety Population[a]

System Organ Class Preferred Term	Nirsevimab N=2,570 n (%)	Placebo N=1,284 n (%)
Bronchiolitis	34 (1.3%)	33 (2.6%)
Pneumonia	17 (0.7%)	12 (0.9%)
Lower respiratory tract infection	16 (0.6%)	10 (0.8%)
Bronchitis	12 (0.5%)	13 (1.0%)
Gastroenteritis	16 (0.6%)	5 (0.4%)

Source: CDS: adae.xpt; Software: R.
[a] Pooled Safety Population: subjects from Trial 03 (infants who received the proposed dose of nirsevimab) and Trial 04 (Primary and Safety Cohorts)
Abbreviations: AE, adverse event; CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects with adverse event; PT, preferred term; SOC, system organ class

Trial 05

A serious adverse event was defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.

Serious adverse events reported at least 0.5% of the total subjects in RSV Season 1 of Trial 05 are shown in Table 43. In the overall population, all SAEs are reported in 2.0% or fewer subjects, and the percentage of subject with each SAE is similar in the two treatment arms. SAEs were more common in the CLD/CHD cohort than in the preterm cohort. This is likely due to the underlying disease that qualified the subjects for enrollment in Trial 05. There was an increase in gastroenteritis in subjects who received nirsevimab in the CLD/CHD cohort compared to subjects who received palivizumab. This difference was not observed in the preterm cohort, and the reason for the difference is unclear. Overall, the percentages of individual SAEs were low and similar between the two treatment arms.

Table 43. Treatment-Emergent Serious Adverse Events Trial 05 – Season 1

	Overall		Preterm		CLD/CHD	
Event Category	NIRS N=614 n (%)	PALI N=304 n (%)	NIRS N=406 n (%)	PALI N=206 n (%)	NIRS N=208 n (%)	PALI N=98 n (%)
Bronchiolitis	12 (2.0%)	4 (1.3%)	5 (1.2%)	0	7 (3.4%)	4 (4.1%)
Gastroenteritis	6 (1.0%)	1 (0.3%	0	1 (0.5%)	6 (2.9%)	0
Bronchitis	5 (0.8%)	2 (0.7%)	3 (0.7%)	1 (0.5%)	2 (1.0%)	1 (1.0%)
Pneumonia	5 (0.8%)	1 (0.3%)	2 (0.5%)	0	3 (1.4%)	1 (1.0%)
RSV bronchiolitis	4 (0.7%)	2 (0.7%)	0	1 (0.5%)	4 (1.9%)	1 (1.0%)
Upper respiratory tract infection	1 (0.2%)	4 (1.3%)	0	2 (1.0%)	1 (0.5%)	2 (2.0%)

Source: adae.xpt; Software: R
Abbreviations: AE, adverse event; CHD, congenital heart disease; CI, confidence interval; CLD, chronic lung disease; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PALI, palivizumab; SAE, serious adverse event; SOC, system organ class.

SAEs were reported in 17 subjects (9.4%) who received nirsevimab in Season 2 of Trial 05 but in none of the subjects who received palivizumab. SAEs reported in more than one subject in the

nirsevimab arm were viral bronchitis (N=3 or 1.7%) and in two subjects (1.1%) each: COVID-19, gastroenteritis, lower respiratory tract infection and upper respiratory tract infection. Although there was an overall increase in SAEs in the nirsevimab arm, the individual types of SAEs varied and there was not an increase in any individual type of SAE.

7.6.1.4. Adverse Events Leading to Treatment Discontinuation: Trials 03 and 04 (Pooled), and Trial 05

Nirsevimab was administered as a single intramuscular dose. As a result, premature discontinuation did not change either the number of subjects dosed or the amount of trial drug administered. Only one subject discontinued a trial prematurely due to an adverse event. That subject experienced a Grade 1 maculopapular rash that appeared on his face and trunk 15 minutes after receipt of a placebo injection in Trial 05. The subject had received 100 mg nirsevimab 60 days earlier, and the placebo injection was given to blind for the use of the active control (palivizumab), which is administered monthly during the RSV season. No subjects in the palivizumab arm of Trial 05 discontinued either the treatment or the trial prematurely because of an adverse event.

Please see Section 7.7.2. for a description of subjects who prematurely discontinued trial due to death.

7.6.1.5. Common Treatment-Emergent Adverse Events: Trials 03 and 04 (Pooled), and Trial 05

Trials 03 and 04

Treatment-emergent adverse events (TEAEs) were defined in Trials 03 and 04 as any AE that started on/after the Day 1 dose and prior to Day 361.

Individual TEAEs reported in Trials 03 and 04 were common childhood illnesses (Table 44). The most commonly reported TEAE in nirsevimab and placebo arms was upper respiratory tract infection. There were no individual TEAEs reported in a higher percentage of nirsevimab subjects with a greater than 2% difference between the nirsevimab and placebo arms. Overall, the frequency of all individual TEAEs was similar between the nirsevimab and treatment arms.

Table 44. Treatment-Emergent Adverse Events Occurring at ≥5% Frequency in the Nirsevimab Treatment Group, Pooled Safety Population[a]

Preferred Term[b]	Nirsevimab N=2570 n (%)	Placebo N=1284 n (%)
Upper respiratory tract infection	818 (31.8%)	384 (29.9%)
Nasopharyngitis	489 (19.0%)	269 (21.0%)
Pyrexia	356 (13.9%)	162 (12.6%)
Diaper dermatitis	256 (10.0%)	115 (9.0%)
Gastroenteritis	244 (9.5%)	110 (8.6%)
Rhinitis	229 (8.9%)	118 (9.2)

Preferred Term[b]	Nirsevimab N=2570 n (%)	Placebo N=1284 n (%)
Nasal congestion	208 (8.1%)	94 (7.3%)
Rhinorrhea	171 (6.7%)	75 (5.8%)
Bronchiolitis	169 (6.6%)	114 (8.9%)
Teething	159 (6.2%)	80 (6.2%)
Conjunctivitis	156 (6.1%)	74 (5.8%)
Otitis media	151 (5.9%)	83 (6.5%)
Viral URTI	148 (5.8%)	68 (5.3%)

Source: CDS: adae.xpt; Software: R.
[a] Pooled Safety Population: subjects from Trial 03 (infants who received the proposed dose of nirsevimab) and Trial 04 (Primary and Safety Cohorts)
[b] Coded as MedDRA preferred terms.
Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; N, number of subjects in treatment arm; n, number of subjects with adverse event; PT, preferred term

Adverse reaction is defined as drug-related adverse event as assessed by an Investigator to be at least possibly related to trial treatment. Adverse reactions are described in Table 45. The percentage of subjects with any treatment-related AE was low and similar in the nirsevimab and placebo arms. Although the percentages of subjects with rash in each arm are low, there were more subjects with rashes in the nirsevimab arm (N=11 or 0.4%) (maculopapular rash in seven subjects, rash in three subjects, and petechia, papular rash, and drug eruption in one each) compared to the placebo arm (N=2 or 0.2%) (papular rash and macular rash in one subject each). There were also three subjects (0.1%) in the nirsevimab arm with injection site reactions; no subjects in the placebo arm had injection site reactions. Overall, the percentages of subjects in the nirsevimab and placebo arms with adverse reactions were similar in the two treatment arms. Although the percentages of subjects in the nirsevimab arm with rashes and with injection site reactions were low, these adverse reactions were reported more commonly with nirsevimab than with placebo; it is possible that rashes and injection site reactions will be reported with nirsevimab in the postmarketing setting.

Table 45. Treatment-Emergent Adverse Reactions, Pooled Safety Population[a]

Preferred Term	NIRS N=2570 n (%)	PBO N=1284 n (%)
Any treatment-related AE	33 (1.3)	18 (1.4)
Rash maculo-papular	7 (0.3)	0
Irritability	4 (0.2)	3 (0.2)
Rash	3 (0.1)	0
Pyrexia	3 (0.1)	3 (0.2%)
Diarrhea	2 (0.08)	0
Injection site pain	2 (0.08)	0
Hypersomnia	1 (0.04)	0
Decreased activity	1 (0.04)	0
Dermatitis	1 (0.04)	0
Drug eruption	1 (0.04)	0
Gastroenteritis	1 (0.04)	0
Injection site swelling	1 (0.04)	0
Petechiae	1 (0.04)	0
Skin hypopigmentation	1 (0.04)	0
Decreased appetite	1 (0.04)	0

Preferred Term	NIRS N=2570 n (%)	PBO N=1284 n (%)
Nasal congestion	1 (0.04)	1 (0.08)
Rash papular	1 (0.04)	1 (0.08)
Somnolence	1 (0.04)	1 (0.08)
Vomiting	0	1 (0.08)
Eczema	0	1 (0.08)
Pharyngitis	0	1 (0.08)
Rash macular	0	1 (0.08)
Anemia	0	1 (0.08)
Constipation	0	1 (0.08)
Erythema	0	1 (0.08)
Fever neonatal	0	1 (0.08)
Upper respiratory tract infection	0	1 (0.08)
Vaccination complication	0	1 (0.08)

Source: CDS: adae.xpt; Software: R
[a] Pooled Safety Population: subjects from Trial 03 (infants who received the proposed dose of nirsevimab) and Trial 04 (Primary and Safety Cohorts)
Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo

Trial 05: First RSV Season

In Trial 05, treatment-emergent adverse events were defined as any AE that started on or after the first RSV season Day 1 dose and prior to the end of the first RSV season (Day 361), or the last day prior to the first dose for the second RSV Season 2 (start of RSV Season 2).

Individual TEAEs reported in more than 5% of subjects in Trial 05, Season 1, are shown in Table 46. The most frequently reported TEAE in the overall trial population, as well as in both the preterm and CLD/CHD cohorts, was upper respiratory tract infection, with similar proportions of subjects with upper respiratory tract infection in the nirsevimab and palivizumab arms in the overall trial population, and in the preterm and CLD/CHD cohorts. The only TEAE in the total population that was reported in more subjects who received nirsevimab than palivizumab with a difference of 1% or more was nasal congestion. Nasal congestion was reported in 6.7% of subjects who received nirsevimab and 4.3% of those who received palivizumab. The increase in nasal congestion was observed in observed in both the preterm and CLD/CHD cohort. The reason for the increased incidence of nasal congestion in subjects who received nirsevimab is unclear. Of note, URTI, nasopharyngitis, and rhinitis were all reported more often in subjects who received palivizumab than in nirsevimab. The differences in all TEAEs for nasal signs and symptoms were small and are not likely to be clinically relevant. In the preterm cohort, no TEAEs besides nasal congestion were observed more frequently in the nirsevimab arm than the palivizumab arm with a difference of more than 1%. In the CLD/CHD cohort, both pyrexia and teething were reported more often in the nirsevimab arm compared to the palivizumab arm with a difference of >1%. The reason for increased incidence of pyrexia and teething in the CLD/CHD cohort alone is unclear. Overall, the percentages of individual TEAEs were similar between the two treatment arms for the total trial population and in the preterm and CLD/CHD cohorts.

Table 46. Adverse Events Reported in >5% of Trial Subjects, Trial 05 – Season 1

	Overall		Preterm		CLD/CHD	
Event Category	**NIRS N=614 n (%)**	**PALI N=304 n (%)**	**NIRS N=406 n (%)**	**PALI N=206 n (%)**	**NIRS N=208 n (%)**	**PALI N=98 n (%)**
Upper respiratory tract infection	148 (24.1%)	78 (25.7%)	109 (26.8%)	55 (26.7%)	39 (18.8%)	23 (23.5%)
Pyrexia	83 (13.5%)	43 (14.1%)	54 (13.3%)	33 (16.0%)	29 (13.9%)	10 (10.2%)
Rhinitis	75 (12.2%)	40 (13.2%)	48 (11.8%)	27 (13.1%)	27 (13.0%)	13 (13.3%)
Nasopharyngitis	57 (9.3%)	39 (12.8%)	36 (8.9%)	20 (9.7%)	21 (10.1%)	19 (19.4%)
Nasal congestion	41 (6.7%)	13 (4.3%)	33 (8.1%)	11 (5.3%)	8 (3.8%)	2 (2.0%)
Viral upper respiratory tract infection	35 (5.7%)	15 (4.9%)	16 (3.9%)	6 (2.9%)	19 (9.1%)	9 (9.2%)
Teething	31 (5.0%)	16 (5.3%)	18 (4.4%)	12 (5.8%)	13 (6.2%)	4 (4.1%)
Bronchiolitis	32 (5.2%)	13 (4.3%)	22 (5.4%)	9 (4.4%)	10 (4.8%)	4 (4.1%)

Source: CDS: adae.xpt; Software: R
Abbreviations: AE, adverse event; CHD, congenital heart disease; CLD, chronic lung disease; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PALI, palivizumab

Adverse reactions, defined as adverse events as determined by the Investigator to be at least possibly related to trial treatment, reported during the first RSV season of Trial 05 are shown in Table 47.

Agitation was reported as an adverse reaction more commonly in the nirsevimab arm than in the palivizumab arm and was associated with nirsevimab administration. Although the preferred term, increased body temperature, was reported more often in the nirsevimab arm, there was no increase in the preferred term, pyrexia in infants who received nirsevimab. Overall, the percentage of subjects with adverse reactions was low and was similar between the nirsevimab and palivizumab arms.

Table 47. Treatment-Emergent Adverse Reactions Trial 05 – Season 1

	Overall		Preterm		CLD/CHD	
Preferred Term	**NIRS N=614 n (%)**	**PALI N=304 n (%)**	**NIRS N=406 n (%)**	**PALI N=206 n (%)**	**NIRS N=208 n (%)**	**PALI N=98 n (%)**
Any treatment-related AE	10 (1.6)	6 (2.0)	6 (1.5)	4 (1.9)	4 (1.9)	2 (2.0)
Agitation	3 (0.5)	0	1 (0.2)	0	2 (1.0)	0
Body temperature increased	2 (0.3)	0	2 (0.5)	0	0	0
Induration	1 (0.2)	0	1 (0.2)	0	0	0
Rash	1 (0.2)	0	0	0	1 (0.5)	0
Rash maculo-papular	1 (0.2)	0	1 (0.2)	0	0	0
Irritability	1 (0.2)	1 (0.3)	1 (0.2)	0	0	1 (1.0)
Pyrexia	1 (0.2)	2 (0.7)	0	2 (1.0)	1 (0.5)	0
Diarrhea	0	1 (0.3)	0	1 (0.5)	0	0
Injection site induration	0	1 (0.3)	0	0	0	1 (1.0)
Rash macular	0	1 (0.3)	0	1 (0.5)	0	0
Vomiting	0	1 (0.3)	0	1 (0.5)	0	0

Source: CDS: adae.xpt; Software: R
Abbreviations: AE, adverse event; CHD, congenital heart disease; CLD, chronic lung disease; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PALI, palivizumab

Trial 05: Second RSV Season

The most common TEAE in Season 2 of Trial 05 was upper respiratory tract infection (See Table 48). All other frequently reported TEAEs were common childhood illness except for COVID-19. Season 2 was conducted during the COVID-19 pandemic and COVID was reported in 5.9% of subjects in the nirsevimab arm and in 7.1% of subjects in the palivizumab arm. The differences in the incidences of TEAEs are larger in Season 2 than in Season 1 or in pooled data from Trials 03 and 04 because of the smaller number of subjects who participated in Season 2, particularly in the palivizumab arm. Although URTI, viral URTI, and rhinorrhea were reported more frequently in the nirsevimab arm, nasopharyngitis and rhinitis were reported more often in the palivizumab arm. This inconsistency in upper respiratory symptoms suggests that the differences are not likely related to the trial drugs. Pyrexia, gastroenteritis, and otitis media were also reported in a higher percentage of subjects who received nirsevimab than who received palivizumab, but the difference between the incidence of each TEAE was less than 1.5% for each. Overall, there was no substantial increase incidence of any individual TEAE in Season 2 of Trial 05.

Table 48. Treatment-Emergent Adverse Events, Trial 05 - Season 2

Event Category	All NIRS N=220 n (%)	PALI/PALI N=42 n (%)
Upper respiratory tract infection	52 (23.6)	7 (16.7%)
Nasopharyngitis	28 (12.7%)	9 (21.4%)
Pyrexia	29 (13.2%)	5 (11.9%)
Rhinitis	26 (11.8%)	6 (14.3%)
Rhinorrhea	17 (7.7%)	2 (4.8%)
Viral URTI	17 (7.7%)	2 (4.8%)
Diarrhea	11 (5.0%)	5 (11.9%)
Gastroenteritis	13 (5.9%)	2 (4.8%)
COVID-19	12 (5.5%)	3 (7.1%)
Acute otitis media	12 (5.5%)	2 (4.8%)

Source: CDS: adae.xpt; Software: R
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PALI, palivizumab

There were no adverse events judged as treatment-related in Season 2 of Trial 05.

7.6.1.6. Adverse Events of Special Interest: Trials 03, 04, and 05

In the three pivotal trials (Trials 03, 04, and 05), information on adverse events of special interest (AESIs) was collected during the 360-day safety follow-up. AESIs in these trials were defined in the protocols as immediate hypersensitivity reactions, including anaphylaxis, immune complex disease, and thrombocytopenia. These adverse events have been reported with palivizumab or with other monoclonal antibodies. We conducted a review of all TEAEs that were consistent with one of these three categories, regardless of causality as judged by the investigator.

Hypersensitivity Reactions, Including Anaphylaxis

There were no reports of anaphylaxis. See Section 7.7.1 for discussion on hypersensitivity reactions, including anaphylaxis and skin reactions (rashes that may represent hypersensitivity events).

Immune Complex Disease

There were no reports of immune complex disease during the clinical trials.

Thrombocytopenia

There were two AESIs of thrombocytopenia reported, both reported in Trial 05, and both subjects received nirsevimab. One event of thrombocytopenia was in a subject with CHD who experienced heparin-induced thrombocytopenia. The other was reported in a subject with CHD who was septic at the time. Neither event was assessed as related to nirsevimab.

7.7. Key Safety Review Issues

7.7.1. Hypersensitivity Reactions, Including Anaphylaxis and Rash

Issue

Allergic reactions have been associated with biologic products, including monoclonal antibodies. Anaphylaxis, hypersensitivity reactions, and rash are a well-known, and often serious, presentations of an allergic reaction to monoclonal antibodies. Therefore, the safety database for nirsevimab was examined carefully to fully characterize allergic reactions to nirsevimab.

Background

Immune-mediated adverse reactions, ranging from anaphylaxis to hypersensitivity skin reactions, are well-known adverse reactions associated with the use of monoclonal antibodies (Pintea et al. 2021). These adverse reactions have been reported during the postmarketing period with palivizumab, a monoclonal antibody against the RSV fusion protein with a similar mechanism of action as nirsevimab.

Anaphylaxis, a form of severe hypersensitivity reaction, can occur within minutes to hours after administration. Anaphylaxis typically involves a cluster of clinical signs and symptoms that may include changes in the skin and/or mucosa, respiratory changes, gastrointestinal symptoms, and/or a decrease in blood pressure. Please see the National Institute of Allergy and Infectious Disease (NIAID) criteria for the diagnosis of anaphylaxis (Sampson et al. 2006).

Skin reactions and rashes have been reported with monoclonal antibody use. Hypersensitivity skin reactions can range from mild macular rash or urticaria, to more severe forms. Severe hypersensitivity skin reactions such as Stevens-Johnson syndrome or toxic epidermal necrolysis may be observed from 2 to 7 days after drug exposure. Other rashes typically occur within 2 to 3

weeks of starting a new medication, but the time of onset could be longer with a drug or product that has a long half-life, such as nirsevimab.

Allergic reactions to drugs can also occur days after drug product administration. Immune complex diseases may occur 1 to 3 weeks after drug exposure and may result in serum sickness, fever, rash, arthralgias, urticaria, glomerulonephritis, or vasculitis (Riedl and Casillas 2003; Isabwe et al. 2018).

Assessment

Nirsevimab was administered on trial Day 1 in the clinical trials. In order to search for hypersensitivity reactions, including anaphylaxis, adverse events with onset on trial Day 1 or 2 were examined by:

- Identification of adverse events consistent with hypersensitivity reactions such as MedDRA preferred terms of anaphylactic reaction, anaphylactoid reaction and drug hypersensitivity in all trials of nirsevimab;
- Analysis of adverse events in the pivotal nirsevimab trials, Trials 03, 04, and 05 using the narrow FDA Medical Queries (FMQs),[1] which is a group of related preferred terms for AEs for hypersensitivity;
- Analysis of adverse events in Trials 03, 04, and 05 using an algorithm developed by FDA Clinical Data Scientists. This algorithm was designed to identify adverse events clusters that fulfilled any of the three criteria for anaphylaxis, as defined by the NIAID and the Food Allergy and Anaphylaxis Network and used for diagnosing anaphylaxis.

No adverse events which were categorized as anaphylaxis, anaphylactic reaction, or hypersensitivity reaction were reported in any of the nirsevimab trials submitted with the BLA. No adverse events occurring within the two days of nirsevimab administration were identified by the FMQ search for hypersensitivity AEs. No cluster of adverse events consistent with the NIAID and Food Allergy Anaphylaxis Network definition for anaphylaxis were identified.

Safety data were also analyzed for hypersensitivity reactions or possible allergic reactions occurring more than 2 days after dosing with trial product. This included a search for angioedema, which is a severe skin and/or mucosal tissue allergic reaction. One adverse event of angioedema was reported in Trial 04, in which a 1-month-old, white, female developed Grade 3

[1] FDA FMQs include acute generalized exanthematous pustules, administration site hypersensitivity, administration site recall reaction, administration site vasculitis, allergic cough, allergic cystitis, allergic gastroenteritis, allergic hepatitis, allergic otitis, allergic respiratory disease, allergic stomatitis, anaphylactic reaction, anaphylactic shock, anaphylactoid reaction, anaphylactoid shock, allergic arthritis, atopic cough, documented hypersensitivity to administered product, drug eruption, drug hypersensitivity, allergic encephalitis or encephalopathy, epidermal necrosis, epidermolysis, eye allergy, fixed eruption, hypersensitivity, hypersensitivity myocarditis, pneumonitis or vasculitis, injection site hypersensitivity, Kounis syndrome, allergic nephritis, Nikolsky's sign, oculo-respiratory syndrome, oral allergy syndrome, allergic pruritis, allergic scleritis, serum sickness, serum sickness-like reaction, Stevens-Johnson syndrome, toxic epidermal necrolysis, Type I, II, II, or IV hypersensitivity, cross sensitivity reaction.

angioedema 142 days after receiving nirsevimab. Angioedema was attributed to a change in formula and judged as unrelated to nirsevimab; the review team agrees with this assessment.

All adverse events of rashes identified in the analysis are discussed in the next section.

Rash (Hypersensitivity Skin Reaction)

Based on the FMQ hypersensitivity search criteria, the proportion of subjects for with rash events that were consistent with a hypersensitivity reaction or allergic reaction was low.

The incidence of all rashes occurring within 14 days of receipt of trial product was assessed for Trials 03, 04, and 05. Rashes that were not considered allergic or part of a hypersensitivity reaction were excluded from the analysis (e.g., diaper dermatitis, insect bites, infantile acne, viral exanthem, and single skin lesions). The two-week time period captures the peak serum concentration of nirsevimab. The proportion of subjects with rash within the 2 weeks after dosing was <1% in both the nirsevimab and the control arm in all three trials. Finally, there was no consistent increase in any specific type of skin reactions, such as dermatitis, rash, erythema or exfoliation in the nirsevimab arm compared to the control arm across the three trials.

In the FMQ search for adverse events related to hypersensitivity during the 360-day follow-up period, three AEs of skin reactions possibly related to nirsevimab were identified. While there were other adverse events of rash identified in this search, all were thought to be due to other causative agents. The rash events, considered at least possibly related to nirsevimab included the following:

- A Grade 2 (moderate) drug eruption was reported on Day 6 postdose in a 4-month-old female enrolled in Trial 04. The drug eruption was judged as related to nirsevimab and was treated with calamine lotion.
- Grade 1 urticaria was reported on Day 20 postdose in a 6.9-month-old female enrolled in Trial 03. The urticaria required treatment with oral antihistamines and a topical steroid and resolved in 5 days.
- Grade 1 urticaria was reported on Day 7 postdose in a 6-month-old female enrolled in Trial 08. The urticaria resolved within 2 days without treatment.

In summary, after an analysis of possible hypersensitivity reactions in the pivotal trials by review of the datasets, the incidence of rash, judged as possible hypersensitivity skin reactions and considered possibly related to nirsevimab was less than 1%, and was only slightly higher in subjects who received nirsevimab compared to placebo.

Another analysis of skin adverse reactions was analysis of skin adverse reactions that were judged by the investigator as related to the trial drug and as skin hypersensitivity reactions. In this analysis, skin adverse events consistent with these criteria were identified in no subjects who received placebo and in six subjects (0.2%) who received nirsevimab in Trials 03 and 04. Skin hypersensitivity reactions that were judged as drug-related were identified in no subjects who received palivizumab and in one subject (0.2%) who received nirsevimab in Trial 05, Season 1. There were no skin adverse events that were identified as drug-related and hypersensitivity reactions in Trial 05, Season 2. All rashes were generalized in distribution. Four of the 7 skin

hypersensitivity rashes occurred on Day 3 or earlier. All but one rash was Grade 1 in severity. One rash, that began on Day 7, was Grade 3; this rash was also temporally related to the infant starting a new probiotic. In this analysis, the percentage of subjects with a skin adverse event judged by the investigator as a drug-related skin hypersensitivity adverse event, the number of adverse events were higher in the nirsevimab arms compared to the control arms. However, the overall percentage of these rashes was low (<1%) and the majority of rashes were mild.

Conclusion

No adverse events of anaphylaxis were reported in any of the clinical trials. In addition, severe or serious skin reactions were uncommon in the trials of nirsevimab, as were rashes that were considered allergic reactions to nirsevimab. The incidence of rashes judged as possibly drug-related was <1% among subjects randomized to either the nirsevimab arm or the control arms over the 360-day safety follow-up period. In addition, the proportion of subjects with rashes was similar in the nirsevimab and control arms in Trials 03, 04, and 05 and there was no substantial increase in rash in the two weeks following administration of nirsevimab. Overall, the incidence of rash in the trials of nirsevimab was low, regardless of the type of analysis performed.

7.7.2. Imbalance in Deaths During Clinical Trials

Issue

Nirsevimab was studied in five pediatric clinical trials. In these trials, there were 12 deaths in subjects who received nirsevimab compared to four deaths in subjects who received the control. The number of deaths in the nirsevimab exceeds what one would expect with the 2:1 randomization in Trials 03, 04, 05.

Background

Subjects in each nirsevimab trials were followed for at least 360 days to monitor safety. In addition, the clinical trials were conducted in multiple countries, including countries in which the health care may vary from that in the United States. The global mortality rate in 2022 was 26.7 deaths per 1,000 live births (Macrotrends 2023). Therefore, it is not surprising to have infant deaths occur during the clinical development of nirsevimab. However, because of the imbalance in the number of deaths between the nirsevimab arms compared to the control arms, a thorough evaluation of these events was conducted.

Assessment

There were 16 deaths during the clinical development of nirsevimab. All available data, including subject-level narratives, were reviewed for each individual subject that died during the trials. As shown in Table 49, 12 deaths were reported in subjects who received nirsevimab and 4 in subjects who received the control. There were no deaths in Trial 02. In Trials 03, 04, and 05, subjects were randomized in a 2:1 ratio to nirsevimab or control; and in these trials, there were 11 deaths in the nirsevimab arms and 4 in the control arms. These numbers are not consistent with the randomization. Trial 08 was a single arm, uncontrolled trial; a single death was reported in this trial.

When the number of deaths was examined by percentage of subjects, the percentage was only slightly higher in the nirsevimab arms. The percentage of deaths was 0.32% in subjects who received nirsevimab (12/3,711) and 0.22% (4/1,797) in subjects who received control. The percentage of deaths (0.3%) was identical in subjects who received 50 mg of nirsevimab and in those who received 100 mg. The number of subjects who received 200 mg in the clinical development program was small; no deaths occurred at this dose.

Table 49. Number (Percentage) of Deaths by Trial

Trial	Nirsevimab Arm	Control Arm
02	0/71	0/18
03	2/968 (0.2%)	3/479 (0.6%)
04	4/1,998 (0.2%)	0/996
05	5/613 (0.8%)	1/304
08	1/60 (1.7%)	--
Total	12/3,710 (.32%)	4/1,797 (0.22%)

Source: BLA 761328, Clinical Study Reports for all studies

The causes of death are provided in Table 50.

Table 50. Causes of Death in Trials of Nirsevimab

Trial	Age at Enrollment	Demographics	Gestational Age (weeks)	Country	Cause of Death	Trial Day of Death	Medical History and other Relevant Information
				Nirsevimab Arms			
03	14 weeks	Black female	31	South Africa	Unknown	123	Previously healthy, found dead in crib
03	1.9 months	White male	32	Estonia	Cardiac failure	97	Subject with undiagnosed pulmonary vein stenosis
04	5 months	Mixed Race female	38	Panama	Skull fracture	285	Hit by car
04	3 months	Black male	37	South Africa	Gastroenteritis	143	Previous episodes of gastroenteritis and history of poor weight gain Dead on arrival to emergency services
04	7 months	Black female	38	South Africa	Gastroenteritis	338	Found lifeless after 3 days of vomiting and diarrhea, Dead on arrival to emergency services
04	1 day	White male	40	Israel	Unknown	140	History of failure to thrive with recurrent hypoglycemia and anemia. Found dead in crib
05	5 weeks	White male	32	Bulgaria	Non-RSV bronchiolitis Cardiac failure	52	History of respiratory distress syndrome and congenital CMV infection. Severe protein calorie malnutrition at time of death
05	7.5 months	White male	29	Ukraine	COVID-19	162	Premature infant (born at 29 weeks GA). Diagnosed with COVID-19 and admitted to ICU but died in hospital.
05	12 weeks	White female	39	Hungary	Bronchopneumonia	19	Subject with coarctation of aorta, congenital kidney disease, hypothyroidism and hypertonia
05	2 months	Hispanic female	38	Mexico	Cardiogenic shock	66	Subject with ASD, VSD, Trisomy 21, hypothyroidism

Trial	Age at Enrollment	Demographics	Gestational Age (weeks)	Country	Cause of Death	Trial Day of Death	Medical History and other Relevant Information
05	6.5 months	White female	38	Russian Federation	Cardiac failure	19	Subject with pulmonary atresia, VSD, congenital artery anomalies
08	10.7 months	White female	39	United States	Tumor hemorrhage	125	Bled into malignant astrocytoma
Control Arms							
03	14 weeks	Black male	32	South Africa	Pericardial effusion	343	Placebo arm: Found not breathing
03	1 month	Black male	30	South Africa	E. coli meningitis	26	Placebo arm: New onset of apnea, diagnosed with *E. coli* meningitis and sepsis and hospitalized. Died in hospital.
03	2 weeks	Black male	33	South Africa	Pneumonia	109	Placebo arm: Treated with traditional African medicine and was dead on arrival to hospital
05	20 days	White female	38	Lithuania	Respiratory insufficiency	155	Palivizumab arm: Subject with PDA, ASD, chromosomal anomaly. Hospitalized with bronchiolitis and progressively deteriorated.

Source: Clinical Study Report for Trials, 03, 04, 05, and 08, Section 14, narratives.
Abbreviations: ASD, atrial septal defect; CMV, cytomegalovirus; COVID-19, coronavirus disease 2019; GA, gestational age; ICU, intensive care unit; PDA, patent ductus arteriosus; RSV, respiratory syncytial virus; VSD, ventricular septal defect

The causes of death were varied and were not related to the same System Organ Class. In the nirsevimab group, the causes of death appeared to be related to a cause other than the drug product for eight subjects: three subjects with congenital cardiac disease, two with untreated gastroenteritis, one with COVID-19, one with a tumor, and one with a skull fracture. Another two subjects died of lower respiratory tract infections. Of these two subjects, one had underlying severe protein calorie malnutrition and the other had multiple underlying conditions. One subject died at home and the other had been removed from the hospital against medical advice. Both deaths were likely related to underlying conditions and not to nirsevimab. Finally, two subjects in the nirsevimab arm died of "unknown" causes after being found dead in their crib. One of these subjects had multiple prior hospitalizations, and his health care provider theorized that this subject had an underlying congenital metabolic or chromosomal anomaly. The other subject was healthy without previous issues. The death was on Day 123 and was not temporally related to receipt of nirsevimab. The circumstances of death are consistent with sudden infant death syndrome; however, the cause is unknown, and the autopsy result was not available for review. The causes of death in the nirsevimab are consistent with the causes of death in the control arms. In the opinion of the reviewer, none of the deaths appeared related to the trial drug. See additional information on death by trial in Section 17.

Conclusion

The number of deaths (N=12) in subjects who received nirsevimab was higher than the number in the control arms (N=4), even after accounting for 2:1 randomization in the trials. The percentage of deaths was slightly higher in the nirsevimab arms (0.32%) compared to the placebo arms (0.22%). However, the percentage of deaths in the trials was much lower than global infant mortality rates reported in 2022 (Macrotrends 2023). The causes of deaths were varied and were not related to a single System Organ Class. The causes of death in the nirsevimab arms were consistent with those in the control arms. The majority of deaths appeared related to causes other than the trial drug product. Other deaths were complicated by underlying conditions. None of the deaths were judged by the study investigators as related to the trial drug product. In the opinion of this reviewer, none of the deaths appeared related to the trial drug.

7.7.3. Pharmacovigilance

Issue

If approved, nirsevimab has the potential for widespread use and will be used for the prevention of disease in a population that includes healthy children, where the risk tolerance for use of an agent is low.

Background

Pharmacovigilance Strategies are plans for coordinated monitoring and assessment of safety information across the Center for Drug Evaluation and Research (CDER) Offices and/or Divisions to enhance postmarketing drug safety surveillance.

Assessment

CDER plans to implement a broad pharmacovigilance strategy for nirsevimab. The key focus areas of the pharmacovigilance strategy are to: 1) identify new safety signal(s); 2) monitor for increased or unusual numbers of reports of a serious adverse event; and 3) monitor for increase in the severity of a serious adverse event. To help identify safety signals, the Agency will focus on unlabeled adverse events with the potential for serious outcomes and labeled adverse events with unexpected characteristics such as an increase in severity.

CDER plans to utilize several platforms to monitor postmarked safety adverse events across several postmarketing surveillance data streams, including:

- Reports submitted to the FDA's Adverse Event Reporting System (FAERS) database, contains spontaneous adverse event reports for human drugs and therapeutic biologics,
- Relevant reports that are submitted to the Vaccine Adverse Event Reporting System (VAERS) will also be screened, as applicable, because of either a concomitantly administered vaccine AE with nirsevimab administration or an error in reporting to VAERS instead of FAERS, since this product may be confused by some to be a vaccine.
- Published medical literature using Embase and PubMed for new safety signals
- Required, periodic safety reports (PSRs) submitted by the manufacturer
- Review of safety data from ongoing clinical trial(s) evaluating nirsevimab

Based on the clinical development program and adverse events associated with monoclonal antibodies, prespecified adverse events of interest during postmarketing drug safety surveillance for nirsevimab include the following:

- Hypersensitivity reactions, including anaphylaxis and angioedema
- Immune complex disease, cytopenias, and thrombocytopenia
- Injection Site Reactions, Serious Cutaneous Adverse Reactions

CDER is also exploring active surveillance approaches for nirsevimab using the Sentinel Distributed Database that can be conducted postapproval on claims-based data sources. Additionally, FDA is collaborating with CDC on safety data collection using near real-time active surveillance platform which encompasses claims-based data sources.

While used for prevention of RSV, nirsevimab is not a vaccine and is being regulated as a drug. Education may be warranted for healthcare providers and caregivers on the appropriate adverse event reporting database for nirsevimab. CDER is in discussion with the CDC to develop educational materials for nirsevimab, similar to the Vaccine Information Statement(s) that are available for routine vaccinations. One of the key purposes of such information sheet would be to familiarize care givers on reporting of adverse events through MedWatch, the platform for adverse events reporting associated with use of drugs and therapeutic biologics.

Conclusions

CDER has determined a pharmacovigilance strategy is necessary to support coordinated monitoring and assessment of safety information from data sources across CDER Offices and/or Divisions and with the CDC. If approved, the Advisory Committee on Immunization Practices (ACIP) will provide clinical guidelines for use of nirsevimab. ACIP recommendations will be factored into the pharmacovigilance strategy, as appropriate. The full details of the pharmacovigilance strategy will be finalized in a separate document within 90 days of marketing approval. The pharmacovigilance strategy may be modified as safety information accumulates during the postmarketing period.

For information on Postmarketing Requirements and Commitments, refer to Section 24.

8. Therapeutic Individualization

8.1. Intrinsic Factors

Body Weight

The population PK analysis identified body weight as a significant covariate on clearance and central volume of distribution. Nirsevimab clearance and volume of distribution increase with increasing body weight. Please refer to Section 14.5 for details.

Age

An effect of postmenstrual age was estimated in the population PK analysis and there is a high correlation between age and body weight in children. In addition to body weight, postmenstrual age was included in the population PK analysis to describe the clearance in children. The analysis indicates clearance increases asymptotically with postmenstrual age with a maturation t½ of 14.8 months. An infant born at 40 weeks GA is estimated to have 64% lower CL at birth (9.2 months postmenstrual age), and 37% lower CL at 1 year of age (21 months postmenstrual age), compared to complete maturation. Please refer to Section 14.5 for details.

Race

Race, including White, Black, Asian, American Indian, Hawaiian/ Pacific Islander, and Multiple, was identified as a statistically significant covariate on the clearance and central volume of nirsevimab in the population PK analysis; however, the estimated effects of race (independent of body weight) were generally small relative to the overall variability. Race had no clinically relevant differences on nirsevimab PK.

Renal Impairment

No clinical studies were conducted to investigate the effect of renal impairment on nirsevimab. As a typical IgG mAb, nirsevimab is not cleared renally due to its large molecular weight, and change in renal function is not expected to influence nirsevimab CL.

Hepatic Impairment

No clinical studies were conducted to investigate the effect of hepatic impairment on nirsevimab PK. Monoclonal antibodies are not primarily cleared via the hepatic pathway and change in hepatic function is therefore not expected to influence nirsevimab CL.

8.2. Drug Interactions

No drug-drug interaction studies were conducted with nirsevimab. Monoclonal antibodies do not typically have significant drug-drug interaction potential, as they do not directly affect cytochrome P450 enzymes and are not substrates of hepatic or renal transporters. Nirsevimab is eliminated by intracellular catabolism and not primarily cleared via hepatic pathways.

8.3. Plans for Pediatric Drug Development

Nirsevimab was studied in two pediatric populations: preterm and term infants born during or entering their first RSV season and infants with CLD and/or CHD during their first and/or second RSV season. Severe RSV lower respiratory tract disease typically occurs with the first RSV infection. Most otherwise healthy children are infected with RSV and experience RSV lower respiratory tract (LRT) disease during their first RSV season, and almost all infants have had an RSV infection by 2 years of age. While the risk of hospitalization due to RSV LRT disease is highest in the second month of life and remains increased for the first 6 to 7 months of life, the risk of MA RSV LRT infection (MA RSV LRTI) extends throughout the first year of life (Hall et al. 2009). The severity of RSV disease decreases after the first year of life when RSV is typically a re-infection with upper respiratory symptoms only. In addition, in subjects older than 12 months of age, primary RSV infection with LRT disease is typically milder because changes in the pulmonary anatomy with lung growth; the larger airways of older children are less likely to have pulmonary flow resistance caused by the edema, cellular sloughing and mucus in the airways that occurs with RSV LRT disease. As a result, children older than 12 months of age have a lower incidence of infection with fewer hospitalizations and less severe disease.

The Centers for Disease Control defines children at high risk of severe RSV LRT disease as those who were premature, who are younger than 2 years of age than 2 years old with chronic lung disease or congenital heart disease, who have weakened immune systems, or who have neuromuscular disorders, including those who have difficulty swallowing or clearing mucus secretions. In the American Academy of Pediatrics (AAP) guidelines for the use of palivizumab, palivizumab use is recommended for the second year of life in only select populations (American Academy of Pediatrics Committee on Infectious and American Academy of Pediatrics Bronchiolitis Guidelines 2014). Although the AAP guidelines state that palivizumab may be considered for prophylaxis in the second year of life, the only condition for which palivizumab prophylaxis is recommended in the second year of life is for children with chronic lung disease who required at least 28 days of supplemental oxygen after birth and who continue to require medical intervention (supplemental oxygen, chronic corticosteroid, or diuretic therapy). Neither the CDC nor the AAP recommend the use of RSV prophylaxis after the second year of life. Therefore, in the high-risk population, the need for RSV prevention is limited to children ≤24 months of age.

Thus, prevention of RSV LRT disease is most appropriate for healthy neonates and infants in their first RSV season, and in those with comorbidities such as CLD of prematurity or hemodynamically significant CHD in children ≤24 months of age.

The Applicant and FDA agree that nirsevimab has been studied in the appropriate population, and the Applicant was granted a Pediatric Research Equity Act waiver for the study of nirsevimab in children older than the age of 24 months.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

Nirsevimab is not indicated for use in females of reproductive potential and in vivo DART studies were not performed. There were no signs of reproductive or developmental toxicity in the 4-week GLP repeat-dose toxicology study in cynomolgus monkeys and no clinically significant binding in the GLP TCR studies with human adult, juvenile, neonatal and fetal tissues.

Clinical Data

Adults were enrolled in a single trial of nirsevimab, Trial 01. Trial 01 was a phase 1, randomized, double-blind, placebo-controlled, dose-escalation, PK and safety trial in healthy adult volunteers from 18 to <50 years of age. Two pregnancies were reported in Trial 01.

- A 28-year-old Black / African American female received a single 3000 mg intramuscular dose of nirsevimab on [redacted] (b) (6). She had a positive pregnancy test on her Day 361 visit on [redacted] (b) (6), which was her last trial visit. Trial personnel were unable to contract this subject after the visit, and the pregnancy outcome is unknown.
- A 23-year-old White female received a single 300 mg intramuscular dose of nirsevimab on [redacted] (b) (6). She had a positive urine pregnancy test on [redacted] (b) (6). This subject delivered a healthy baby on [redacted] (b) (6).

These are the only available clinical data for pregnancies in the clinical trials of nirsevimab. There are too few human pregnancies in this trial to reach any conclusions regarding safety. We note, however, that nirsevimab, should this BLA were approved, would be indicated in infants and children up to 24 months of age, so any use in persons of childbearing potential would be considered unapproved use. The package insert will state that nirsevimab is not indicated for use in females of reproductive potential.

9. Product Quality

The Office of Pharmaceutical Quality, CDER, has assessed BLA 761328 with respect to Chemistry, Manufacturing, and Controls (CMC) and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such, OPQ recommends approval of BLA 761328 for Beyfortus (nirsevimab-alip) manufactured by AstraZeneca Pharmaceuticals, Inc., from a product quality perspective. Beyfortus will be supplied as a single-dose pre-filled syringe (PFS) with sub-assembly (SA) in two presentations: 50 mg (0.5 mL) or 100 mg (1.0 mL) of nirsevimab per PFS. The data submitted in this

application are adequate to support the conclusion that the manufacture of Beyfortus is well-controlled and leads to a product that is pure and potent. It is recommended that this product be approved for human use under conditions specified in the package insert.

The CMC postmarketing commitment (PMC) and postapproval quality agreements between OPQ and the Sponsor are listed below should be included in the action letter:

1) Postmarketing commitment for real world shipping studies for the final drug product in the proposed container closure system, covering all transportation routes. Two study reports will be provided by December 2023.
2) Postapproval stability commitment to conduct stability testing on a minimum of one commercial Drug Substance lot per presentation per year for each calendar year during which Drug Product is manufactured.
3) Postapproval stability commitment to conduct stability testing on a minimum of one commercial Drug Product lot (in the PFS-SA presentations) per year for each calendar year during which Drug Product is manufactured.

9.1. Device or Combination Product Considerations

Not applicable.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

Human Subjects Protection

The Applicant states that the clinical trials were conducted in accordance with the principals of the "Declaration of Helsinki" and with International Conference on Harmonisation good clinical practice requirements. The trials were reviewed and approved by the appropriate Ethics Committees and Institutional Review Boards. The trials were conducted according to FDA requirements, under investigational new drug application regulations (IND 118524).

Clinical Site Inspection

Inspection sites were chosen from the three main trials, Trials 03, 04, and 05. Four sites were selected, one South African site that enrolled subjects in all three trials, one Hungarian site for Trial 03 only, one Panamanian site for Trial 04 only, and one U.S. site for Trial 05 only. The sites chosen were based on enrollment, efficacy outcome, number of adverse events, and previous inspection history.

On inspection of the four trial sites, minor protocol deviations were identified, but OSI reviewers determined that the trials were conducted adequately and that the data from these sites were acceptable in support of the application.

Financial Disclosure

The Applicant adequately disclosed financial interests /arrangements with clinic investigators as recommended in the guidance for industry, Financial Disclosure by Clinical Investigators (February 2013) (see Section 25), and by 21 CFR 54.4. The Applicant provided financial disclosure information for 92% of the investigators (2,422 of 2,620) in Trials 03, 04, and 05.

The investigator financial disclosures do not raise questions about the integrity of the data. The primary efficacy endpoints were predefined in the protocols and were not vulnerable to investigator bias. In addition, all three trials were randomized, controlled, and double-blind, which would minimize the potential for investigator bias. Finally, only 2 investigators of 2,620 total investigators had financial interests or arrangements with the Applicant, and these investigators controlled <1% of trial subjects. One of these investigators enrolled a single subject in Trial 04. The other investigator enrolled one subject in Trial 05, but that subject was a screen failure.

In conclusion, the likelihood that trial results were biased based on financial interests is minimal and should not affect the approvability of the application.

11. Advisory Committee Summary

An Antimicrobial Drugs Advisory Committee Meeting (AMDAC) was held on June 8, 2023. The committee comprised of AMDAC standing members, and additional temporary voting members with subject matter expertise in pediatrics; pediatric infectious diseases, pulmonology, emergency medicine, hospital medicine; and neonatology.

The Agency convened this Advisory Committee (AC) meeting to discuss whether available data support a favorable benefit-risk assessment for the use of nirsevimab for the prevention of respiratory syncytial virus (RSV) lower respiratory tract (LRT) disease in neonates and infants born during or entering their first RSV season, and in children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season. Specifically, the committee was asked to opine or vote on the following:

1) (Vote): Is the overall benefit-risk assessment favorable for the use of nirsevimab for the prevention of RSV lower respiratory disease in neonates and infants born during or entering their first RSV season?
2) (Discuss): Please comment on the benefits and risks for nirsevimab when assessed by chronological and gestational age groups. Discuss the population or subpopulation for whom nirsevimab administration in the first RSV season would be most appropriate.
3) (Vote): Is the overall benefit-risk assessment favorable for the use of nirsevimab for the prevention of RSV lower respiratory tract disease in children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season?
4) (Discuss): In the context of potential, future availability of maternal RSV vaccine to protect infants from RSV disease during their first RSV season, what additional data may be helpful to inform future recommendations regarding the use of nirsevimab in infants born to mothers who received RSV vaccination?

After considering the presentations from both the Applicant and the FDA, the responses to clarifying questions, and the open public hearing, the committee voted/discussed their responses to the aforementioned questions or discussion points. The committee unanimously voted that the overall benefit-risk assessment favors the use of nirsevimab for the prevention of RSV lower respiratory disease in neonates and infants born during or entering their first RSV season. With respect to considerations regarding chronological and gestational age, the committee generally advised that the product should not be limited to certain chronological or gestational ages for the Season 1 indication. The committee felt strongly that the ACIP and other guideline committees (e.g., AAP) could provide additional recommendations on the age considerations, if needed. Regarding the benefit-risk assessment for nirsevimab for Season 2 indication in certain pediatric patients, the majority (19/21) of the committee members agreed that the overall assessment was favorable for the use of nirsevimab for the prevention of RSV lower respiratory tract disease in children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season. Finally, the committee opined that, should a maternal RSV vaccine be licensed, postapproval studies (including nonclinical and comparative effectiveness studies) will be important to address questions related to use of nirsevimab among infants whose mother received RSV vaccination.

For full information on the advisory committee meeting, including event materials, refer to the Meeting of the Antimicrobial Drugs Advisory Committee Meeting Announcement.

III. Additional Analyses and Information

12. Summary of Regulatory History

On May 10, 2013, MedImmune submitted a pre-investigational new drug (PIND) meeting request to discuss the development program for nirsevimab, anti-Respiratory Syncytial Virus (RSV) human IgG1κ monoclonal antibody. The FDA provided advice on the preclinical development plans, the design of the proposed phase 1 clinical trial and the overall clinical development plan for nirsevimab (also known as MED18897).

On March 11, 2014, the initial IND application for nirsevimab was submitted under IND 118524. The IND application proposed to evaluate the nirsevimab for the prevention of RSV lower respiratory tract disease in all infants entering their first RSV season, and children with chronic lung disease (CLD) or congenital heart disease (CHD) entering their first and second RSV season. The FDA determined the application was safe to proceed and the study-may-proceed letter was issued April 24, 2014.

On January 26, 2015, Fast Track Designation Request was submitted and on March 27, 2015, the request was granted for the prevention of lower respiratory tract infection caused by RSV infection in infants and young children.

On February 4, 2016, MedImmune requested an End of Phase (EOP) 1/Pre-Phase 2 meeting to discuss specific trial design aspects of the phase 2b/phase 3 clinical development program for nirsevimab for the prevention of lower respiratory tract infection caused by RSV infection. The meeting was held on April 7, 2016, and the FDA provided advice on the planned phase 2b trial, including the size of the safety database, dose selection rationale, statistical evaluation and viral resistance testing plans, as well as the needed nonclinical toxicology data.

On October 24, 2016, MedImmune submitted the initial Pediatric Study Plan (iPSP) for nirsevimab along with a request for waiver of pediatric studies for the pediatric age group of 2 to <17 years of age. The Agreed Initial Pediatric Study Plan (iPSP) was issued on April 28, 2017, including 3 proposed clinical trials to support the efficacy, safety, and dosing of nirsevimab for all infants entering their first RSV season, and high-risk preterm infants and children up to 2 years of age with CLD or CHD entering their second RSV season; a request for waiver for children older than 24 months of age was considered acceptable.

On December 3, 2018, Breakthrough Therapy Designation Request was submitted and on February 1, 2019, the request was granted for the proposed indication of the prevention of RSV lower respiratory tract disease healthy preterm infants entering their first RSV season, and children up to 24 months of age with CLD or CHD.

On February 26, 2019, an EOP 2 meeting was held to discuss the results of the phase 2b trial and key trial design aspects for the phase 3 programs, Trials 04 and 05. The following topics were discussed:

- Dose selection (Trial 05, second RSV season): The FDA had some concerns with respect to the proposed dose for the second RSV season –200 mg nirsevimab, regardless of body weight. Because of the wide range of possible body weights, smaller infants may experience higher nirsevimab exposure (on a mg/kg consideration) and may potentially experience more adverse events, while the dose may be inadequate for larger infants. FDA agreed with the proposed 200 mg dose for the second season in the CHD/CLD population with provision for increased monitoring for the smaller children.
- Study Design (Trial 05): The FDA recommended an interim analysis to confirm that the appropriate dose and endpoint definitions were appropriate.
- Safety Database: The FDA requested a safety database of at least 3,000 infants exposed to nirsevimab, including premature infants and infants/children with CLD and CHD from the phase 2/3 trials.
- The FDA did not agree with the pooled analysis of the secondary efficacy endpoint(s) (b) (4)

On March 9, 2020, the FDA provided feedback on MedImmune's approach to bridging nirsevimab from the current registrational trial presentation (vial) to the commercial presentation (prefilled syringe) in response to January 13, 2020, Type B CMC meeting request.

On March 23, 2020, MedImmune requested review of their use-related risk analysis (URRA) and justification for not submitting Human Factors (HF) validation study results. On May 15, 2020, based on the review of the submitted URRA and the justification for not submitting HF validation study results, MedImmune was informed that inclusion of the HF validation study results in the nirsevimab BLA was not required.

On October 7, 2020, MedImmune requested a Type B Breakthrough Therapy Guidance Meeting to provide an update on the status of the ongoing nirsevimab clinical development program that had been impacted by the COVID 19 pandemic and to obtain FDA's feedback on an updated clinical package for nirsevimab that will be submitted to support a marketing application and revised statistical analysis plans for the Trials 04 and 05. At that point, MedImmune had paused enrollment in Trials 04 and 05 due to the COVID-19 pandemic. On December 2, 2020, a Type B Breakthrough Therapy Guidance Meeting was held. It was agreed that the analysis of efficacy in Trial 04 could be based on the 1,478 subjects randomized and dosed prior to the pause in enrollment (Primary Cohort). The FDA again requested that the safety database for nirsevimab include at least 3,000 infants and children, of which at least 2,000 subjects should be late preterm or term infants. After the meeting, MedImmune proposed enrolling an additional cohort in Trial 04 to collect safety data only (Safety Cohort). In the revised Trial 04 protocol, MedImmune stated that there was "no intent to pool the efficacy data from the Safety Cohort with that from the Primary Cohort." Efficacy was considered an exploratory endpoint in the Safety Cohort of Trial 04.

On August 10, 2021, the proposed proprietary name, Beyfortus, was found acceptable and the proprietary name was conditionally granted.

On August 17, 2021, the FDA's written responses regarding the final drug product comparability strategy proposed by MedImmune were provided in response to June 25, 2021, Type B CMC, Breakthrough Therapy Guidance Meeting Request.

On September 7, 2021, the FDA issued a sponsor name/address change correspondence in response to a notification from MedImmune on changing corporate name and/or address from MedImmune, LLC to AstraZeneca Pharmaceuticals LP.

On July 26, 2022, a Type B pre-BLA meeting was held between the FDA and AstraZeneca to discuss the content and format of the BLA submission. Key discussion points are as follows:

- The FDA agreed that the clinical data AstraZeneca planned to submit with the BLA would support review of nirsevimab for prevention of RSV lower respiratory tract disease. The exact indication will be a review issue.
- The FDA stated that the efficacy results from Trials 03 and 04 should not be pooled because of the difference in infant GA enrolled in the two trials, and different risks for severe RSV disease or hospitalization due to RSV.
- The FDA and AstraZeneca agreed on pooling safety data from subjects in Trial 03 who received the proposed nirsevimab dose and subjects in Trial 04.

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

The nonclinical safety studies conducted to support nirsevimab were originally submitted to and reviewed under IND 118524. All pertinent studies were also submitted to the present BLA and are reviewed in the following sections.

13.2. Individual Reviews of Studies Submitted With the New Drug Application

The nonclinical safety studies conducted to support nirsevimab were originally submitted to and reviewed under IND 118524. All pertinent studies were also submitted to the present BLA and are reviewed in the following sections.

13.3. Pharmacology (Primary and Secondary)

Nirsevimab is a recombinant human immunoglobulin G1 kappa (IgG1κ) mAb that binds the prefusion conformation of the RSV fusion (F) protein. It was derived from a human mAb D25 that was isolated directly from human B cells and binds the prefusion conformation of the RSV F protein. D25 was affinity optimized for RSV neutralization potency in vitro to generate 1G7.

Nirsevimab differs from 1G7 by a 3 amino acid substitution, M257Y/S259T/T261E (M252Y/S254T/T256E, according to the EU numbering system), referred to as "YTE", in the heavy chain CH2 fragment crystallizable (Fc) region of the mAb. The YTE modification was added to the mAb to prolong the terminal half-life ($t_{1/2}$) of the antibody in humans. The activity of nirsevimab was evaluated in the cotton rat model, in vivo. Please refer to the virology review for more information.

13.4. Safety Pharmacology

Safety pharmacology of nirsevimab was assessed as a component of the GLP 4-week repeat dose toxicology study in cynomolgus monkeys (Study #1468-038). In that study, ECG parameters assessed predose and 24 hours postdose on Days 1 and 29 and prior to the recovery necropsy, showed no adverse nirsevimab-related effects following 50 mg/kg IV, 300 mg/kg IV, or 300 mg IM once weekly for five total doses. In addition, there were no clinical signs nor microscopic findings indicating any nirsevimab-related effects on the CNS or respiratory system in monkeys. Please refer to the GLP 4-week toxicology study review in Section 13.6.1 for more information.

13.5. Pharmacokinetics

The pharmacokinetics/toxicokinetics of nirsevimab were evaluated in the GLP 4-week intravenous and intramuscular repeat-dose toxicology study in cynomolgus monkeys (study #1468-038), and the toxicokinetic parameters from this study are presented in Section 13.6.1.

The pharmacokinetics of nirsevimab were also evaluated following intravenous dosing in an additional single-dose study in cynomolgus monkeys (study #R10933-PK-2007). The objective of this study was to compare levels of nirsevimab to other anti-RSV F mAbs (MEDI-524 and MEDI-557; in development previously for prevention/treatment of RSV, in order to benchmark nirsevimab against mAbs for which human data was available) in serum, bronchoalveolar lavage (BAL) and nasal wash (NW) samples collected after intravenous (IV) infusion to male cynomolgus monkeys.

Five animals per group were dosed with a single dose of 50 mg/kg nirsevimab, 150 mg/kg nirsevimab, 50 mg/kg MEDI-524 or 150 mg/kg MEDI-557. Clinical signs, food consumption and body weights were evaluated; in addition, bioanalysis and pharmacokinetic evaluation of serum, BAL fluid, and NW fluid were conducted.

Nirsevimab serum exposures were higher than MEDI-524 and MEDI-557 at equivalent doses (over 72 hours; Table 51). Tmax of all the mAbs was 0.0208 days (about 30 minutes). Nirsevimab was detected in both NW and BAL in all animals at all sampled time points. The partitioning of nirsevimab in BAL was within 2 to 3-fold of the other mAbs (at 72 hours; Table 52). The partitioning of nirsevimab in NW was variable and was within 4-fold of the other mAbs at 24 hours and within 4 to 18-fold of other mAbs at 72 hours Table 53.

Table 51. Mean Nirsevimab (MEDI8897), MEDI-524 and MEDI-557 Pharmacokinetic Parameters

Parameters	MEDI-524	MEDI-557	MEDI8897	
Dose (mg/kg)	50	150	50	150
Tmax (day)	0.0208 (0.00)	0.0208 (0.00)	0.0208 (0.00)	0.0208 (0.00)
Cmax (µg/mL)	1100 (134)	4000 (458)	1570 (64.9)	4520 (540)
AUC_{0-3} (day·µg/mL)	1710 (130)	6690 (535)	3100 (167)	9910 (1050)
Dose Normalized AUC_{0-3} ((day·µg/mL)/mg)	10.2 (1.28)	13.9 (1.51)	19.3 (1.37)	19.8 (1.48)

Values are presented as mean, n = 5 (standard deviation)
AUC0-3 = area under the curve from time 0 to 3 days; Cmax = maximum observed concentration;
Tmax = time at which Cmax was observed.

Source: Sponsor table; study R10933-PK-200; pages 18

Table 52. Mean Nirsevimab (MEDI8897), MEDI-524 and MEDI-557 BAL Concentrations and Their Mean Partition of Serum Concentrations

Drug	Dose (mg/kg)	Time (hour)	Mean BAL Conc. (ng/mL)[a]	Mean Serum Conc. (µg/mL)[a]	Mean Partition, % of Serum[a]	Relative Partition (8897:524 /557)	Relative Partition (524/557: 8897)	Relative Raw BAL (8897:524 /557)	Relative Absolute BAL (524/557: 8897)
MEDI8897	50	72	256.5	855.1	0.03	0.48	2.1	1.3	0.8
MEDI-524		72	192.7	307.2	0.0627				
MEDI8897	150	72	455.6	2753.9	0.0165	0.34	2.9	0.6	1.8
MEDI-557		72	803.3	1653.4	0.0486				

[a] Geometric mean (n =5); BAL: bronchoalveolar lavage; Conc. = concentration.

Source: Sponsor table; study R10933-PK-200; page 19

Table 53. Mean Nirsevimab (MEDI8897), MEDI-524 and MEDI-557 Nasal Concentrations and Their Mean Partition of Serum Concentrations

Drug	Dose (mg/kg)	Time (hour)	Mean NW Conc. (ng/mL)*	Mean Serum Conc. (µg/mL)*	Mean Partition, % of Serum*	Relative Partition (8897:524 /557)	Relative Partition (524/557: 8897)	Relative Raw NW (8897:524 /557)	Relative Raw NW (524/557: 8897)
MEDI8897	50	24	78.4	999.9	0.0078	0.23	4.3	0.4	2.4
		72	62.3	855.1	0.0073	0.06	18.1	0.2	6.5
MEDI-524		24	185.5	548.5	0.0338				
		72	405.6	307.2	0.132				
MEDI8897	150	24	347	3216.1	0.0108	0.23	4.4	0.3	2.9
		72	285	2753.9	0.0103	0.28	3.6	0.5	2.2
MEDI-557		24	1001.4	2105.5	0.0476				
		72	613	1653.4	0.0371				

* Geometric mean (n=5); NW = Nasal Wash; Conc = concentration.

Source: Sponsor table; study R10933-PK-200; page 20

13.6. Toxicology

13.6.1. General Toxicology

Nirsevimab: A 1-Month Repeat Dose IV/IM Toxicity Study in Cynomolgus Monkeys With a 25-Week Recovery Period (Study #1468-038):

Key Study Findings

- NOAEL =300 mg/kg IV or IM
  - 300 mg/kg IV: Day 29 C_{max} =13360 µg/mL; AUC_{1-31}=208500 µg•day/mL
  - 300 mg/kg IM: Day 29 C_{max} =4982 µg/mL; AUC_{1-31}=92380 µg•day/mL
- Minor, nonadverse effects were observed in clinical pathology parameters (increased APTT, globulin and total protein) and the spleen (red pulp macrophage hypertrophy/hyperplasia) at 300 mg/kg IV and/or IM.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 54. GLP 4-Week Monkey Intravenous/Intramuscular Toxicity Study Methods

Study Features and Methods	Details
Dose and frequency of dosing	Weekly for 4 weeks (5 injections total)
Route of administration	Either intravenous or intramuscular
Formulation/vehicle	(b) (4) mM Histidine/ Histidine-HCl, (b) (4) mM Arginine-HCl, (b) (4) mM Sucrose, (b) (4) % PS80 pH 6.0
Species/strain	Cynomolgus monkeys
Number/sex/group	See Table 55
Age	2 to 4 years old
Satellite groups/unique design	All animals were dosed once weekly for 4 weeks (Days 1, 8, 15, 22 and 29). Main group animals were euthanized on Day 32. Recovery animals were euthanized on Day 169.
Deviation from study protocol affecting interpretation of results	None

Source: Reviewer analysis
Abbreviations: GLP, good laboratory practice; HCl, hydrochloric acid; pH, potential of hydrogen; PS80, polysorbate 80

Table 55. Group Assignments

Group Assignments						
Group Number	Dose Level (mg/kg)	Dose Volume	Dose Concentration (mg/mL)	Route	Number of Animals	
					Male	Female
1	0/0	5 mL/kg 3 mL[b]	0/0	IV IM[a]	6[c]	6[c]
2	50	5 mL/kg	10	IV	3	3
3	300	5 mL/kg	60	IV	6[c]	6[c]
4	300	3 mL[b]	100	IM	6[c]	6[c]

[a]Animals received IM injection prior to IV administration.
[b]Fixed dose and volume
[c]Three animals were maintained for a 25-week recovery period.

Source: Sponsor table; page 10
Abbreviations: GLP, good laboratory practice; IM, intramuscular; IV, intravenous

Table 56. GLP 4-Week Monkey Intravenous/Subcutaneous Toxicity Study Findings

Parameters	Major Findings
Mortality	Assessed twice daily. No premature deaths.
Clinical signs & local tolerability	Examined at least once daily. No test article-related findings.
Injection site	Examined at least once daily. No clear intolerability at injection sites noted. Reddened skin noted in all groups. Increased incidence/severity of transient erythema (nonsevere) and/or edema (very slight to slight) noted in HD IV &/or IM groups. Finding may be a normal foreign protein response.
Body weights	Measured once weekly. No test article-related findings.
Food consumption	Measured qualitatively twice daily. No test article-related findings (as part of clinical observation).
Ophthalmoscopy	Conducted pretest and prior to the terminal and recovery necropsies. No test article-related findings.
Physical Exam	Conducted pretest, on Days 2, 31 (prior to the terminal necropsy), 57, 85, and 169 (prior to the recovery necropsy). No test article-related findings.
Electrocardiography & blood pressure	Conducted pretest and pre-test and 24 hours (±15 minutes) postdose on Days 1 and 29, and prior to the recovery necropsy. No test article-related findings.
Hematology/Coagulation	Blood samples collected pre-test and Days 8, 31 (prior to the terminal necropsy), 57, 85, and 169 (prior to the recovery necropsy). The significant changes in APTT observed on Day 8 through Day 169 in females receiving 300 mg/kg (IV) were not regarded as meaningful based on their small magnitude (approximately 10% increase) and overlap with individual control animal and pretest values.
Clinical chemistry	Blood samples collected pre-test and Days 8, 31 (prior to the terminal necropsy), 57, 85, and 169 (prior to the recovery necropsy). On Day 31 there was an increase in globulin (+40% relative to controls; +17% relative to pretest) in males receiving 300 mg/kg (IV). In addition, total protein levels were mildly increased (less than 20%) in males receiving 300 mg/kg IM. These findings were considered test article-related but were not associated with effects on leukocytes and had no microscopic correlates hence they were not regarded as

Reference ID: 5208810

Parameters	Major Findings
	biologically relevant. They may have been in part, related to the presence of the test article which is an immunoglobulin. At the recovery intervals (Days 57-169) globulin values continued to be mildly (up to 25%) increased in males receiving 300 mg/kg (IV) and were also statistically increased (up to 50%) in the 300 mg (IM) group. In addition, total protein levels were also mildly increased (less than 20%) in males receiving 300 mg/kg IV or IM. These observations similarly did not have other correlative findings suggesting these were not biologically relevant effects. The effects were not considered adverse due to small magnitude and lack of biologically relevant effect.
Urinalysis	Urine samples collected pre-test and Days 8, 31 (prior to the terminal necropsy), 57, 85, and 169. No test article-related findings.
Gross pathology	Evaluated at necropsy on Days 32 and 169. No test article-related findings.
Organ weights	Evaluated at necropsy on Days 32 and 169. No test article-related findings.
Histopathology Adequate battery: Yes Peer review: Yes	Evaluated at necropsy on Days 32 and 169. Microscopic changes in the spleen (red pulp macrophage hypertrophy/hyperplasia) were noted at terminal necropsy (one female and two males receiving 300 mg IM). This microscopic finding is commonly seen with the administration of foreign proteins to monkeys and may be related to the clearance of antibodies from circulation. At recovery necropsy, only gross lesions were examined microscopically. These microscopic observations were considered incidental, unrelated to treatment and thus nonadverse.
Immunogenicity	Blood samples (approximately 2 mL) were collected from all animals prior to dosing on Days 1 and 29 and on Days 57, 85, 113, 141, and 169 (prior to the recovery necropsy) via the femoral vein for anti-drug antibodies analysis. No animals tested ADA positive during the treatment phase. Four of 18 animals tested ADA positive during the recovery phase with variable impact on TK.

Parameters	Major Findings
Toxicokinetics	*Blood Samples and Nasal Wash Collection* Blood samples collection time is described in Table 57. Nasal wash samples were collected prior to the terminal and recovery necropsies from three animals/sex/group. Mean nirsevimab nasal concentrations on Day 31 were 227.3 ng/mL, 346.2 ng/mL and 160.92 ng/mL following the administration of 50 mg/kg IV, 300 mg/kg IV and 300 mg IM, respectively. *Nasal to Serum Partition Results* The mean nasal to serum partition ratio of nirsevimab was 0.000091, 0.000038, and 0.000032 following 50 mg/kg IV, 300 mg/kg IV and 300 mg IM, respectively. Mean nirsevimab nasal concentrations on Day 169 for all the recovery phase animals in the control (0 mg/kg IV/IM), 300 mg/kg IV and 300 mg IM groups were below the limit of quantification. Results not shown in table. *Serum TK* On day 29, AUC values increased less than dose-proportionally between 50 and 300 mg/kg IV. AUC values of nirsevimab were lower after IM administration than after IV administration of 300 mg/kg (Table 58). Nirsevimab serum exposure was similar between males and females in all groups, except in 300 mg/kg IV group, males had slightly higher exposures during the treatment phase. In the recovery phase, males had slightly higher exposures compared to females in the 300 mg/kg IV group and females had slightly higher exposures in the 300 mg IM group compared to males, probably due to ADA. It should be noted that sample size was small (N=3-6 per sex per dose group) and dissimilarity in exposures between sexes might not reflect true differences. Mean $t_{1/2}$ was 40.45 and 39.91 days, following administration of 300 mg/kg IV and 300 mg IM, respectively.

Source: Reviewer analysis
Abbreviations: ADA, anti-drug ant body; APTT, activated partial thromboplastin time; AUC, area under the concentration-time curve; HD, high dose; IV, intravenous; IM, intramuscular; N, number of subjects; $t_{1/2}$, terminal half-life; TK, toxicokinetic

Table 57. Blood Collection Times for Toxicokinetic Analysis

Group(s)	Study Day	Interval
1 through 3	1 and 29	Pre-dose, EOI, 12 and 24 hrs post end of infusion[a]
4	1 and 29	Predose, 12, and 24 hrs post dose
1 through 4	3, 4, 6, 31, 32, 33, 35, 37, 43, 57, 71, 85, 99, 113, 141, and 169	Once/day
1 through 3	8, 15, and 22	Predose, EOI[a] and 3 days post dose
4	8, 15, and 22	Predose, 24 hrs and 3 days post dose

[a] Collection times for animals in Group 1 were calculated based on the IV dose time.
EOI = within 2 minutes of the end of the infusion

Source: Sponsor table; page 20

Table 58. Toxicokinetic Parameters From the GLP 4-Week Monkey Toxicity Study: After the First Dose

Parameter	Group 2 (50 mg/kg IV)	Group 3 (300 mg/kg IV)	Group 4 (300 mg IM)
T_{max} (day)	0.104 (0.204)	0.021 (0)	2.333 (1.67)
C_{max} (µg/mL)	1678 (339.8)	8782 (3401)	1774 (397.9)
C_{trough} (µg/mL)	834 (220)	3556 (545.3)	1287 (196.4)
$AUC_{(1-8)}$ (µg·day/mL)	5909 (736.9)	30040 (4324)	9334 (1329)

Values are presented as mean (standard deviation); AUC_{1-8}= area under the curve over the dosing interval Day (1-8); C_{max} = maximum observed concentration; C_{trough} = observed trough concentration; T_{max} = time at which C_{max} was observed

Source: Sponsor tables; pages 829-830

Table 59. Toxicokinetic Parameters From the GLP 4-Week Monkey Toxicity Study: Day 29

Parameter	Group 2 (50 mg/kg IV)	Group 3 (300 mg/kg IV)	Group 4 (300 mg IM)
$T_{max,5}$ (day)	0.014 (0.011)	0.099 (0.187)	1 (0.798)
$C_{max,5}$ (µg/mL)	3772 (910.7)	13360 (2704)	4982 (863.4)
$C_{trough,4}$ (µg/mL)	2495 (609.2)	7204 (4033)	4078 (1010)
C_{31} (µg/mL)	2702 (720.1)	8105 (1776)	4383 (820.3)
AUC_{29-31} (µg·day/mL)	5852 (972.5)	19710 (3518)	8513 (1382)
AUC_{1-31} (µg·day/mL)	61590 (7370)	208500 (43270)	92380 (9428)
$AI.C_{max}$	2.36 (0.86)	1.65 (0.47)	2.93 (0.74)
$AI.C_{trough}$	3.15 (0.46)	2.07 (1.19)	3.2 (0.85)
$t_{1/2}$	NC[a]	40.45 (14.86)	39.91 (7.442)

Values are presented as mean (standard deviation). a No recovery phase animals in 50 mg/kg IV group

$AI.C_{max}$ = Accumulation index for C_{max}; $AI.C_{trough}$ = Accumulation index for C_{trough}; AUC_{29-31} = area under the curve from Day 29 to Day31; (C_{31})=last measured concentration on Day 31; AUC_{1-31} = cumulative area under the curve from Day 1 to Day 31; $C_{max,5}$ = maximum observed concentration after 5th dose on Day 29; $C_{trough,4}$ = trough concentrations after 4th dose on Day 22; NC = not calculated (No recovery phase cohort in Group 2); $T_{max,5}$ = time of C_{max} after fifth dose on Day 29; $t_{1/2}$ = terminal elimination half-life.

Source: Sponsor tables; pages 829-830

13.6.2. Genetic Toxicology

Genotoxicity studies have not been conducted with nirsevimab. In accordance with ICH S6(R1), genotoxicity studies are not required for biologics as they are not anticipated to interact with DNA or other genetic material.

13.6.3. Carcinogenicity

Carcinogenicity studies have not been conducted with nirsevimab. A weight of evidence approach was conducted in accordance with the FDA's recommendations and applicable ICH guidelines for establishing the carcinogenic potential of nirsevimab and did not reveal any specific carcinogenic risk. Available scientific literature, the mode of action, nonclinical and clinical data of nirsevimab suggested a low potential for off-target toxicity and no evidence of pre-neoplastic lesions.

13.6.4. Reproductive Toxicology

Dedicated in vivo DART studies have not been conducted with nirsevimab. In accordance with ICH S6(R1), DART studies are generally not needed for biologics to exogenous targets. In

addition, no male or female reproductive toxicities were observed in the 4-week repeat-dose toxicology study in cynomolgus monkeys (study # 1468-038), and no off-target binding was observed in the tissue cross-reactivity studies in either normal adult human tissues (study # 20046491) or in select human fetal, neonatal and juvenile tissues (study # 20060018). Lastly, nirsevimab is not indicated for use in females of reproductive potential

13.6.5. Other Toxicology/Specialized Studies

A Tissue Cross-Reactivity Study of Nirsevimab in Normal Human Tissues (Study #MEDI8897-6007-2013; Testing Facility Study #20046491)

The potential cross-reactivity of nirsevimab was evaluated in cryosections of normal adult human tissues (3 donors/tissue) at concentrations up to 40 µg/mL. Positive (SK epitope UV resin spot slides) and negative controls (Intron A UV-resin spot slides) produced appropriate responses; the mAb negative control article (human IgG antibody directed against HIV; r347-YTE) also produced an appropriate response (no binding). No binding was observed with nirsevimab any tissue observed under the conditions of this study.

A Tissue Cross-Reactivity Study of Nirsevimab in Selected Juvenile, Neonatal, and Fetal Human Tissues (Study #MEDI8897-6014-2014; Testing Facility Study #20060018)

The bladder, thymus and uterus were the only tissues investigated for juvenile aged children (2 to 6 months old). Neonatal tissues included adrenal gland, esophagus, eye, heart, intestine, kidney, liver, lung, muscle, pituitary, skin, small intestine, spinal cord, spleen, stomach and testes. Fetal tissues included adrenal gland, blood vessels, bone marrow, esophagus, eye, heart, kidney, large intestine, liver, lung, mammary gland, muscle, ovary, pancreas, skin, small intestine, spleen, stomach and thyroid. There was one donor per tissue for each of the three juvenile tissues examined, one donor per tissue for each of the sixteen neonatal tissues examined and one to three donors per tissue for each of the 21 fetal tissues examined. An indirect immunoperoxidase procedure was performed. Staining was performed with the test article nirsevimab and the negative control r347-YTE at 1.5 and 30 µg/mL. The positive control was SK epitope UV-resin spot slides, and the negative control material was Intron A for Injection UV-resin spot slides. The positive control yielded moderate to intense staining with nirsevimab at both concentrations and no staining with the isotype control. No staining of spot material was observed with nirsevimab or r347-YTE for the negative control material. All evaluated human tissues stained positive for β2-microglobulin, a minor Class 1 determinant expressed on many cell types and strongly expressed on the endothelium (positive control for tissue inclusion).

13.7. Excipients/Impurities/Degradants

No excipient-related issues with the nirsevimab drug product have been identified. All impurities are categorized into process and product impurities and may arise from raw materials, manufacturing and/or degradation. Overall, the proposed specifications, or lack of specifications, are considered acceptable from a pharmacology/toxicology perspective.

Please refer to the product quality review for more information on the specifications for control of nirsevimab drug substance.

13.8. Extractables/Leachables

No concerns from the pharmacology/toxicology perspective.

13.9. Individual Reviews of Studies Submitted to the NDA

Not applicable.

14. Clinical Pharmacology

14.1. In Vitro Studies

This BLA does not include in vitro study reports for review by clinical pharmacology.

14.2. In Vivo Studies

14.2.1. Trial 01

Title

A phase 1, randomized, double-blind, placebo-controlled, dose-escalation study to evaluate the safety, tolerability, and pharmacokinetics of nirsevimab in healthy adults

Trial Design and Treatment

Healthy adult subjects were randomized to receive nirsevimab as a single fixed dose of 300, 1000, or 3000 mg IV (n=6 for each nirsevimab dose level), or 100 mg (n=6) or 300 mg (n=78) IM or placebo. Intensive pharmacokinetic (PK) samples (predose, 0.5 hour, 8 hours, 2, 4, 6, 8, 15, 22, 31, 61, 91, 121, 151, 181, 271, 361 days postdose) were collected from each subject. All subjects were followed for approximately 360 days for safety after dosing.

Results

Following a single IV dosing, nirsevimab exposure, C_{max} and AUC, increased in a dose proportional manner over the dose range of 300 mg to 3000 mg (Table 60). After IM administration, mean C_{max} and AUC increased less than dose proportionally across the tested dose range (100 mg and 300 mg). T_{max} after IM dosing was 6−10 days, and the mean t½ ranged from 85−103 days. Nirsevimab bioavailability in the 300-mg IM cohort in comparison with 300-mg IV cohort was 77.3%.

Table 60. Mean Nirsevimab Serum Single-Dose PK Parameters in Healthy Adult Subjects

Cohort	t_{max} [day(s)], %CV, n	C_{max} (µg/mL), %CV, n	$AUC_{0\text{-}inf}$ (day*µg/mL), %CV, n	$t_{1/2}$ (days), %CV, n	CL/F*(mL/day), %CV, n	$V_z/F^{\dagger}$(L), %CV, n
300 mg IV n = 6	0.08 172 n = 6	97.0 21.9 n = 6	6714.8 21.7 n = 5	117 19.6 n = 5	46.1 17.3 n = 5	7.7 24.8 n = 5
1000 mg IV n = 6	0.06 0 n = 6	333.8 22.4 n = 6	25320 17 n = 5	92.0 12.6 n = 5	40.3 15.4 n = 5	5.4 26.7 n = 5
3000 mg IV n = 6	0.21 62.7 n = 6	1163 23.8 n = 6	63580 10.4 n = 5	89.8 18.2 n = 5	47.6 10.6 n = 5	6.1 18.5 n = 5
100 mg IM n = 6	5.5 71.4 n = 6	20.4 29.4 n = 6	2249.1 17.9 n = 5	103 11.3 n = 5	45.5 15.4 n = 5	6.8 24.5 n = 5
300 mg IM n = 78	9.4 78.4 n = 78	47.5 26.2 n = 78	5193.7 32.1 n = 75	85.3 30.8 n = 75	64.6 37.7 n = 75	7.4 34 n = 75

Source: CSR Table 11.4.4.1-1
Abbreviations: $AUC_{0\text{-}inf}$, area under the curve from time 0 to infinity; CL, clearance; C_{max}, maximum concentration; %CV, percentage coefficient of variation; F, bioavailability following IM administration; IM, intramuscular; IV, intravenous; n, number of subjects; $t_{1/2}$, terminal half-life; T_{max}, time to reach maximum concentration; Vz, volume of distribution

14.2.2. Trial 02

Title

A phase 1b/2a randomized, double-blind, placebo-controlled, dose-escalation study to evaluate the safety, tolerability, and pharmacokinetics of nirsevimab, a monoclonal antibody with an extended half-life against Respiratory Syncytial Virus, in healthy preterm infants

Trial Design and Treatment

This is a first-time-in-infants trial with a dose escalation design. Trial subjects were healthy preterm infants born between 32 weeks 0 days and 34 weeks 6 days gestational age. Three sequential dose-escalation cohorts (4:1 randomization of nirsevimab to placebo per cohort) were administered a single IM dose of 10, 25, or 50 mg nirsevimab. Sparse PK samples (predose, 8, 31, 151, 361 days postdose) were collected.

Results

After a single IM injection on Day 1, peak nirsevimab concentrations were observed about 7 days postdose in all dose groups. Drug exposures increased in an approximately dose-proportional manner from 25−50 mg nirsevimab IM dose. The mean terminal t½ in serum ranged from 62.5 to 72.9 days across the tested IM doses. On Day 151 postdose, 87% of the nirsevimab serum concentrations following the 50 mg IM dose were above 6.8 µg/mL.

Reference ID: 5208810

Table 61. Mean (%CV) Nirsevimab Pharmacokinetic Parameters by Dose in Trial 02

MEDI8897 Dose	T_{max} (day)	C_{max} (µg/mL)	AUC_{1-151} (day*µg/mL)	AUC_{∞} (day*µg/mL)	$t_{½}$ (day)	CL/F (mL/day)	Vz/F (mL)
10 mg IM (n=8)	7.04 0.969 N=5	23.2 40.0 N=5	1940 41.7 N=5	2450 N=1	72.9 N=1	4.08 N=1	429 N=1
25 mg IM (n=31)	7.04 5.60 N=29	30.9 33.8 N=29	2260 39 N=29	4320 24.8 N=6	66.2 11.8 N=6	6.05 21.7 N=6	581 27.4 N=6
50 mg IM (n=32)	6.93 7.91 N=31	71.7 22.1 N=31	5470 26.4 N=31	7510 25.0 N=14	62.5 15.0 N=14	7.01 22.4 N=14	633 26.6 N=14

Source: CSR Table 11.4.4.1-1
Abbreviations: AUC_{1-151}, area under the concentration-time curve over the interval of day 1 to day 151; CL/F, extravascular clearance after IM administration; C_{max}, maximum concentration; IM, intramuscular; n, number of subjects; $T_{1/2}$, terminal half-life; T_{max}, time to reach maximum concentration; Vz/F, extravascular volume of distribution after IM administration

14.2.3. Trial 03

Title

A phase 2b, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of nirsevimab, a monoclonal antibody with an extended half-life against Respiratory Syncytial Virus, in healthy preterm infants

Trial Design and Treatment

The trial population was healthy preterm infants born between 29 weeks 0 days and 34 weeks 6 days gestational age entering their first RSV season who would not receive RSV prophylaxis (e.g., palivizumab) based on the American Academy of Pediatrics (AAP) or other local or national guidelines. Subjects were randomized 2:1 to receive a 50 mg intramuscular (IM) dose of nirsevimab (n~1,000) regardless of weight or placebo (n~500). Randomization was stratified by the northern and southern hemispheres and by subject age at the time of randomization (i.e., ≤3 months, >3 to ≤6 months, and >6 months). Enrollment of infants >6 months of age was limited to approximately 500 subjects. Sparse PK samples (predose, 91, 151, 361 days postdose) were collected from each subject. Intensive PK samples were collected in 48 infants who received nirsevimab. All infants were followed for approximately 360 days after dosing.

Results

The serum concentrations decayed monoexponentially beyond the Day 91 sampling timepoint. On Day 151, nirsevimab serum concentrations in 97.8% of the enrolled subjects were above 6.8 µg/mL. Table 62 summarized nirsevimab serum concentration by nominal sampling time. Noncompartmental analysis was performed based on the intensive PK data collected in the 48 infants. The results are shown in Table 63. The estimated apparent mean half-life was 59.3 days (Table 63), which is similar to previously observed data from Trial 02.

Reference ID: 5208810

Table 62. Summary of Nirsevimab Serum Concentrations by Nominal Sampling Time

Nominal Time Point-Dose (Day)	N	Mean (SD) Serum Concentration (µg/mL)
90	883	35.9 (10.9)
150	849	18.9 (7.4)
360	771	2.1 (1.1)

Source: CSR Table 28
Abbreviations: N, number of subjects; SD, standard deviation

Table 63. Nirsevimab Serum Pharmacokinetic Parameters (N=48)

Parameter	N	Mean[a] (%CV)
$AUC_{0-\infty}$ (day*µg/mL)	48	5176.3 (35.0)
CL/F (mL/day)	48	9.7 (36.1)
$t_{1/2}$ (day)	48	59.3 (9.6)

Source: CSR Table 29
Abbreviations: %CV, percentage coefficient of variation; N, number of subjects

14.2.4. Trial 04

Title

A phase 3 randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of nirsevimab, a monoclonal antibody with an extended half-life against Respiratory Syncytial Virus, in healthy late preterm and term infants (MELODY)

Trial Design and Treatment

Trial subjects were late preterm and term infants born ≥35 weeks 0 days gestational age and entering their first RSV season. The trial is ongoing for safety data collection and comprises two cohorts: a Primary Cohort (efficacy cohort, 1490 randomized subjects) and a complementary Safety Cohort (1522 randomized subjects). In each cohort, subjects were randomized at a 2:1 ratio to receive a single fixed intramuscular (IM) dose of nirsevimab (50 mg for infants weighing <5 kg or 100 mg for infants weighing ≥5 kg) or placebo. Randomization was stratified by the northern and southern hemispheres and by subject age at the time of randomization (≤3.0 months, >3.0 to ≤6.0 months, and >6.0 months). Enrolment of subjects >6.0 months of age was limited to approximately 500 subjects. Sparse PK samples (predose, 31, 151, 361 days postdose) were collected from each subject. All subjects are followed for approximately 510 days after dosing. In this application, PK data through Day 361 are available for the Primary Cohort, and PK data are available through Day 151 for the Safety Cohort.

Results

Nirsevimab serum concentrations decreased monoexponentially beyond the Day 31 sampling time point in both weight groups. Table 64 summarizes nirsevimab serum concentrations by weight group.

Table 64. Summary of Nirsevimab Serum Concentrations (µg/mL) by Weight Group at Scheduled Sampling Time in Primary Cohort

Group	Summary statistic	Scheduled time					
		Baseline	Day 8	Day 15	Day 31	Day 151	Day 361
Weight < 5 kg (N = 799)	n	572	32	131	349	257	312
	n < LLOQ	571	0	4	1	3	33
	Arithmetic mean	NC	101.07	92.15	74.49	19.62	2.19
	Arithmetic SD	NC	18.97	29.26	18.52	7.77	1.36
	Geometric mean	NC	99.49	78.13	70.77	17.66	1.76
	Geometric CV%	NC	17.9	119.7	45.1	62.0	79.1
Weight ≥ 5 kg (N = 585)	n	924	44	167	684	396	463
	n < LLOQ	922	0	1	0	1	27
	Arithmetic mean	NC	175.27	118.82	117.35	31.14	3.76
	Arithmetic SD	NC	20.71	45.12	28.40	13.65	2.48
	Geometric mean	NC	174.07	105.30	112.88	27.89	2.93
	Geometric CV%	NC	12.0	71.9	33.3	59.9	90.6

Subjects < 5 kg received 50 mg nirsevimab, subjects ≥ 5 kg received 100 mg nirsevimab.
Arithmetic mean and other statistics are derived from planned visit day ± 14 days.

Source: CSR Table 47
Abbreviations: CV, coefficient of variation; LLOQ, lower limit of quantification; N, number of subjects; n, number of subjects in specific group; SD, standard deviation

14.2.5. Trial 05

Title

A phase 2/3 randomized, double-blind, palivizumab-controlled study to evaluate the safety of nirsevimab, a monoclonal antibody with an extended half-life against Respiratory Syncytial Virus, in high-risk children (MEDLEY)

Trial Design and Treatment

Trial 05 is an ongoing palivizumab-controlled trial in infants at higher risk for RSV severe disease who are eligible to receive palivizumab when entering their first or second RSV season. This review summarizes available PK data in the current submission. Palivizumab eligible infants entering their first RSV season were enrolled to two cohorts: (i) preterm cohort, including approximately 600 preterm infants born <35 weeks of gestational age without CLD/CHD, or (ii) CLD/CHD cohort, including approximately 300 infants with CLD of prematurity or hemodynamically significant CHD. For preterm and CLD/CHD Cohorts in RSV Season 1, subjects were randomized 2:1 to either nirsevimab or palivizumab. Subjects in the nirsevimab group in RSV Season 1 received a single fixed IM dose of nirsevimab, 50 mg for infants weighing <5 kg or 100 mg for infants weighing ≥5 kg. For CLD/CHD Cohort in RSV Season 2, subjects were rerandomized to either nirsevimab or palivizumab. Subjects in the nirsevimab group in RSV Season 2 received a single fixed IM dose of 200 mg nirsevimab. Sparse PK samples (predose, 31, 151, 361 days postdose) were collected from each subject. All subjects are followed for approximately 510 days after dosing. PK data are available in the current

Reference ID: 5208810

submission through Day 361 in RSV Season 1 (both cohorts) and at least through Day 151 in RSV Season 2 in CLD/CHD cohort.

Results

In RSV Season 1, subjects in the same weight group (i.e., <5 kg or ≥5 kg) had comparable serum nirsevimab concentrations (Table 65) regardless of cohorts. Nirsevimab serum concentrations are lower in subjects weighing <5 kg compared to serum concentrations in subjects weighing ≥5 kg (Table 65).

Table 65. Summary of Nirsevimab Serum Concentrations (µg/mL) by Weight Group, and Preterm and CLD/CHD Cohort by Scheduled Sampling Time Through 360 Days Post First Dose in Season 1 – As-Treated Population (Season 1)

Group	Summary statistic	Scheduled time					
		Baseline	Day 8	Day 15	Day 31	Day 151	Day 361
Preterm cohort Weight < 5 kg (N = 243)	n	1	13	129	95	175	198
	n < LLOQ	1	0	0	1	1	13
	Arithmetic Mean	NQ	127.38	97.13	82.66	21.98	2.54
	Arithmetic SD	NC	21.97	23.26	23.46	8.05	1.46
Preterm cohort Weight ≥ 5 kg (N = 162)	n	0	3	84	65	120	138
	n < LLOQ	0	0	0	1	0	5
	Arithmetic Mean	NA	180.96	142.28	108.83	34.50	4.36
	Arithmetic SD	NA	35.01	27.27	32.99	10.33	3.55
CLD/CHD cohort Weight < 5 kg (N = 101)	n	0	5	50	46	77	87
	n < LLOQ	0	0	2	0	1	16
	Arithmetic Mean	NA	101.96	96.99	85.44	23.90	2.51
	Arithmetic SD	NA	22.71	39.39	19.20	13.03	2.00
CLD/CHD cohort Weight ≥ 5 kg (N = 107)	n	0	3	42	55	94	91
	n < LLOQ	0	0	0	1	0	8
	Arithmetic Mean	NA	157.08	130.27	104.87	36.20	4.53
	Arithmetic SD	NA	23.86	48.78	33.13	16.51	6.54

Source: CSR table 30
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; LLOQ, lower limit of quantification; N, number of subjects; n, number of subjects in specific group; SD, standard deviation

In RSV Season 2, with nirsevimab administered as a single fixed 200 mg IM dose, nirsevimab serum concentrations were overall higher than those in Season 1 (Table 65 and Table 66).

Table 66. Summary of Nirsevimab Serum Concentrations (µg/mL) by Scheduled Sampling Time Through At Least 150 Days Post First Dose in Season 2 for 200 mg Fixed Dose – As-Treated Population (Season 2)

Summary statistic	Scheduled time					
	Baseline	Day 8	Day 15	Day 31	Day 151	Day 361
Subjects with CLD/CHD (N = 220)						
n	168	11	97	108	192	79
n < LLOQ	24	0	3	4	5	9
Arithmetic Mean	3.30	260.11	179.84	153.96	52.27	6.65
Arithmetic SD	2.73	49.23	64.44	71.96	24.92	4.90

Source: CSR table 31
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; LLOQ, lower limit of quantification; N, number of subjects; n, number of subjects in specific group; SD, standard deviation

14.2.6. Trial 08

Title

A phase 2, open-label, uncontrolled, single-dose study to evaluate the safety and tolerability, pharmacokinetics, and occurrence of antidrug antibody for nirsevimab in immunocompromised children ≤24 months of age

Trial Design and Treatment

Trial 08 is an ongoing open-label, uncontrolled, single-arm trial in immunocompromised children who were ≤24 months of age at the time of dose administration. Sixty subjects were enrolled and received treatment with nirsevimab. The dose received by subjects was dependent upon their age and body weight at the time of dosing: 50 mg in subjects with body weight <5 kg or 100 mg in subjects with body weight ≥5 kg in their first year of life, and 200 mg in their second year of life. Sparse PK samples (predose, 31, 151, 361 days postdose) were collected for each RSV season. This review summarizes interim available PK data collected through at least Day 151 for 60 enrolled subjects. The Applicant plans to enroll a total of 100 subjects for Trial 08.

Results

Mean nirsevimab serum concentrations were higher in those subjects who received 200 mg nirsevimab than in those who received 50 mg or 100 mg nirsevimab, with substantial overlap between the two groups.

Table 67. Summary of Serum Concentrations (ug/mL) of Nirsevimab As-Treated Population (interim data)

Dosing level	Summary statistic	Scheduled visit				
		Baseline	Day 8*	Day 31	Day 151	Day 361
Nirsevimab 50mg/100mg (N=36)	n	35	15	36	25	6
	n <LLOQ	35	0	1	1	1
	Geometric mean	0.25	139.24	66.85	21.79	2.51
	Geometric CV (%)	0.00	22.26	156.33	146.28	183.45
	Geometric SD (gSD)	1.00	1.25	3.04	2.91	3.37
	Geometric mean - gSD	0.25	111.76	21.99	7.48	0.75
	Geometric mean + gSD	0.25	173.48	203.26	63.52	8.45
	Arithmetic mean	0.25	142.41	85.74	28.33	3.68
	SD	0.00	31.17	37.69	14.36	2.41
	Median	0.25	143.08	84.73	28.94	3.74
	Min	0.25	99.923487	0.25	0.25	0.25
	Max	0.25	190.21498	168.40017	67.412464	6.637815
Nirsevimab 200mg (N=24)	n	24	11	24	23	4
	n <LLOQ	23	0	0	0	0
	Geometric mean	0.30	206.79	147.92	30.72	5.41
	Geometric CV (%)	101.73	16.58	32.11	75.40	71.75
	Geometric SD (gSD)	2.32	1.18	1.37	1.96	1.90
	Geometric mean - gSD	0.13	175.40	108.14	15.71	2.84
	Geometric mean + gSD	0.69	243.81	202.33	60.09	10.32
	Arithmetic mean	0.89	209.35	154.51	36.45	6.31
	SD	3.12	34.35	43.80	17.70	3.95
	Median	0.25	210.32	158.19	41.88	5.50
	Min	0.25	164.006113	76.299945	5.912068	2.874244
	Max	15.532915	260.543115	243.941878	58.721995	11.381248

CV = Coefficient of variation, gSD = Geometric SD, LLOQ = Lower limit of quantification (0.5 ug/mL), N = Number of subjects as per actual dose administered, n = Number of subjects in analysis, NA = Not applicable, NC = Not calculated, PK = Pharmacokinetics, SD = Standard deviation.
* Day 8 data is only collected for Japanese subjects.
Geometric mean - gSD = exp(mean(log(PK Concentration)) - SD(log(PK Concentration))).
Geometric mean + gSD = exp(mean(log(PK Concentration)) + SD(log(PK Concentration))).

Source: CSR Table 14.4.1.1

14.3. Bioanalytical Method Validation and Performance

PK assay: bioanalytical methods for the measurement of nirsevimab concentrations in human serum.

Nirsevimab concentrations in human serum were determined with validated enzyme-linked immunosorbent assay (ELISA) based method. Two methods were fully validated, CTVR-0101 and ICD 817, at two sites. The capture reagent and the detection reagent bind to two distinct sites on the variable region of nirsevimab. The capture antibody partially directed against the paratope of nirsevimab. The detection reagent is a full anti-paratope antibody. Thus, the PK assay measures a combination of free and partially free nirsevimab in serum. We reviewed the method validation reports and in study assay performances as shown in Table 68 below. The ligand binding assay method validations and in study assay performance are acceptable.

Table 68. Bioanalytical Method Life Cycle Information

Parameter	Method Validation #1	Method Validation #2	D5290C00003	D5290C00004	D5290C00005	D5290C00008
Analyte	Nirsevimab	Nirsevimab	Nirsevimab	Nirsevimab	Nirsevimab	Nirsevimab
Validation type	Validation	Validation	In study	In study	In study	In study
eCTD number and link	5.3.1.4 link	5.3.1.4 link	5.3.5.1 link	5.3.1.4 link	5.3.1.4 link	5.3.1.4 link
Method ID	CTVR-0101	ICD 817	CTVR-0101	ICD 817	ICD 817	ICD 817
Site	MedImmune GxP Testing Lab	(b) (4)	MedImmune GxP Testing Lab	(b) (4)		
Matrix	Human serum	Human (adult and infant) serum	Infant serum	Infant serum	Infant serum	Infant serum
Platform and format	ELISA: Nirsevimab is captured by monoclonal anti-MEDI8897 antibody coated on a microtiter plate. A second anti-MEDI8897 idiotypic monoclonal antibody that carried a biotin label is used to bind to a site on nirsevimab distinct from the capture antibody. Bound biotin molecules are detected by addition of streptavidin horseradish peroxidase (SA-HRP) conjugate. Tetramethylbenzidine (TMB) enzyme substrate is added to generate a colorimetric reaction that is measured at a wavelength of 450 nm.					
MEDI8897 Reference Standard	RSN889713H (Expiration date: August 15, 2018)	PRS8897A (Expiration date: January 31, 2022)	RSN889713H (Expiration date: August 15, 2018)	PRS8897A (Expiration date: January 31, 2022)	PRS8897A (Expiration date: January 31, 2022)	PRS8897A (Expiration date: January 31, 2022)
Anti-MEDI8897 coat antibody	P15112Jun13NS (Expiration date: June 12, 2023)	P35260Feb19RC (Expiration date: February 6, 2029)	P15112Jun13NS (Expiration date: June 12, 2023)	P35260Feb19RC (Expiration date: February 6, 2029)	P35260Feb19RC (Expiration date: February 6, 2029)	P35260Feb19RC (Expiration date: February 6, 2029)
Biotin-Anti-MEDI8897 detection antibody	CR92375.192 (Expiration date: February 24, 2019)	ML02960-63 (Expiration date: August 28, 2024)	CR92375.192 (Expiration date: February 24, 2019)	ML02960-63 (Expiration date: August 28, 2024)	ML02960-63 (Expiration date: August 28, 2024)	ML02960-63 (Expiration date: August 28, 2024)
Calibration range	0.13 µg/mL to 96 µg/mL (including 2 lower anchor and 2 higher anchor points)					
Quantitation range	0.5 µg/mL to 32 µg/mL					

Source: The reviewer analysis and summary based on Validation Reports of CTVR-0101 and ICD 817, and Bioanalytical Reports of Trials 03, 04, 05, 08.
Abbreviations: eCTD, electronic common technical document; ELISA, enzyme-linked immunosorbent assay; GxP, good practice; ICD, International Classification of Diseases; ID, identification; (b) (4); SA-HRP, streptavidin horseradish peroxidase; TMB, tetramethylbenzidine

Table 69. Summary Method Validation of CTVR-0101 Method at MedImmune Site

Parameter	Summary
Bioanalytical method validation report name	CTVR-0101
Method description	ELISA: Nirsevimab is captured by monoclonal anti-MEDI8897 antibody coated on a microtiter plate. A second anti-MEDI8897 idiotypic monoclonal antibody that carried a biotin label is used to bind to a site on nirsevimab distinct from the capture antibody. Bound biotin molecules are detected by addition of streptavidin horseradish peroxidase (SA-HRP) conjugate. Tetramethylbenzidine (TMB) enzyme substrate is added to generate a colorimetric reaction that is measured at a wavelength of 450 nm.
Minimum required dilutions (MRDs)	500
Source & lot of reagents	Nirsevimab: RSN889713H Anti-nirsevimab: P15112Jun13NS Biotin-anti-MEDI8897: CR92375.192 Normal human serum: BRH662060 - BRH662064, BRH662015 - BRH662019, BRH815901, BRH726936
Calibration curve performance Accuracy and precision	0.13 (anchor), 0.25 (anchor), 0.5, 1, 2, 4, 8, 16, 32, 64 (anchor), and 96 (anchor) µg/mL Accuracy (for quantitation range standard, %bias) ranged from –1.9 to 1.7% Precision (for quantitation range standard, %CV) ranged from 2.3 to 5.4%
QC performance Accuracy and precision	5 QC concentrations 0.5, 1.5, 6, 24, 32 µg/mL Accuracy (%bias) ≤12.3% Precision (%CV) ranged from 5.4 to 19.4% Total error (%) (defined as Accuracy (%bias) + Precision (%CV)) ranged from 11.8 to 31.6%
Incurred Sample Reanalysis (ISR)	Acceptable (the relative percent difference (absolute value) between the ISR result and the original result was less than or equal to (≤) 30% for at least 66.7% of the samples retested.)
Matrix qualification and selectivity	100% of the unspiked pooled (from 10 individuals) human serum samples measure below the LLOQ of the assay. At least 80% of the spiked pooled (from 10 individuals) human serum samples measure within ±20% of the mean value of the three spiked samples of pooled serum.
Dilution linearity and hook effect	At least 75% of the diluted samples in the validated range of the standard curve have absolute bias and imprecision levels of less than or equal to ≤20%. No hook effect is evident in this assay up to a concentration of 485 µg/mL nirsevimab.
Plate coating stability	Stable up to 7 days after coating and storage at 5°C ±3°C
Room temperature stability	Stable in serum at room temperature for up to 20 hours
Freeze thaw stability	Stable up to 5 freeze/thaw cycles
Long term storage	Up to 692 days in human serum stored at –80±10°C Up to 122 days in human serum stored at –20±5°C

Source: The reviewer analysis based on Validation Report of CTVR-0101
Abbreviations: %CV, percentage coefficient of variation; C, Celsius; ELISA, enzyme-linked immunosorbent assay; ISR, Incurred Sample Reanalysis; LLOQ, lower limit of quantification; QC, quality control; SA-HRP, streptavidin horseradish peroxidase; TMB, tetramethylbenzidine

Table 70. Summary Method Validation of ICD 817 Method at (b) (4) Site

Parameter	Summary
Bioanalytical method validation report name	ICD 817
Method description	ELISA: Nirsevimab is captured by monoclonal anti-MEDI8897 antibody coated on a microtiter plate. A second anti-MEDI8897 idiotypic monoclonal antibody that carried a biotin label is used to bind to a site on nirsevimab distinct from the capture antibody. Bound biotin molecules are detected by addition of streptavidin horseradish peroxidase (SA-HRP) conjugate. Tetramethylbenzidine (TMB) enzyme substrate is added to generate a colorimetric reaction that is measured at a wavelength of 450 nm.
Minimum required dilutions (MRDs)	500
Source & lot of reagents	Nirsevimab: PRS8897A Anti-nirsevimab: P35260Feb19RC Biotin-anti-MEDI8897: ML02960-63 Normal human serum: NB20165-34-01, BRH1497575 - BRH1497593, HMN8578, HMN8579
Calibration curve performance Accuracy and precision	0.13 (anchor), 0.25 (anchor), 0.5, 1, 2, 4, 8, 16, 32, 64 (anchor), and 96 (anchor) µg/mL Accuracy (for quantitation range standard, %bias) ranged from –2.3 to 2.0% Precision (for quantitation range standard, %CV) ranged from 1.8 to 3.9%
QC performance Accuracy and precision	5 QC concentrations 0.5, 1.5, 6, 24, 32 µg/mL Accuracy (%bias) ≤12.3% Precision (%CV) ranged from 5.4 to 19.4% Total error (%) ranged from 11.8 to 31.6%
Incurred sample reanalysis	Acceptable (the relative percent difference (absolute value) between the ISR result and the original result was less than or equal to (≤) 30% for at least 66.7% of the samples retested.)
Matrix qualification and selectivity	100% of the unspiked pooled (from 10 pediatric individuals) human serum samples measure below the LLOQ of the assay. At least 80% of the spiked pooled (from 10 individuals) human serum samples measure within ±20% of the mean value of the three spiked samples of pooled serum.
Dilution linearity and hook effect	At least 75% of the diluted samples in the validated range of the standard curve have absolute bias and imprecision levels of less than or equal to ≤20% (≤25% at the LOQ). No hook effect is evident in this assay up to a concentration of 510 µg/mL nirsevimab.
Plate coating stability	Not evaluated
Room temperature stability	Stable in serum at room temperature for up to 48 hours
Freeze thaw stability	Stable up to 6 freeze/thaw cycles

Parameter	Summary
Long term storage	Up to 35 days in human serum stored at –80±10°C Stability in human serum stored at –20±5°C (not evaluated)
Antibody interference	The quantification assay for nirsevimab (at all tested concentrations) was not tolerant at goat anti-MEDI8897 concentrations ≥1000 ng/mL.
Cross-laboratory assay performance	Cross-laboratory performance of the assay was evaluated by the analysis of 30 samples containing MEDI8897 at concentrations unknown to (b) (4). Average Relative Percent Difference (%) is 8.4% (range: 0–15.2%)

Source: The reviewer analysis based on Validation Report of ICD 817
Abbreviations: %CV, percentage coefficient of variation; C, Celsius; ELISA, enzyme-linked immunosorbent assay; ICD, International Classification of Diseases; ISR, Incurred Sample Reanalysis; LLOQ, lower limit of quantification; MRD, minimum required dilution; (b) (4); QC, quality control; SA-HRP, streptavidin horseradish peroxidase; TMB, tetramethy benzidine

14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety

14.4.1. Evaluation of Effects of ADA on PK

14.4.1.1. Summary of Clinical Trials

Immunogenicity of nirsevimab was evaluated in four phase 1/2 trials (Trial 01, 02, 03, and 08), one, phase 2/3 trial (Trial 05) and one, phase 3 trial (Trial 04). Trials 03, 04, and 05 provided the primary data for Immunogenicity Specimen (IS) analysis to assess the impact of immunogenicity on PK. Trial 03 and the Primary Cohort of Trial 04 were completed and provided the main data to inform the immunogenicity labeling. Trial 05 is an ongoing study in two RSV seasons, which provides information in the high-risk population; immunogenicity data up to one year were available for analysis and data from the second RSV season are incomplete.

Table 71 contains (1) the information for the three clinical trials assessing immunogenicity, and (2) a high-level summary of study results for PK (concentration on Day 361).

The overall study design in terms of treatment duration, sampling time, and sample size was adequate to assess the anti-drug antibody (ADA) impact on PK. The reviewer independently analyzed the ADA, NAb (neutralizing antibody), and anti-YTE incidence and verified the Applicant's reported values. The reviewer's analysis results aligned with the Applicant's.

Table 71. Summary of Clinical Trials Information and Immunogenicity Incidence

Clinical Trial Information	Trials			
	Trial 03	Trial 04	Trial 05	
Status	Completed	Ongoing (data available up to 361 days postdose)	Ongoing (Season 1 data available up to 361 days postdose)	
Dose regimen (single IM dose)	50 mg	50 or 100 mg	Season 1: 50 or 100 mg	Season 2: 200 mg
Sampling time	Predose, Day 91, 151, 361	Predose, Day 31, 151, 361	Predose, Day 31, 151, 361	
Number of subjects who received	572	987 (efficacy cohort)	RSV Season 1: 614	RSV Season 2:

Clinical Trial Information	Trials			
	Trial 03	Trial 04	Trial 05	
recommended dose of nirsevimab			(Preterm and CHD/CLD cohorts combined)	180 (CLD/CHD cohort only)
Applicant reported ADA incidence at Day 361	3.3% (16/492)	7% (55/830)	6% (32/534)	TBD
Applicant reported NAb incidence at Day 361	0% (0/16)	22% (12/55)	6% (2/32)	TBD
Applicant reported anti-YTE incidence at Day 361	94% (15/16)	96% (53/55)	97% (31/32)	TBD

Source: Table 6. Overview of Immunogenicity Results and Sampling Time Points by Study (Integrated Summary of Immunogenicity)
Abbreviations: ADA, anti-drug ant body; CHD, congenital heart disease; CLD, chronic lung disease; NAb, neutralizing antibody; RSV, respiratory syncytial virus; TBD, to be determined; YTE, amino acid substitution

Table 72. Mean (SD) Serum Concentrations of Nirsevimab at Day 361 in Trials 03, 04, 05

	Trials			
	Trial 03 (N=771)	Trial 04 (<5 kg, N=799; ≥5 kg, N=585)	Trial 05 RSV Season 1 - Preterm Cohort (<5 kg, N=243; ≥5 kg, N=162)	Trial 05 RSV Season 2 (N=TBD)
Nirsevimab mean (SD) concentration at Day 361 (µg/mL)	2.1 (1.1)	2.2 (1.36); 3.8 (2.5)	2.5 (1.5); 4.4 (3.6)	TBD

Source: CSR
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus; SD, standard deviation; TBD, to be determined

14.4.1.2. ADA Impact on Safety

As shown in Table 71, the percentages of subjects who were ADA positive was low across the three, main clinical trials. The types and frequency (proportion) of adverse events were compared between subjects who were ADA negative at baseline and positive at Day 361 and subjects who were ADA negative at both timepoints. While no differences in types and frequency of adverse events were observed, it is difficult to reach any definitive conclusions due to the small number of subjects who were ADA positive at Day 361.

14.4.1.3. Highlight of Key Characteristics of Immunogenicity Assays Relevant to this Review

The two versions of validated ADA assays are deemed to display adequate sensitivity. However, the nirsevimab concentrations of most samples collected before or on Day 151 postdose exceeded the drug tolerance limit of the ADA assays. Thus, the ADA status of these samples is not reliable. Therefore, only data from Day 361 are reliable to evaluate the impact of ADA on PK. Table 73 shows several assay characteristics that are relevant for the analysis.

Table 73. Summary of Key Assay Characteristics Related to Immunogenicity Assessment

Characteristic	Trials	
	Trial 03	Trial 04 and 05
Assay validation report number (ADA and NAb)	CTVR-0147	RMUD2
ADA assay sensitivity (ng/mL)	16.87	5.43
ADA assay drug tolerance	10 µg/mL in the presence of 125 ng/mL of PC	12.5 µg/mL in the presence of 100 ng/mL of PC

Source: Integrated Summary of Immunogenicity
Abbreviations: ADA, anti-drug ant body; NAb, neutralizing ant body; PC, placebo-controlled

14.4.1.4. Methods for Evaluating the Effect of Immunogenicity on PK of Nirsevimab

To evaluate the impact of immunogenicity on PK, we compared the observed concentrations in two groups (ADA+ and ADA-) in subjects with time-matched PK and ADA data using Immunogenicity Specimen (IS) tool developed in-house (FDA). At each timepoint, nirsevimab concentrations were summarized by ADA+ and ADA- groups. Graphically, ADA- group was presented in boxplot and ADA+ group was presented as a line graph. The average concentrations of nirsevimab (ADA+ and ADA- groups) were calculated at each timepoint and graphically presented. Summary of nirsevimab concentrations by ADA status (ADA+ or ADA-) were tabulated along with the average concentration by timepoint as well as the number of subjects. For the statistical comparisons of concentration data between ADA+ and ADA- groups, the geometric mean ratio (GMR) of ADA+/ADA- and the corresponding 90% confidence interval (CI) were also presented graphically.

14.4.1.5. Effect of Immunogenicity on PK of Nirsevimab

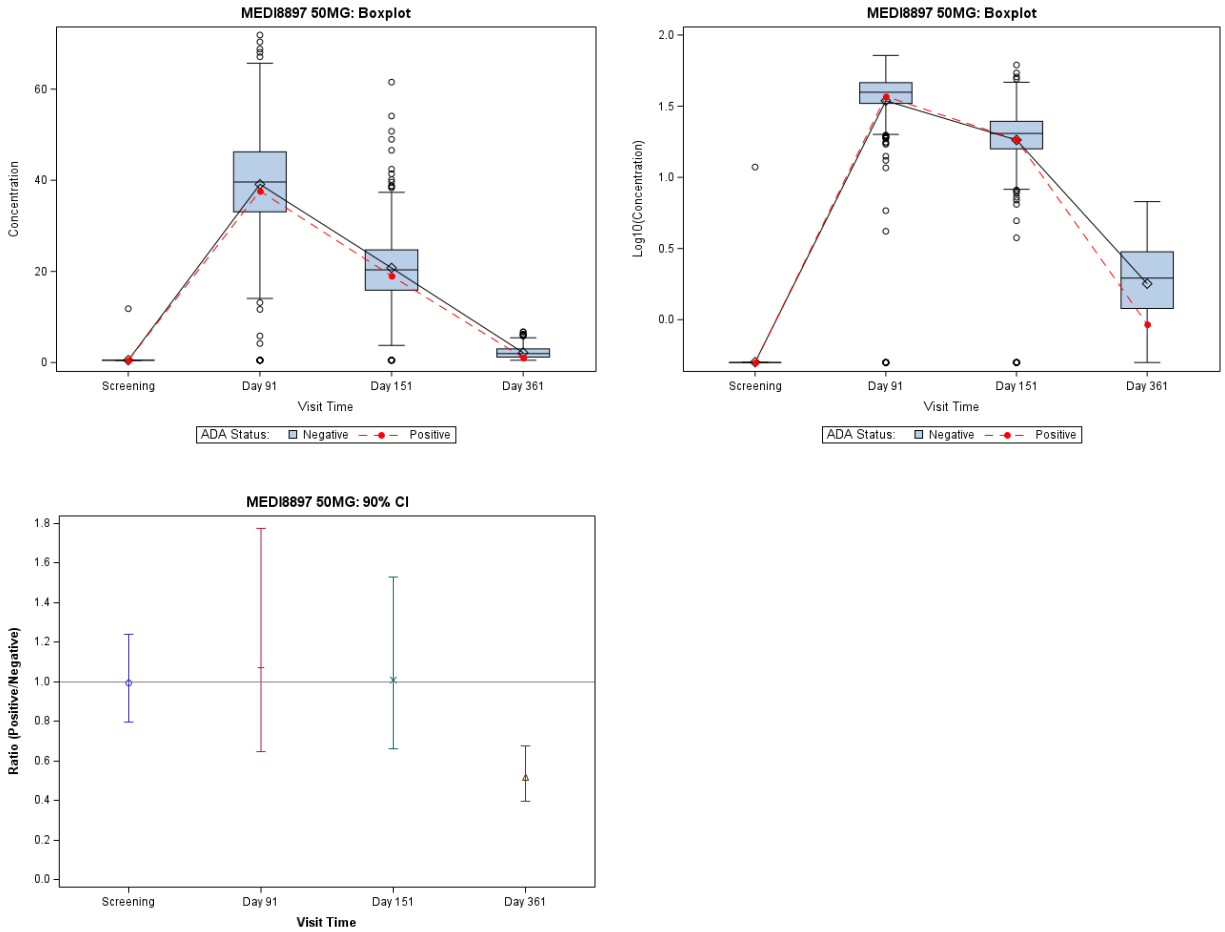
The analysis results for Trials 03, 04, and 05 were summarized by trial below. The results indicate that the development of ADA against nirsevimab is associated with a reduced systemic concentration of nirsevimab, and the effect of ADA was observed starting on Day 151 even with the assay uncertainty caused by drug tolerance.

Trial 03

Figure 11 and Table 74 show that ADA+ group has a lower nirsevimab concentration than ADA- group indicating that ADA had a negative effect on PK of nirsevimab at Day 361. The upper limit of 90% CI of GMR value was <1 and the GMR was 0.52 at Day 361.

In Trial 03 (n=551) the impact of ADA on PK, i.e., lower concentrations in ADA+ group, was observed on Day 361 (Figure 11). The left and middle panels of Figure 11 show nirsevimab concentration of the ADA+ and ADA- samples up to day 361 in linear and semi-log scales. The right panel shows the 90% CI of GMR (ADA+/ADA-) of drug concentration at each visit. See Table 74 for a tabular summary.

Figure 11. Top Left and Right Box Plot Analysis of Drug Concentration From ADA Positive and ADA Negative Samples During the Treatment Period (361 Days) in Linear and Semi-Log Scales, Respectively. Bottom Box Plot: 90% CI of GMR of Drug Concentration at Each Visit



Source: Reviewer analysis
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio

Table 74. Summary of Geometric Mean Nirsevimab Concentration by ADA Status at Each Trial Visit in Subjects Who Received the Proposed Dose of Nirsevimab, Trial 03

Visit #	Treatment Day	Total N	Nirsevimab Concentration (µg/mL); Geometric Mean				GMR (90%CI)
			ADA+ Group	N	ADA- Group	N	ADA+/ADA-
2	Screening	551	0.50	1	0.50	550	1 (NA)
3	91	513	37.02	6	34.52	507	1.07 (0.6,1.7)
4	151	500	18.4	7	18.3	493	1 (0.7,1.5)
5	361	491	0.93	16	1.80	475	0.52(0.4,0.7)

Source: Reviewer analysis
NA: 90%CI not reported for time points with 1 subject in the ADA+ group
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio; N, number of subjects

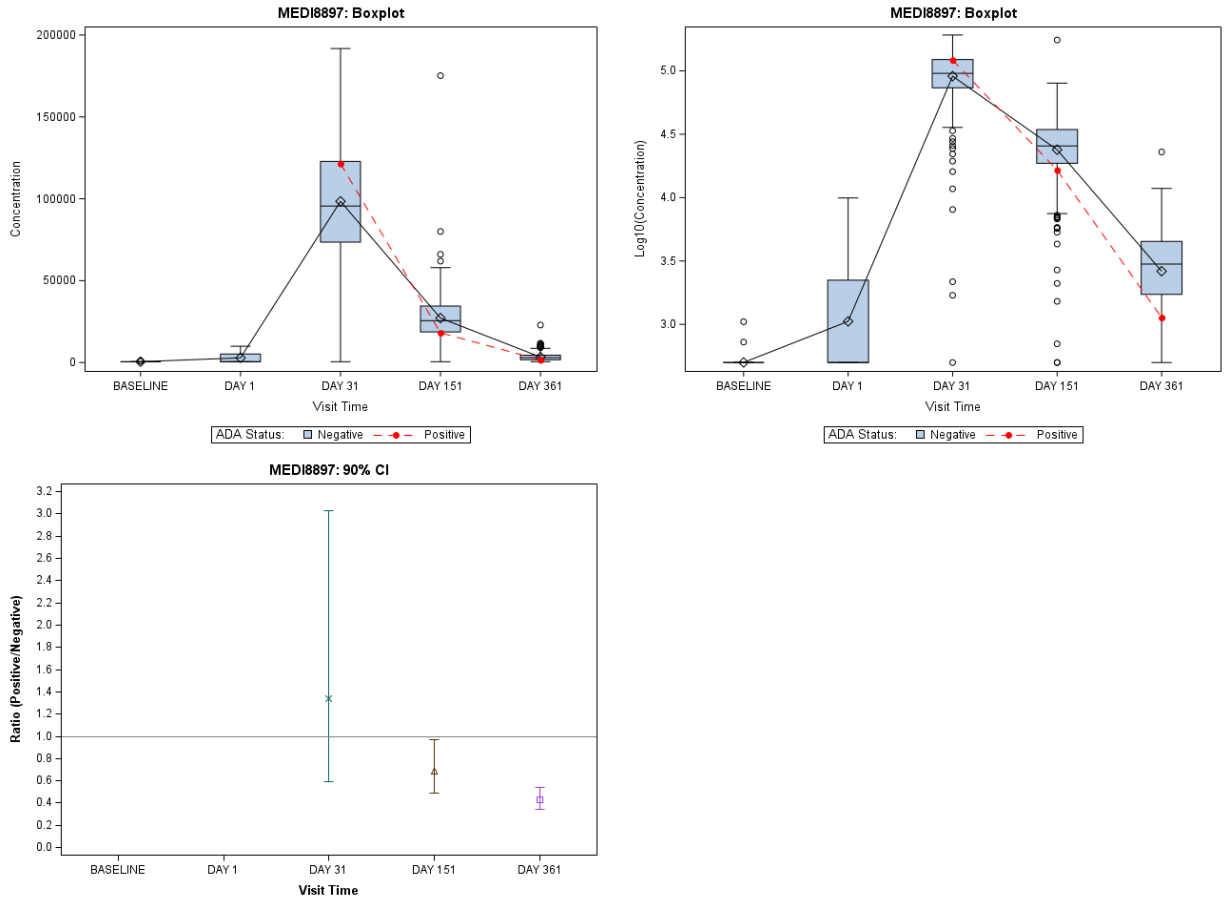
Trial 04

Figure 12 and Table 75 show that ADA+ group had a lower nirsevimab concentration than ADA- group at Day 151 and 361 indicating that ADA had a negative effect on PK of nirsevimab.

Based on the data over the trial duration of 361 days, the upper limit of 90% CI of GMR values were <1 for the last two timepoints.

In Trial 04 (n=777) the impact of ADA on PK, i.e., lower concentrations in ADA+ group, was observed starting from day 151 visit (Figure 12). The left and middle panels in Figure 12 show the drug concentration up to day 361 for the ADA+ and ADA- population in linear and semi-log scales. The right panel shows the 90% CI of GMR of drug concentration at each visit. See Table 75 for a tabular summary.

Figure 12. Left and Right Top Panels: Box Plot Analysis of Drug Concentration From ADA-Negative Samples and Line Graph of ADA-Positive Samples During the Treatment Period (361 Days) in Linear and Semi-Log Scales, Respectively. Bottom Panel: 90% Confidence Interval of GMR of Drug Concentration at Each Visit



Source: Reviewer analysis
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio

Table 75. Summary of Geometric Mean Nirsevimab Concentration by ADA Status at Each Trial Visit, Trial 04

Visit #	Treatment Day	Total N	Nirsevimab Concentration (µg/mL); Geometric Mean				GMR (90%CI)
			ADA+ Group	N	ADA- Group	N	ADA+/ADA-
2	Baseline	777	NA	0	0.50	777	NA
3	1	4	NA	0	1.10	4	NA
4	31	583	123.43	1	90.82	582	1.34 (NA)
5	151	510	16.48	8	23.85	502	0.70 (0.5, 1)
6	361	566	1.13	34	2.63	532	0.40 (0.3, 0.5)

Source: Reviewer analysis
NA: GMR and 90%CI not reported for time points with 0 and 1 subject, respectively, in the ADA+ group
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio; N, number of subjects

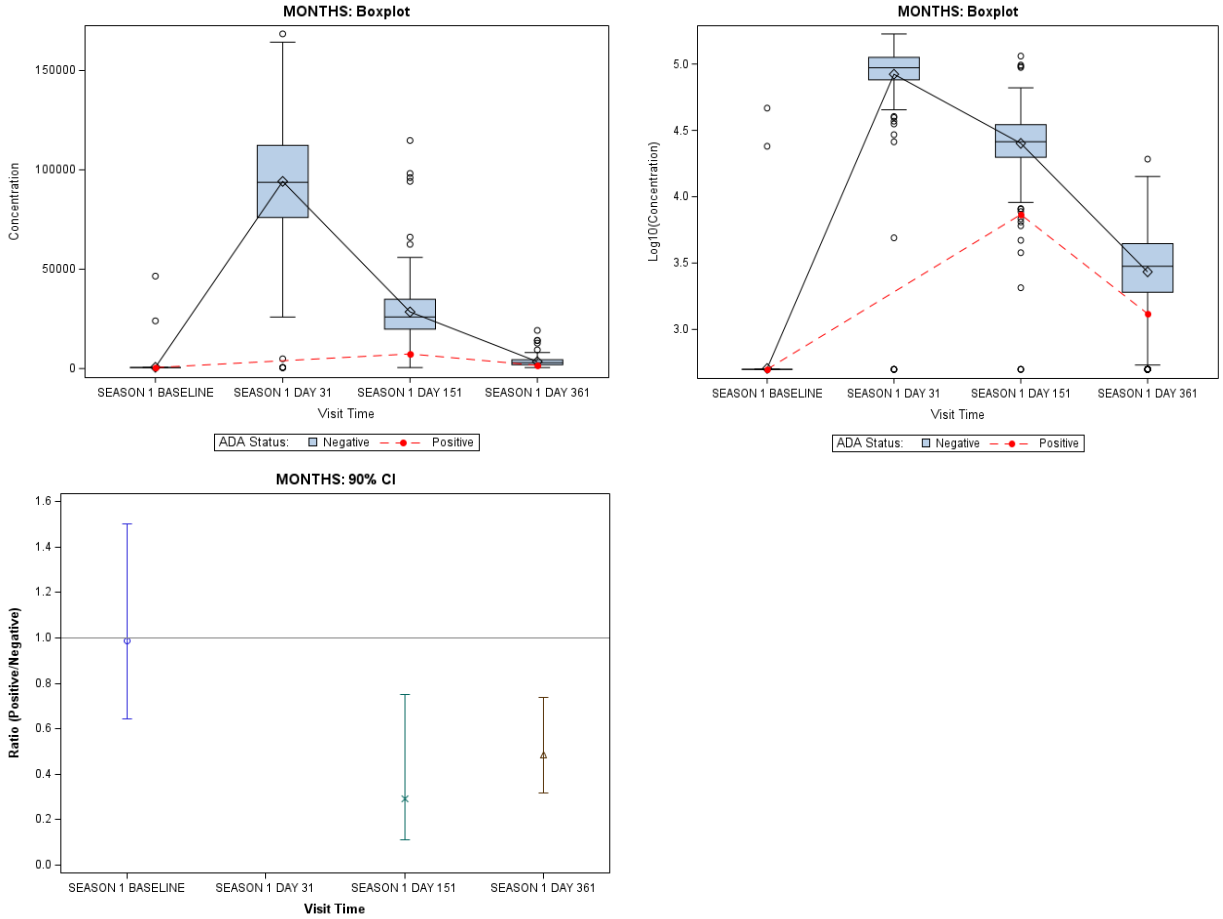
Trial 05

First RSV Season

Figure 13 and Table 76 showed that ADA+ group had a lower nirsevimab concentration than ADA- group at Day 151 and 361 indicating that ADA had a negative effect on PK of nirsevimab. Based on the data over the trial duration of 361 days, the upper limit of 90% CI of GMR values were <1 at the last two timepoints.

In Trial 05 first RSV Season (n=541) the impact of ADA on PK, i.e., lower concentrations in ADA+ group, was observed starting from Day 151 visit (Figure 13). The left and middle panels in Figure 13 showed the drug concentration up to Day 361 for the ADA+ and ADA- population in linear and semi log scale. The right panel showed the 90% CI of GMR of drug concentration at each visit. See Table 76 for a tabular summary.

Figure 13. Left and Right Top Panels: Box Plot Analysis of Drug Concentration From ADA-Negative Samples and Line Graph of ADA-Positive Samples During the Treatment Period (361 Days) in Linear and Semi-Log Scale, Respectively. Bottom Panel: 90% Confidence Interval of GMR of Drug Concentration at Each Visit



Source: Reviewer analysis
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio

Table 76. Summary of Geometric Mean Nirsevimab Concentration by ADA Status at Each Trial Visit for Season 1, Trial 05

Visit #	Treatment Day	Total N	Nirsevimab Concentration (µg/mL); Geometric Mean				GMR (90%CI)
			ADA+ Group	N	ADA- Group	N	ADA+/ADA-
2	Baseline	541	0.50	1	0.51	540	0.99 (NA)
3	31	259	NA	0	83.64	259	NA
4	151	426	7.35	1	25.23	425	0.30 (NA)
5	361	203	1.31	9	2.71	194	0.48 (0.3,0.7)

Source: Reviewer analysis
NA: GMR and 90%CI not reported for time points with 0 and 1 subject, respectively, in the ADA+ group
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio; N, number of subjects

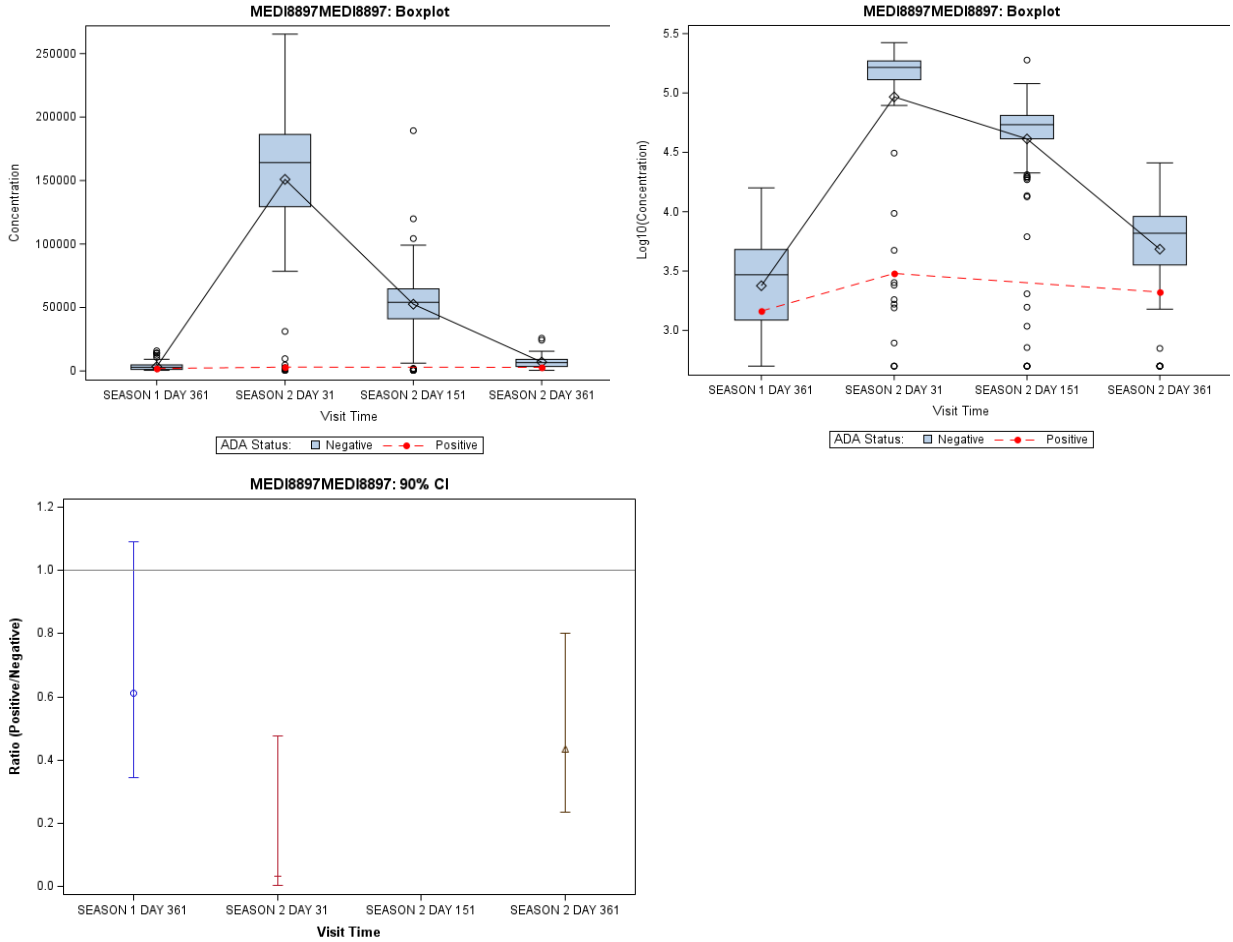
Second RSV Season

Given the interim data in this ongoing trial, Figure 14 and Table 77 show that ADA+ group has a lower nirsevimab concentration than ADA- group at Day 31 and 361 on Season 2 indicating that ADA has a negative effect on PK of nirsevimab. Based on the available data over the trial

duration of 361 days, the upper limit of 90% CI of GMR values were <1 at the Day 31 and 361 timepoints. It's worth noting that the observed GMRs are smaller for visits in the second RSV season (Table 77) when compared to those for the first RSV season (Table 76).

In Trial 05 second RSV season (n=172) the impact of ADA on PK, i.e., lower concentrations in ADA+ group, is observed starting from day 31 visit (Figure 14). The left and middle panels in Figure 14 show the drug concentration up to day 361 for the ADA+ and ADA- groups in linear and semi-log scales. The right panel shows the 90% CI of GMR of drug concentration at each visit. The results indicate that the drug concentration in samples is lower in ADA+ group. See Table 77 for a tabular summary of nirsevimab geometric mean concentration in Trial 05 in the second RSV season.

Figure 14. Left and Right Top Panels: Box Plot Analysis of Drug Concentration From ADA-Negative Samples and Line Graph for ADA-Positive Samples During the Treatment Period (361 Days) in Linear and Semi-Log Scale, Respectively. Bottom Panel: 90% Confidence Interval of GMR of Drug Concentration at Each Visit



Source: Reviewer analysis
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio

Table 77. Trial 05 - Summary of Geometric Mean Nirsevimab Concentration by ADA Status at Each Trial Visit for Season 2

			Nirsevimab Concentration (µg/mL); Geometric Mean				GMR (90%CI)
Visit #	Treatment Day	Total N	ADA+ Group	N	ADA- Group	N	ADA+/ADA-
2	Season 1, Day 361	172	1.46	7	2.38	165	0.60 (0.3,1.1)
3	31	90	3.02	1	92.68	89	0.03 (NA)
4	151	158	NA	0	41.01	158	NA
5	361	63	2.10	9	4.84	57	0.40 (0.2,0.8)

Source: Reviewer analysis
NA: GMR and 90%CI not reported for time points with 0 and 1 subject, respectively, in the ADA+ group
Abbreviations: ADA, anti-drug ant body; CI, confidence interval; GMR, geometric mean ratio; N, number of subjects

***Reviewer's Comment**: The review team conducted independent analyses to assess whether the decrease of nirsevimab concentration due to ADA may decrease the expected efficacy. The worst-case scenario was chosen (i.e., lower bound of the 90% CI which is 0.3) for the calculation. Mean nirsevimab serum concentrations on Day 151 were 19.6±7.8 µg/mL in subjects <5 kg and 31.1±13.7 µg/mL in subjects ≥5 kg, respectively. Consequently, the worst-case scenario for a subject <5 kg with mean nirsevimab serum concentration and ADA+ is 5.9 µg/mL, which is slightly lower than EC90 (6.8 µg/mL). The worst-case scenario for a subject ≥5 kg with mean nirsevimab serum concentration and ADA+ is 9.3 µg/mL, which is above EC90 (6.8 µg/mL). Due to the low incidence (i.e., 7%) of ADA+ and projection in the worst-case scenarios, although the development of ADA against nirsevimab reduced nirsevimab serum concentration, from a clinical pharmacology perspective, it may not have a clinically significant effect on efficacy. However, we also acknowledged some information gaps, especially the time difference between primary clinical endpoint (i.e., on Day 151) and ADA identification (i.e., only on Day 361 due to drug interference in the ADA assay), and low occurrence of MA RSV LRTI in subjects receiving nirsevimab due to the nature of the proposed indication, prevention of RSV lower respiratory tract disease. According to FDA Guidance Immunogenicity Information in Human Prescription Therapeutic Protein and Select Drug Product Labeling (February 2023), if the clinical effect(s) of anti-drug antibodies on a product's effectiveness is unknown (e.g., methodology is adequate, but the data are too limited to assess any association of the anti-drug antibodies with changes in effectiveness), this uncertainty should be conveyed in the Immunogenicity subsection. Therefore, we recommend the labeling language as shown below in bold in the 12.6 Immunogenicity subsection. We defer the clinical assessment of safety and efficacy to the clinical review team.*

Because of the low occurrence of ADA and MA RSV LRTI in clinical trials, the effect of these ADA on the safety and/or effectiveness of Beyfortus is unknown.

14.5. Pharmacometrics Assessment

Executive Summary

The Pharmacometrics review is aimed at evaluating the extrapolation of efficacy from the trials in otherwise healthy term and preterm infants (Trials 03 and 04) to the higher risk populations in Trial 05. Trial 05 was originally designed to compare nirsevimab to palivizumab in higher risk subjects with congenital heart disease, chronic lung disease, or prematurity (including less than 29 weeks of gestation). The trial stopped enrollment early and few to no MA RSV LRTI events

were observed during the first and second RSV seasons, respectively. The Pediatric Research Equity Act of 2003 established that extrapolation of efficacy may be considered from one pediatric population to another. Given similarities in disease pathophysiology, and because nirsevimab's target (the virus molecule) is the same regardless of the subject's age or medical comorbidities, efficacy extrapolation approach was sought to establish evidence of effectiveness for nirsevimab to prevent lower respiratory tract RSV disease in pediatric patients who remain vulnerable to RSV disease during their second RSV season. Because of the similarities in disease pathophysiology and drug mechanism of action, it is reasonable to expect the therapeutic concentrations of nirsevimab to also be the same for each population. Therefore, an exposure matching approach was taken to support extrapolation of efficacy for nirsevimab administration in the high-risk pediatric patients during their second year of life (second RSV season).

The pharmacometrics review is divided into two sections. The first discusses the Applicant's Population PK model that was utilized to generate nirsevimab exposure parameters for each individual, and the exposure matching comparisons that were utilized to support bridging of efficacy to the higher risk populations in Trials 05. The second section discusses the exposure response analyses that were utilized to support selection of the proposed dosing regimen.

14.5.1. Population PK Analysis

14.5.1.1. Review Summary

In general, the Applicant's population PK analysis is considered acceptable for generating individual exposures utilized for the exposure matching analysis.

The Applicant's model is also considered acceptable for generating individual exposure metrics for the exposure-response analyses and for the descriptive labeling of nirsevimab PK. The Applicant's analyses were verified by the reviewer, with no significant discordance identified.

Aside from the primary role in supporting exposure matching, the developed model was used to support the current submission as outlined in Table 78.

Table 78. Specific Comments on Applicant's Final Population PK Model

Goal of the Analysis	Utility of the Final Model	Reviewer's Comments
Support Applicant's proposed labeling statements about intrinsic and extrinsic factors		
Intrinsic factor	"No clinically significant differences in the pharmacokinetics of nirsevimab-alip were observed based on race or vulnerability to severe RSV disease (i.e., CLD, CHD, GA <29 weeks, or immunocompromised states)."	The statement is acceptable. Covariate analysis on the Applicant's final model did not suggest these factors had a meaningful effect on nirsevimab PK.
Extrinsic factor	Dose Proportionality: The PK of nirsevimab-alip is dose-proportional following a single IM administration	The Applicant's population PK database included doses ranging from 10 mg to 3000 mg. Sensitivity

Goal of the Analysis	Utility of the Final Model	Reviewer's Comments
	of doses ranging from 25 mg (0.5 times the lowest approved recommended dosage) to 200 mg in pediatric subjects.	analysis allowing for nonlinear CL or Vd did not reveal any dose-proportional effect beyond the linear PK defined in the structural model.
Derive exposure metrics for exposure-response analyses	$AUC_{baselineCL}$, $AUC_{0\text{-}365}$, $C_{151,pred}$	The Applicant's final model is generally acceptable for generating exposure metrics for exposure-response analyses. Parameters appear to be estimated with acceptable precision and shrinkage on CL is reasonable at 10%.

Source: Reviewer analysis
Abbreviations: $AUC_{baselineCL}$, area under the concentration-time curve based on clearance at baseline; $AUC_{0\text{-}365}$, area under the concentration-time curve from Day 0 to Day 365; $C_{151,pred}$, predicted concentration on Day 151; CHD, congenital heart disease; CLD, chronic lung disease; GA, gestational age; PK, pharmacokinetic; RSV, respiratory syncytial virus; Vd, volume of distribution

14.5.1.2. Introduction

The primary objectives of Applicant's analysis were to:

- Characterize the population pharmacokinetics (PK) of nirsevimab in data from healthy adults and children and immunocompromised children.
- Generate individual exposure metrics from the phase 3 trials to be used for subsequent exposure-response analyses
- Evaluate the exposures predicted for the proposed dosing regimens in patients from Trial 05, the population to which the Applicant has proposed extrapolation.

14.5.1.3. Model Development

Data

The analyses were based on PK data from six studies trials. The study design, study population, and timing of blood samples varied among the 6 clinical trials. Brief descriptions of the trials included are presented in Table 79.

The final NONMEM data file for analysis contained 9597 PK observations from 3133 subjects. Table 80 and Table 81 provide summary statistics of the baseline demographic covariates in the analysis dataset.

Table 79. Summary of Trials With PK Sampling Included in Population PK Analysis

Study Number, Phase	Subject Population	Supporting analysis, Number of Subjects Dosed	Dose and Regimen	Sampling[a]
D5290C00001 Phase 1 Completed	Healthy adults	Final analysis Treated: 102 Placebo: 34	SAD of nirsevimab IV: 300, 1000, and 3000 mg SAD of nirsevimab IM: 100 and 300 mg or placebo	PK Pre-dose and 8 hours postdose on Day 1, and Days 2, 4, 6, 8, 15, 22, 31, 61, 91, 121, 151, 181, 271, and 361 ADA Pre-dose and Days 15, 31, 91, 181, 271, and 361
D5290C00002 Phase 1b/2a Completed	Healthy preterm infants born 32 to <35 weeks GA entering their first RSV season	Final analysis Treated: 71 Placebo: 18	SAD dose of nirsevimab IM: 10, 25, and 50 mg or placebo	PK Screening and Days 8, 31, 151, 361 ADA Screening and Days 31, 151, and 361
D5290C00003 Phase 2b Completed	Healthy preterm infants born 29 to <35 weeks GA entering their first RSV season[b]	Final analysis, as-treated Total: 1447 Nirsevimab: 968 (572 infants <5 kg dosed per Phase 3 regimen) Placebo: 479	Single 50 mg IM dose of nirsevimab or placebo	PK Screening and Days 91, 151, and 361 ADA Screening and Days 91, 151, and 361 Efficacy medically attended RSV confirmed LRTI

Study Number, Phase	Subject Population	Supporting analysis, Number of Subjects Dosed	Dose and Regimen	Sampling[a]
				through 150 days post dose
D5290C00004 (MELODY) Phase 3 Ongoing	Healthy late preterm and term infants born ≥ 35 weeks GA, entering their first RSV season	Primary analysis, as-treated Total: 1478 Nirsevimab: 987 Placebo: 491 [c]All subjects, as-treated Total: 2994 Nirsevimab: 1998 Placebo: 996	Single 50 mg IM dose of nirsevimab for infants <5 kg or 100 mg IM dose of nirsevimab for infants ≥5 kg or placebo	PK Screening or Day 1 pre-dose and Days 8 (Japan only) and 15 (EU only) or Days 31 (Non-EU), 151, and 361 ADA Screening, Day 31, 151 and 361. Efficacy medically attended LRTI (inpatient and outpatient) due to RT-PCR confirmed RSV through 150 days after dosing

Study Number, Phase	Subject Population	Supporting analysis, Number of Subjects Dosed	Dose and Regimen	Sampling[a]
D5290C00005 (MEDLEY) Phase 2/3 Ongoing	Preterm infants born ≤35 weeks GA, and infants with CLD of prematurity and/or CHD	Season 1, as-treated Total: 918 Preterm cohort: Nirsevimab: 406 Palivizumab: 206 CLD/CHD cohort: Nirsevimab: 208 Palivizumab: 98 Season 2, as-treated: Nirsevimab: 220 Palivizumab: 42	Season 1: Single 50 mg IM dose of nirsevimab for infants <5 kg or single 100 mg IM dose of nirsevimab for infants ≥5 kg followed by 4 monthly IM doses of placebo or 15 mg/kg IM palivizumab 5 monthly doses Season 2 (CHD/CLD cohort only): 200 mg IM dose of nirsevimab or 15 mg/kg IM palivizumab 5 monthly doses	PK Season 1 and 2[d] Screening or Day 1 pre-dose (Season 1 only) and Days 8 (Japan only) and 15 (EU only) or Days 31 (Non-EU), 151, and 361 ADA Season 1 and 2[d] Screening (Season 1 only), Day 31, Day 151, Day 361
D5290C00008 (MUSIC) Phase 2 Ongoing	Infants ≤24 months with a diagnosis of combined immunodeficiency, antibody deficiency or other immunodeficiency	Total: 60 36 subjects entering their 1st RSV season and 24 subjects entering their 2nd RSV season	1st RSV season: 50 mg IM dose of Nirsevimab for infants < 5 kg or 100 mg IM dose of Nirsevimab for infants ≥ 5 kg. 2nd RSV season: 200 mg IM dose of Nirsevimab	PK Screening or day 1 pre-dose and days 8 (Japan only), 31, 151, and 361. ADA Screening, Day 31, 151 and 361.

[a] PK/ADA at unscheduled visits in Studies D5290C00003, MELODY, and MEDLEY (hospitalization). Blood samples for PK and ADA were collected from all subjects hospitalized with lower respiratory tract infection within approximately 2 days following hospital admission.
[b] Preterm infant population was not eligible to receive palivizumab per local guidelines. In EU, infants entering their first RSV season were to be ≤ 8 months of age.
[c] Primary cohort and Safety cohorts combined, limited PK data available from the Safety cohort at database lock (29 April 2022).
[d] Data available through at least Day 151 in Season 2 at database lock (31 May 2022).
Abbreviations: ADA=antidrug antibody; CHD=congenital heart disease; CLD=chronic lung disease; EU=European Union; GA=gestational age; IM=intramuscular; IV=intravenous; LRTI=lower respiratory tract infection; MA RSV LRTI=medically attended RSV-confirmed lower respiratory tract infection; PK=pharmacokinetic(s); RSV=respiratory syncytial virus; RT-PCR=reverse transcription-polymerase chain reaction; SAD=single ascending dose
Source: Applicant's Population PK report, Table 1

Reference ID: 5208810

Table 80. Summary of Baseline Demographic Continuous Covariates for Analysis

Covariate	Covariate	D5290C00001 (N = 102)	D5290C00002 (N = 71)	D5290C00003 (N = 916)	MELODY (N = 963)	MEDLEY Season 1 (N = 593)	MEDLEY Season 2 (N = 191)	Total (N = 2836)
Baseline weight (kg)[a]	Mean (SD%)	78.0 (14.9%)	6.81 (1.89%)	4.58 (1.91%)	5.51 (1.84%)	4.73 (1.87%)	9.88 (1.65%)	7.98 (14.0%)
	Median (Q1, Q3)	77.5 (67.3,88.2)	7.00 (5.53,8.05)	4.40 (2.90,6.00)	5.50 (4.00,6.80)	4.60 (3.10,6.10)	9.70 (8.90,10.9)	5.30 (3.60,7.10)
	Min, max	48.6, 110	1.90, 10.3	1.60, 11.1	1.80, 11.5	1.80, 12.2	6.10, 15.7	1.60, 110
Postmenstrual age at dosing (months)	Mean (SD%)	387 (94.6%)	14.3 (2.64%)	10.8 (2.20%)	11.9 (2.28%)	11.3 (2.48%)	23.8 (2.49%)	25.8 (72.1%)
	Median (Q1, Q3)	375 (305,460)	14.4 (12.4,16.6)	10.5 (9.00,12.3)	11.5 (10.0,13.3)	10.7 (9.20,12.9)	23.5 (21.8,25.4)	11.5 (9.60,13.9)
	Min, max	229, 606	8.60, 18.3	7.40, 19.7	8.20, 20.3	7.70, 19.8	19.4, 31.9	7.40, 606

Source: ASTR-CP-2207-MEDI8897-bla-EDA-plots-music-22Jul2022.Rmd

[a] There were 4 subjects with imputed baseline weight. D5290C00002 (N = 1), D5290C00003 (N = 2), MEDLEY Season 1 (N = 1).

Abbreviations: max=maximum; min=minimum; N=number of subjects with available information; PK=pharmacokinetic; Q1=first quartile; Q3=third quartile; SD=standard deviation

Source: Applicant's Population PK report, Table 7

Table 81. Summary of Baseline Demographic Categorical Covariates for Analysis

Covariate		D5290C00001 (N = 102)	D5290C00002 (N = 71)	D5290C00003 (N = 916)	MELODY (N = 963)	MEDLEY Season 1 (N = 593)	MEDLEY Season 2 (N = 191)	Total (N = 2836)
CHD/CLD	CHD	0 (0%)	0 (0%)	0 (0%)	0 (0%)	65 (11.0%)	58 (30.4%)	123 (4.3%)
	CLD	0 (0%)	0 (0%)	0 (0%)	0 (0%)	137 (23.1%)	132 (69.1%)	269 (9.5%)
	No CHD or CLD	102 (100%)	71 (100%)	916 (100%)	963 (100%)	391 (65.9%)	1 (0.5%)	2444 (86.2%)
ADA	Negative	87 (85.3%)	51 (71.8%)	874 (95.4%)	896 (93.0%)	558 (94.1%)	183 (95.8%)	2649 (93.4%)
	Positive	15 (14.7%)	20 (28.2%)	42 (4.6%)	67 (7.0%)	35 (5.9%)	8 (4.2%)	187 (6.6%)
Race	White	46 (45.1%)	8 (11.3%)	649 (70.9%)	510 (53.0%)	466 (78.6%)	160 (83.8%)	1839 (64.8%)
	Black or African American	56 (54.9%)	41 (57.7%)	184 (20.1%)	272 (28.2%)	56 (9.4%)	11 (5.8%)	620 (21.9%)
	Asian	0 (0%)	1 (1.4%)	4 (0.4%)	35 (3.6%)	36 (6.1%)	12 (6.3%)	88 (3.1%)
	American Indian or Alaskan Native	0 (0%)	1 (1.4%)	0 (0%)	56 (5.8%)	9 (1.5%)	0 (0%)	66 (2.3%)
	Native Hawaiian or Pacific Islander	0 (0%)	0 (0%)	8 (0.9%)	6 (0.6%)	3 (0.5%)	0 (0%)	17 (0.6%)
	Multiple	0 (0%)	2 (2.8%)	11 (1.2%)	12 (1.2%)	6 (1.0%)	3 (1.6%)	34 (1.2%)
	Other	0 (0%)	18 (25.4%)	59 (6.4%)	69 (7.2%)	17 (2.9%)	5 (2.6%)	168 (5.9%)
	Missing	0 (0%)	0 (0%)	1 (0.1%)	3 (0.3%)	0 (0%)	0 (0%)	4 (0.1%)
Japanese	Japanese	0 (0%)	0 (0%)	0 (0%)	32 (3.3%)	24 (4.0%)	10 (5.2%)	66 (2.3%)

	Non-Japanese	102 (100%)	71 (100%)	916 (100%)	931 (96.7%)	569 (96.0%)	181 (94.8%)	2770 (97.7%)

Source: ASTR-CP-2207-MEDI8897-bla-EDA-plots-music-22Jul2022.Rmd
Notes: Numeric columns formatted as count (% of total). One MEDLEY Season 2 subject (20047730001) was not CHD or CLD (Down's syndrome) and was excluded for extrapolation.
Abbreviations: ADA=antidrug antibody; CHD=congenital heart disease; CLD=chronic lung disease; N=number of subjects with available information

Source: Applicant's Population PK report, Table 8

Base Model

The final base model was a linear two-compartment PK model, first-order absorption, and first-order elimination from the central compartment. The effect of weight was included as a fixed allometric exponent on CL/F, Vc/F, Vp/F, and Q/F and the effect of maturation on CL was modeled using an asymptotic exponential function:

$$CL_i = CL_{pop} * \left(\frac{WT_i}{70}\right)^{\theta 1} * \left(1 - (1 - \beta_{CL}) * e^{\left(-\left(PAGE_i - \left(\frac{40}{4.35}\right)\right) * \frac{LN(2)}{T50_{CL}}\right)}\right) * e^{\eta_{CL}}$$

where CL_i is the individual CL and CL_{pop} is the typical clearance. β_{CL} denotes the fractional change in the clearance of a premature infant with respect to a term infant, $T50_{CL}$ denotes the corresponding maturation half-life of the parameter with respect to that of an adult, $PAGE_i$ represents the sum of gestational age and postnatal age in months for each infant, ηCL is the individual clearance between-subject variability, θ_1 is the estimated clearance allometric scaling exponent, and gestational age for adults was imputed to 40 weeks.

Inter-individual variability (IIV) was modelled assuming a log-normal distribution for patient level random effects. Residual variability was modeled as additive plus proportional on the dependent variable. Model evaluation and selection of the base model were based on standard statistical criteria of goodness-of-fit such as a decrease in the minimum objective function value (OFV), accuracy of parameter estimation (i.e., 95% confidence interval excluding 0), successful model convergence, and diagnostic plots.

Covariate Analysis

Covariate parameters, including body weight, postmenstrual age, race, Japanese ethnicity, chronic lung disease, chronic heart disease, and ADA were evaluated.

The Applicant had the following conditions if a covariate were to be included in the model:

- The covariate was available in at least 80% of subjects.
- For categorical covariates, a minimum number of 15 subjects was in each category.
- If covariates (other than weight and age, which were included a priori) show a correlation of >0.5, only one of the correlated covariates was included in the formal analysis. This was either the covariate with the strongest influence, as determined by exploratory graphical analysis or the variable that was most meaningful from a clinical, biological, or practical perspective. Continuous covariates were preferred over categorized covariates with the same meaning.

The previous popPK model included effects of body weight on CL and V, postmenstrual age on CL, race effects on CL, and ADA effect on CL. The covariate selection was performed using a backward elimination process. The likelihood ratio test was used to evaluate the significance of incorporating or removing fixed effects into the population model based on significance level that were set a priori at a significance level 0.001. During the backward elimination process, covariates were removed from the model one at a time if their deletion led to insignificant model deterioration. The most insignificant covariate was removed first, and the procedure was repeated until no further insignificant covariate relationship was detected. Following backward deletion, sensitivity analyses including univariate testing of selected covariates (CHD, CLD), Japanese origin, and ADA (time-varying) were conducted. The likelihood ratio test was used to evaluate the significance of incorporating fixed effects into the population model based on significance level that were set a priori at a significance level 0.001.

14.5.1.4. Final Model

The parameter estimates for the final covariate model are listed in Table 82. The goodness-of-fit plots for the final covariate model for all data are shown in Figure 15 and Figure 16. The Visual Predictive Check (VPC) plots for the final covariate model is shown in Figure 17 for all data stratified by trial, in Figure 18 stratified by dose and in Figure 19.

Table 82. Parameter Estimates, RSE and Bootstrap (95% CI) for the Final Model

Parameters	Estimates	%RSE	Bootstrap 95% CI
Clearance (CL, mL/day) [a]	38.8	6	29.9, 43.9
Central volume of distribution (V2, mL) [a]	1980	10	567, 2760
Intercompartmental clearance (Q, mL/day) [a]	709	9	462, 872
Peripheral volume of distribution (V3, mL) [a]	2400	5	1980, 2790
Absorption rate constant (KA, day^{-1}) [b]	0.401	7	0.206, 0.504
Bioavailability (F)	0.839	7	0.627, 0.939
Fractional clearance (BETACL) [c]	0.364	6	0.312,3 0.416
Maturation half-life (TCL, months) [c]	14.8	9	10.9, 20.0
Body weight effect on clearances (CL, Q) [d]	0.589	3	0.526, 0.646
Body weight effect on volumes (V2, V3) [d]	0.84	1	0.806, 0.863
Race effect on clearance (Black or African American, Other) [e]	CL_{pop}* (1 + 0.132)	8	0.111, 0.153
Race effect on clearance (Asian, Ame.Ind. or Ala.Nat., Multiple races) [e]	CL_{pop}* (1 - 0.0894)	30	-0.123, -0.0545
Race effect on volume of distribution (Asian, Ame.Ind. or Ala.Nat., Multiple races) [e]	$V2_{pop}$* (1 - 0.226)	24	-0.580, -0.141
Season 2 effect on clearance	CL_{pop}* (1 - 0.122)	7	-0.159, -0.0825
Categorical ADA effect (yes or no per subject) on CL	CL_{pop}* (1 + 0.124)	12	0.0890, 0.158
Random Effects	**Estimates (%CV)**	**%RSE [%Shrinkage]**	**Bootstrap 95% CI**
IIV on CL	26	2 [10%]	24, 27
ηV2-ηCL correlation	r = 0.785	-	
IIV on V2	43	4 [29%]	32, 90
IIV on KA	44	8 [83%]	16, 78
Residual Error	**Estimates**	**%RSE**	
Proportional error	21%	1	20, 22

Source: run443.lst, sumo-run443.txt

[a] Parameter estimates for a 70 kg adult. The derived parameters for an infant of 5 kg, 11.1 months postmenstrual age are CL = 3.42 mL/day, V2 = 216 mL, Q = 150 mL/day, and V3 = 261 mL.

[b] Absorption $t_{½}$ (ln(2)/KA) = 1.7 days.

[c] Maturation function: 1-(1-BETACL) *exp (-((postmenstrual age) -(40/4.35)) *log(2)/TCL)

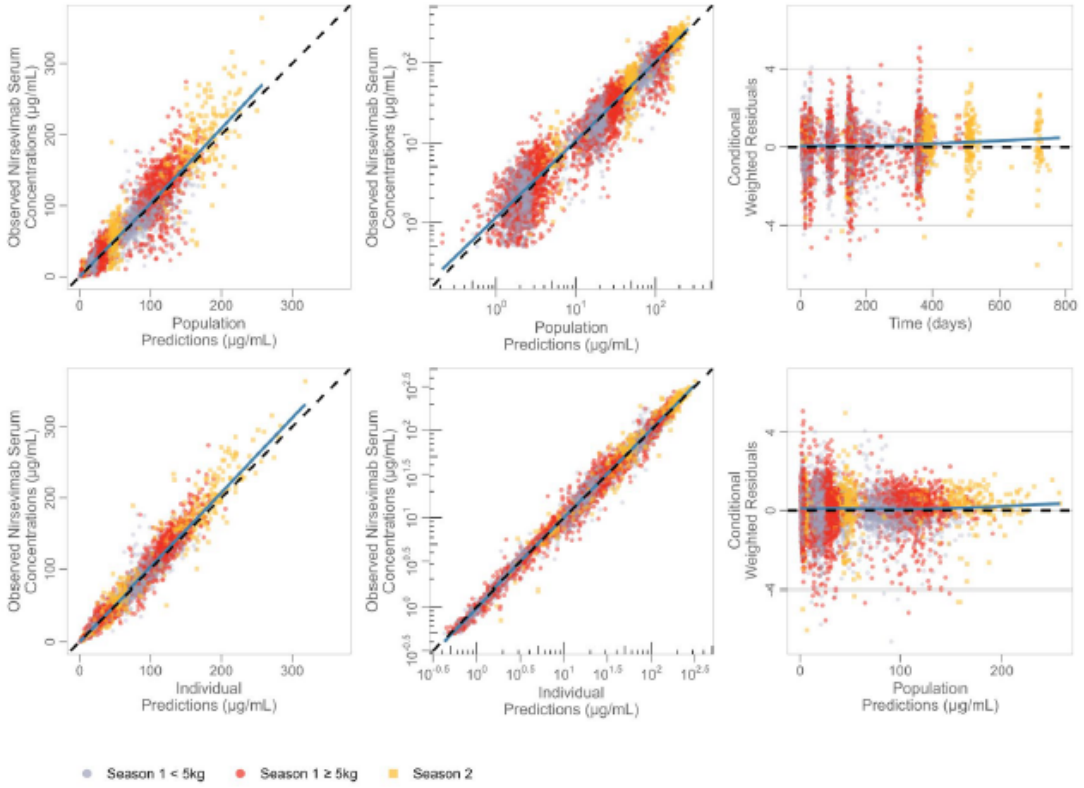
[d] Weight effect function: $\left({}^{WT}/_{70}\right)^{parameter\ estimate}$

[e] Reference group is White or Native Hawaiian/Pacific Islander.

Abbreviations: η=eta; %CV=percent coefficient of variation; %RSE=percent relative standard error; ADA=antidrug antibody; CI=confidence interval; CL_{pop}=typical CL; IIV=inter-individual variability; r=correlation coefficient; $t_{½}$=half-life; $V2_{pop}$=typical V2; V2=Vc = central volume; V3=Vp = peripheral volume, WT=body weight

Source: Applicant's Population PK report, Table 9

Figure 15. Goodness-of-Fit Plots for Pediatric Subjects in the Final Covariate Model by Weight Group and Season



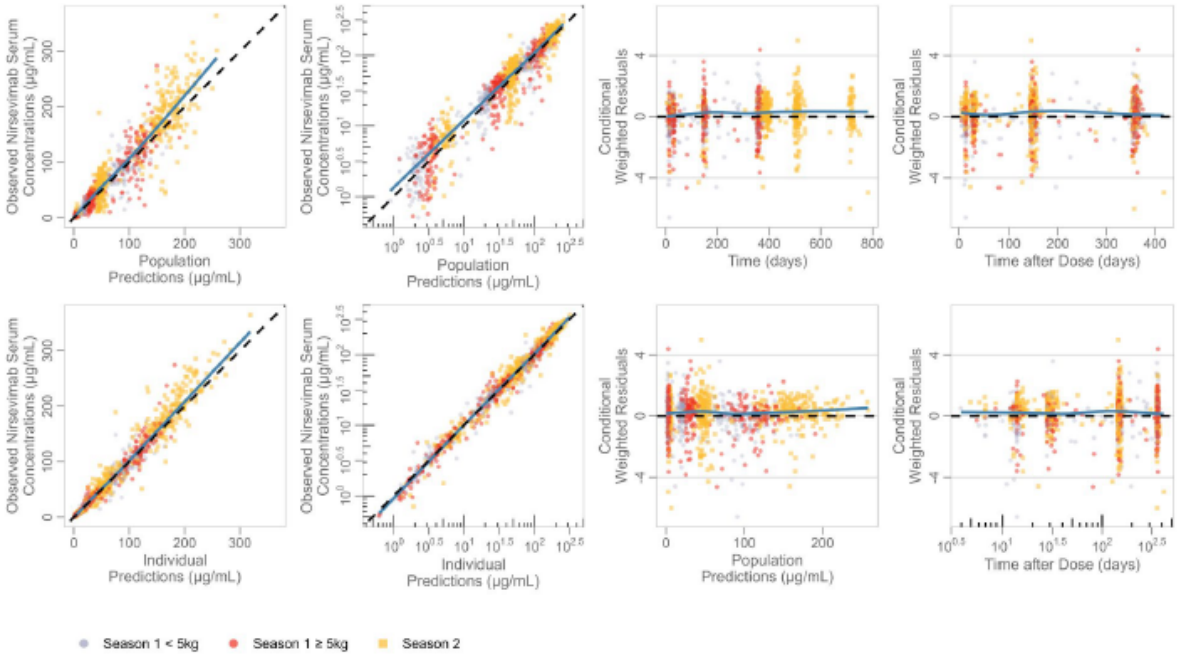
Source: ASTR-NIRSE-run-plots-bla-Aug2022.R (run443)

Notes: Dots are individual data points for pediatric subjects, and solid blue lines are smoothed LOESS lines. The dashed lines in columns 1 and 2 are lines of identity. In the 2 plots on the right, horizontal lines are reference lines.

Abbreviations: GOF=goodness-of-fit; LOESS=locally weighted smoothing

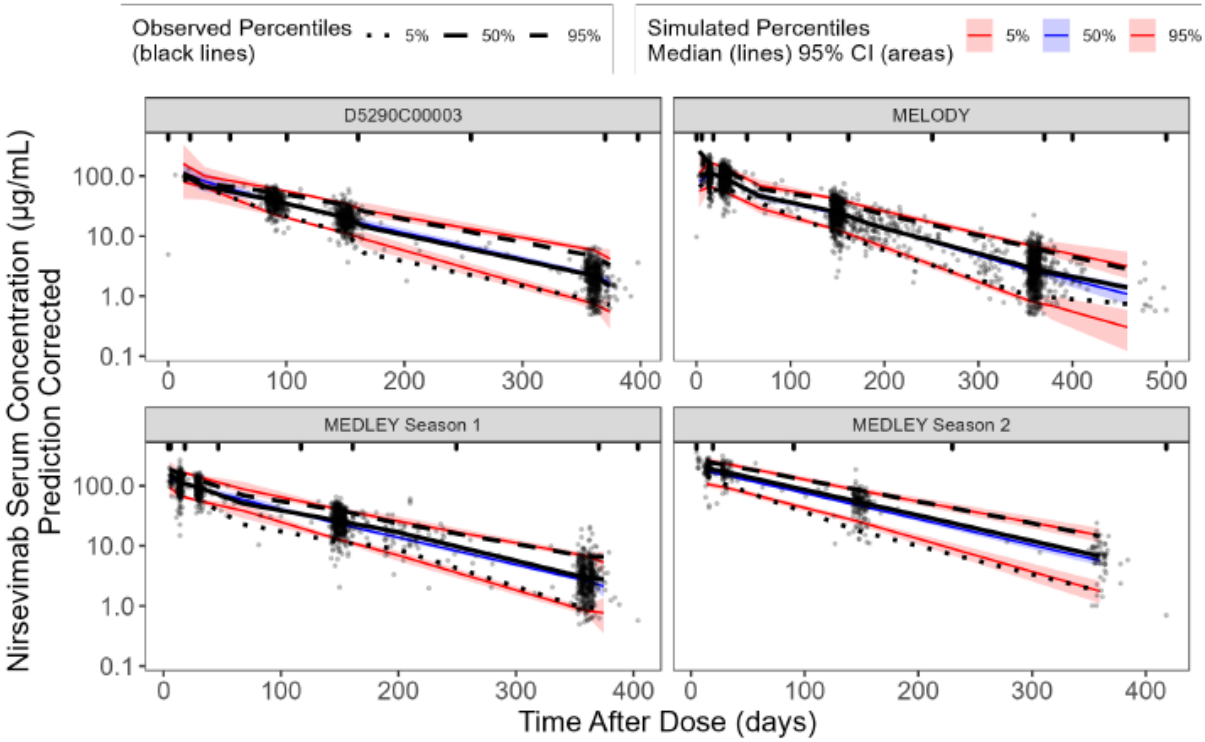
Source: Applicant's Population PK report, Figure 6

Figure 16. Goodness-of-Fit Plots for Pediatric Subjects With CHD/CLD in the Final Covariate Model by Weight Group and Season



Source: ASTR-NIRSE-run-plots-bla-Aug2022.R (run443)
Notes: Dots are individual data points for pediatric, and solid blue lines are smoothed LOESS lines. The dashed lines in columns 1 and 2 are lines of identity. In the 2 plots on the right, horizontal lines are reference lines.
Abbreviations: CHD=congenital heart disease; CLD=chronic lung disease; GOF=goodness-of-fit; LOESS=locally weighted smoothing
Source: Applicant's Population PK report, Figure 7

Figure 17. Prediction-Corrected VPC Plots for Final Covariate Model Stratified by Trial



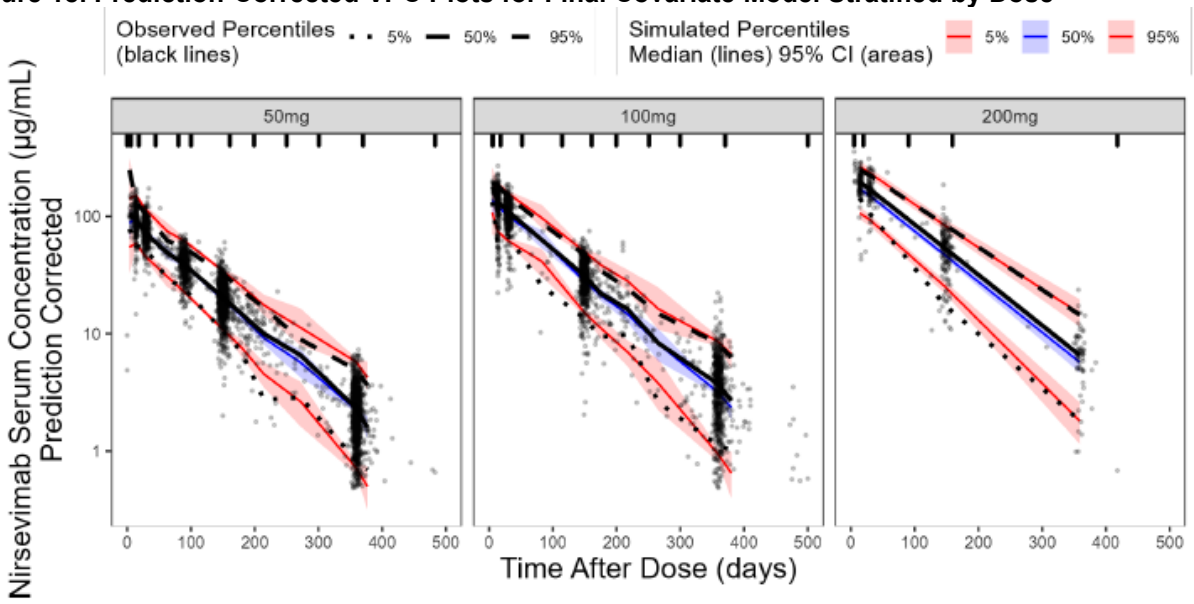
Source: ASTR-NIRSE-run-plots-bla-Aug2022.R (run443)

Notes: Black dots are observed data points for pediatric subjects with weight <5kg receiving 50mg, weight ≥5kg receiving 100mg in Season 1, or subjects receiving 200mg in Season 2. Black solid line is the observed median. Black dotted and dashed lines are observed 5th and 95th percentiles. The blue shaded area is the 95% PI of the simulated median (blue line), and pink shaded areas are the 95% PI of the simulated 5th and 95th percentiles (red lines). Black tick marks at the top of each panel denote bins.

Abbreviations: CI=confidence interval; PI=prediction interval; VPC=visual predictive check

Source: Applicant's Population PK report, Figure 15

Figure 18. Prediction-Corrected VPC Plots for Final Covariate Model Stratified by Dose



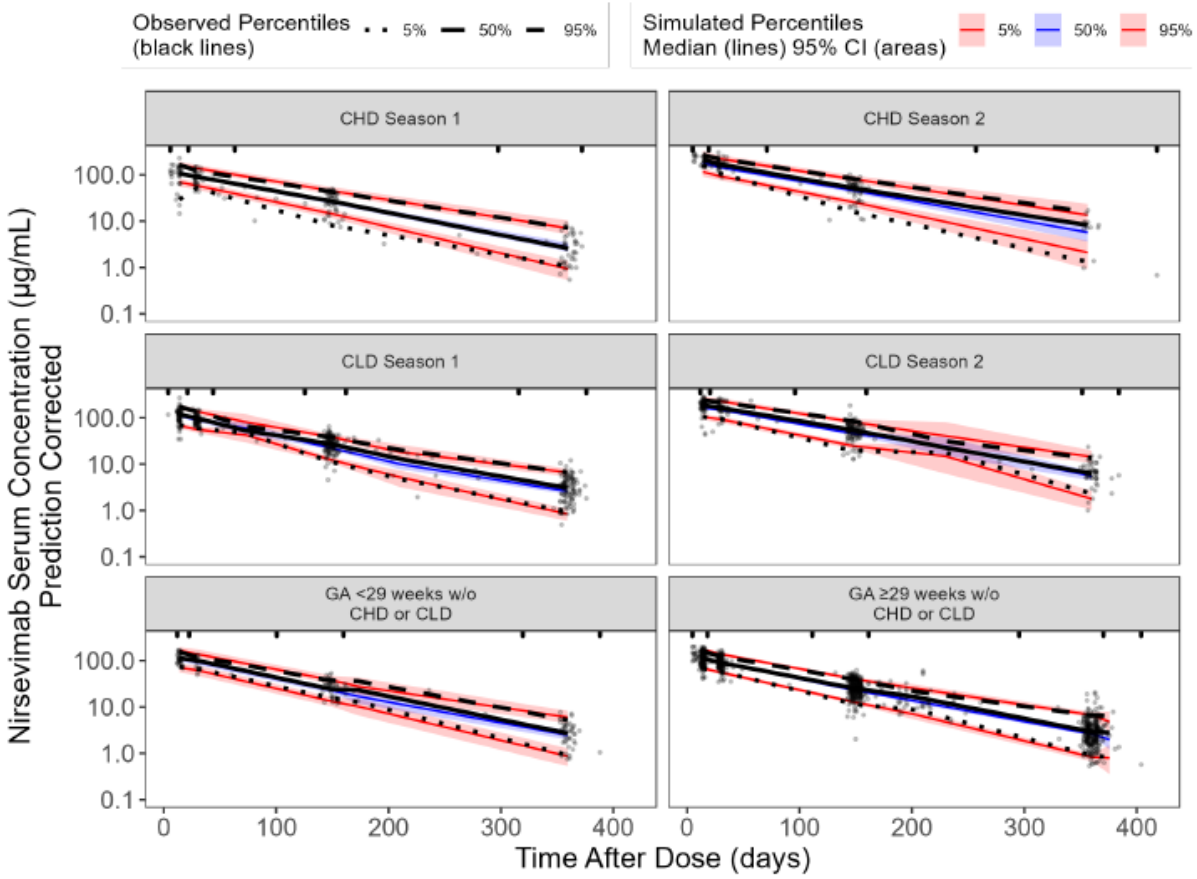
Source: ASTR-NIRSE-run-plots-bla-Aug2022.R (run443)

Notes: Black dots are observed data points for pediatric subjects with weight <5kg receiving 50mg, weight ≥5kg receiving 100mg in Season 1, or subjects receiving 200mg in Season 2. black solid line is the observed median. Black dotted and dashed lines are observed 5th and 95th percentiles. The blue shaded area is the 95% PI of the simulated median (blue line), and pink shaded areas are the 95% PI of the simulated 5th and 95th percentiles (red lines). Subjects with weight >5kg receiving 50mg and weight <5kg receiving 100mg are not included.

Abbreviations: CI=confidence interval; PI=prediction interval; VPC=visual predictive check

Source: Applicant's Population PK report, Figure 16

Figure 19. Prediction-Corrected VPC Plots for Final Covariate Model Stratified by Subgroups and RSV Season in Trial 05



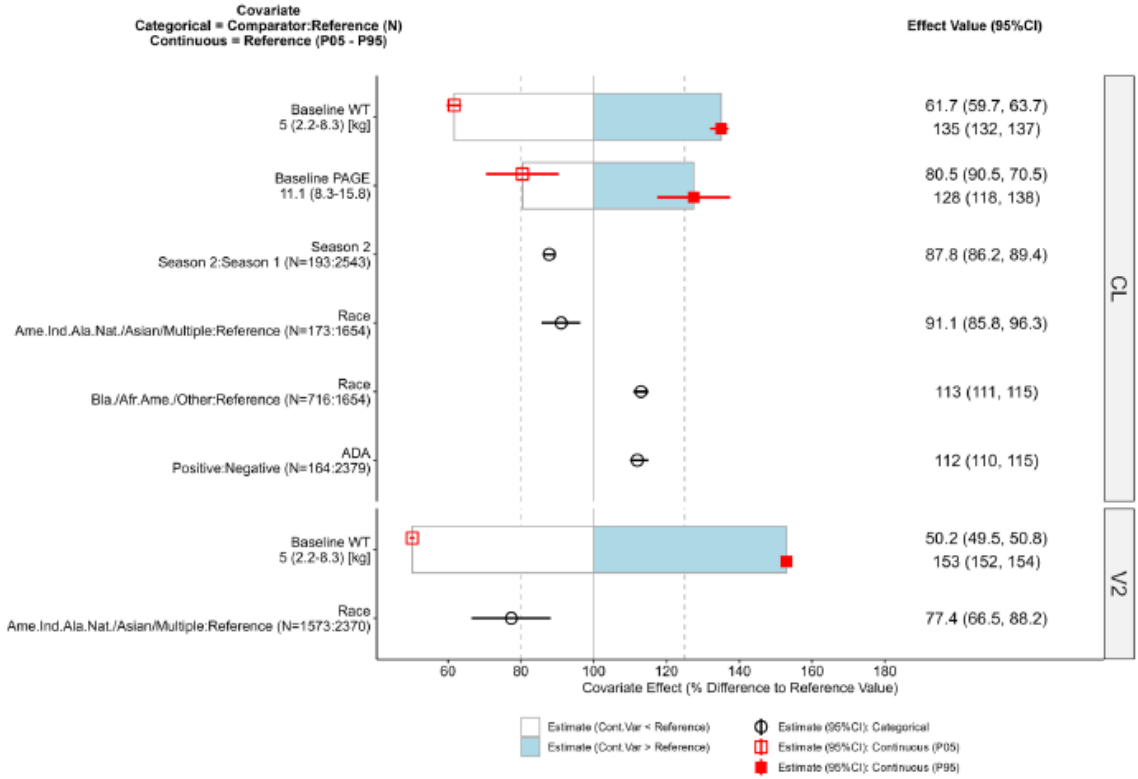
Source: ASTR-NIRSE-run-plots-bla-Aug2022.R (run443)

Notes: Black dots are observed data points for pediatric subjects with weight <5kg receiving 50mg, weight ≥5kg receiving 100mg in Season 1, or subjects receiving 200mg in Season 2. Black solid line is the observed median. Black dotted and dashed lines are observed 5th and 95th percentiles. The blue shaded area is the 95% PI of the simulated median (blue line), and pink shaded areas are the 95% PI of the simulated 5th and 95th percentiles (red lines). Subjects with weight >5kg receiving 50mg and weight <5kg receiving 100mg are not included. The panel for GA <29 weeks w/o CHD or CLD contains only Season 1 subjects. The panel for GA ≥29 weeks w/o CHD or CLD contains Season 1 subjects and a single subject from Season 2 ([redacted] (b) (6)) without CHD or CLD,

Abbreviations: CHD=congenital heart disease; CI=confidence interval; CLD=chronic lung disease; GA=gestational age; PI=prediction interval; VPC=visual predictive check; w/o=without

Source: Applicant's Population PK report, Figure 17

Figure 20. Covariate Effects in Pediatric Subjects for the Final PPK Model

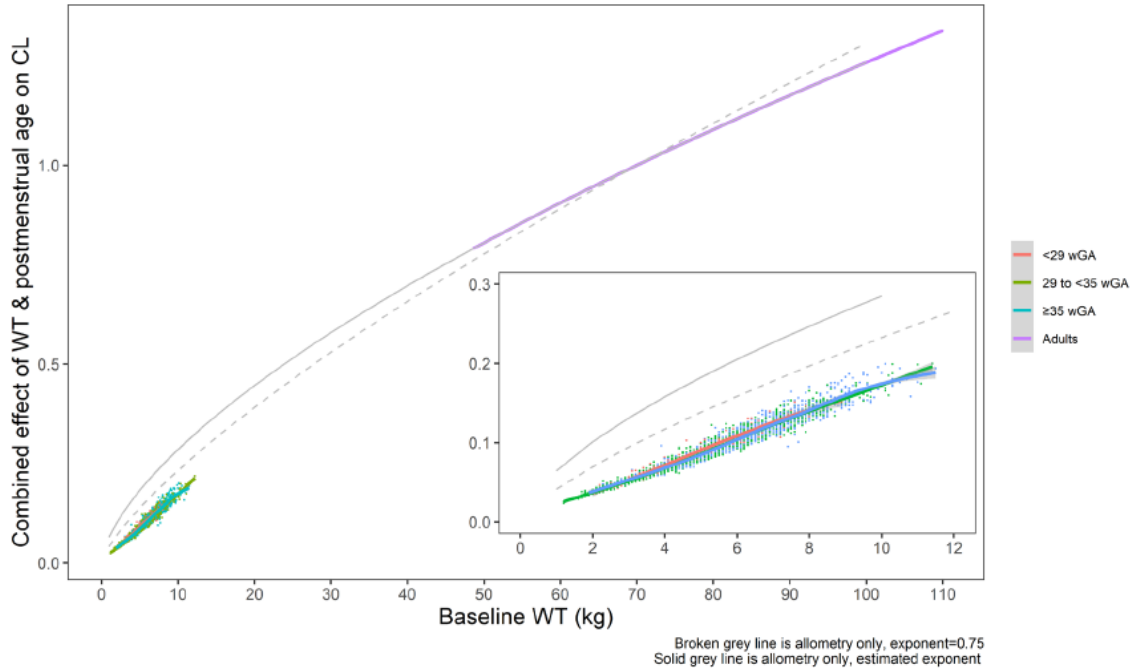


Source: ASTR-NIRSE-run-plots-bla-Aug2022.R (run443)
Notes: Vertical dashed lines are the range of effect considered to be clinically insignificant. Reference race= White or Native Hawaiian/Pacific Islander;
Abbreviations: ADA=antidrug antibody; Ame.Ind./Ala.Nat.=American Indian or Alaskan Native; Bla./Afr.Ame.=Black or African American; CI=confidence interval; CL=clearance; N=number of subjects with available information; P05=5th percentile; P95=95th percentile; PAGE=postmenstrual age; popPK=population pharmacokinetic; V2=Vc=central volume of distribution; WT=body weight; P05=5th percentile, P95=95th percentile

Source: PopPK analysis report 2022 Figure 10

In infants and neonates, the addition of a maturation effect on CL was important for the assessment of nirsevimab CL. The Applicant's plot in Figure 21 illustrates the allometric relationship for CL as a function of weight shown by the solid line and CL values for adults (in purple). The dashed line represents the common allometric relationship with a fixed exponent for clearance of 0.75. Whereas the blue points indicate the CL versus BW relationship in pediatric subjects. In addition to a weight relationship, the maturation effect based on postmenstrual age was incorporated for pediatric subjects as noted above in the model equation describing nirsevimab CL (see the Base Model Section). This maturation of CL helped explain the difference in part from traditional and previously developed allometric relationships for nirsevimab in older subjects.

Figure 21. Weight and Age Effect on Nirsevimab CL for Infants and Adults

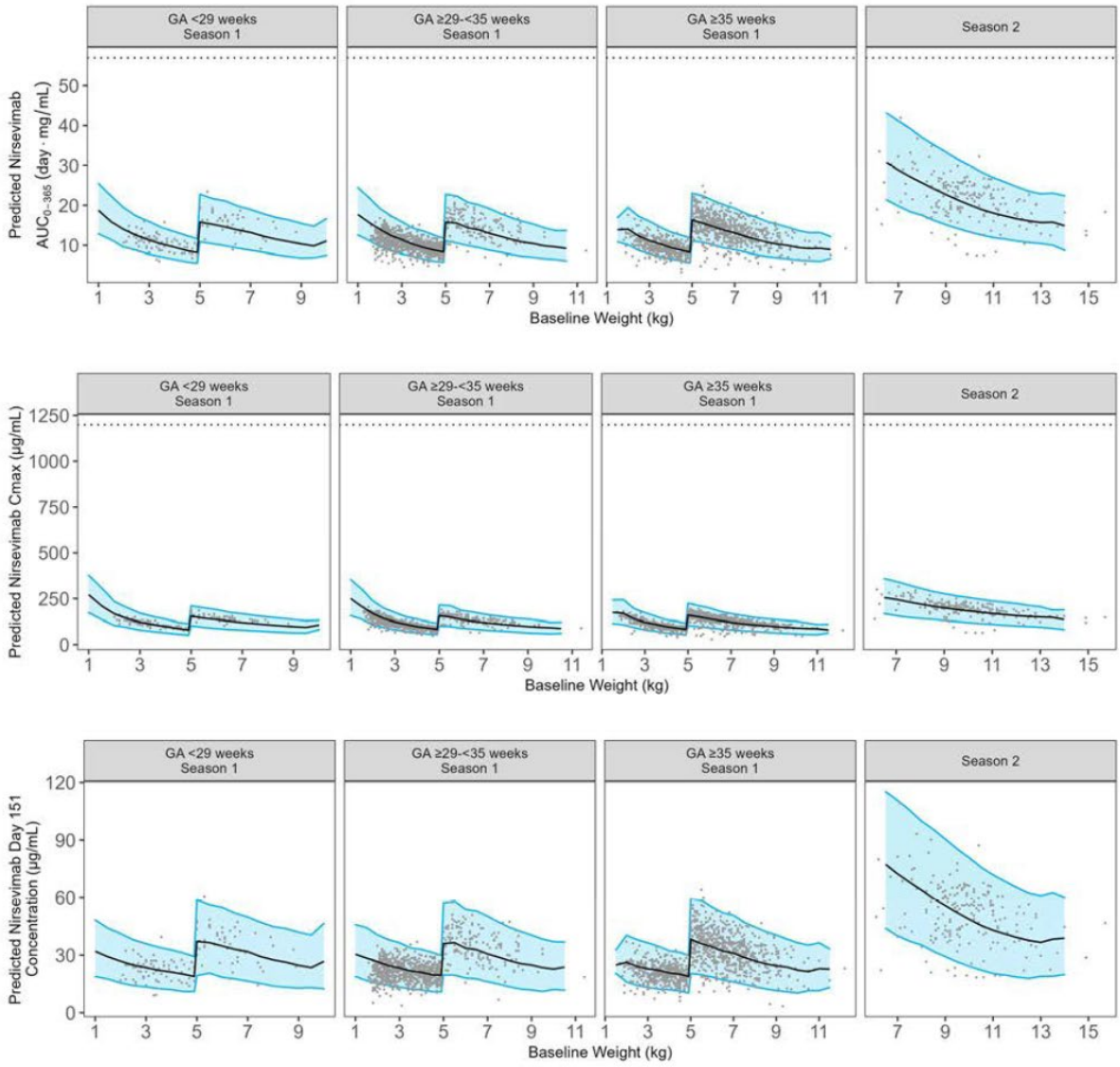


Source: ASTR-NIRSE-run-plots.R
Notes: Dots are population predicted effects for infants in studies D5290C00003, MELODY, and MEDLEY, colored by GA group, and solid colored lines are smoothed LOESS lines. The solid gray line is the effect of body weight alone, the broken gray line is body weight effect based on a fixed 0.75 exponent.
Abbreviations: CL=clearance; wGA=weeks gestational age; LOESS=locally weighted smoothing; WT=weight

Source: Applicant's Population PK Model Report Erratum, Figure 21.

Figure 22 depicts nirsevimab exposures (AUC- top row, C_{max} – middle row, and Day 151 concentration – bottom row) stratified by age (indicated for each panel) and plotted against weight (x-axis for each plot). The dashed reference line is the upper limit of exposures observed in adults. Increases in exposures at 5 kg are for the dose increase from 50 mg to 100 mg for patients over 5 kg. Season 2 also has higher exposures related to the 200 mg dose. The effect of maturation or chronological age can also be seen further in Figure 23 in looking across each panel over the same age range. Caution should be used when interpreting this plot as the Panels and x-axis are the same type of variable.

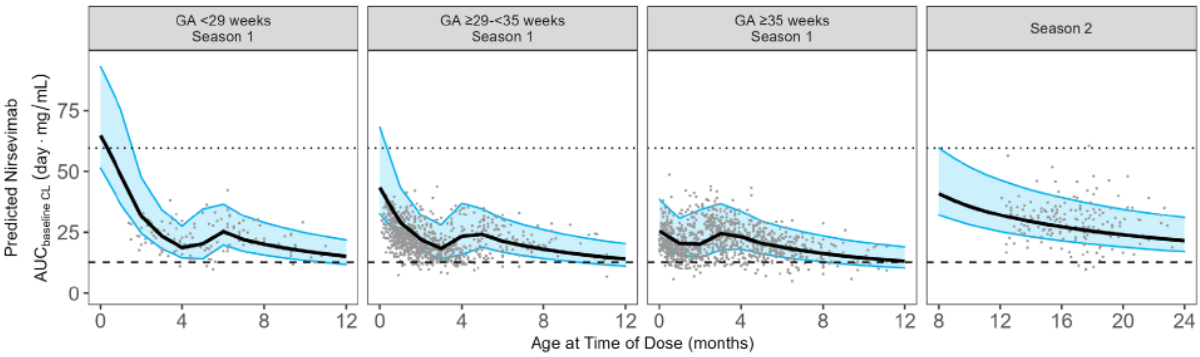
Figure 22. Predicted Exposure Versus Weight at the Time of Dose by Gestational Age Group and Season With 90% Prediction Intervals (Blue)



Blue bands are covering the 5th to 95th percentiles of the predictions for weight-band dosing in Season 1, 200 mg in Season 2. Solid black lines are the predicted medians. Grey dots are individual predictions based on post-hoc parameters from the final model for subjects in Study 3 < 5kg, MELODY and MEDLEY. Dotted black lines are the median post hoc predicted exposure achieved in adults following 3000 mg IV dose (AUC_{0-365}: 57.0 day*mg/mL; C_{max}: 1140 µg/mL).
AUC_{0-365} = predicted area under the serum concentration-time curve from Days 0 to 365 derived using densely predicted concentration-time curves from the final model; C_{max} = maximum serum concentration; GA = gestational age.

Source: Applicant's Summary of Clinical Pharmacology, Figure 4.

Figure 23. Predicted $AUC_{baselineCL}$ vs. Age at the Time of Dose by Gestational Age Group and Season With 90% Prediction Intervals (Blue)



Notes: Season 2 simulations assume no prior dosing in Season 1. Data presented is based on simulated virtual population with weight band dosing. Blue bands are the 20% to 90% prediction intervals. Solid black lines are the predicted medians for weight-band dosing. Grey dots are individual AUC derived from posthoc CL at time of dose and dose, for subjects in Study D5290C00003 <5kg, MELODY and MEDLEY. Dashed black line is the AUC Exposure-Response threshold (12.8 day•mg/mL). Dotted black line is the median $AUC_{baseline\ CL}$ achieved in adults (59.5 day•mg/mL following 3000 mg IV dose).

Abbreviations: AUC=area under the serum concentration-time curve derived from dose and CL values at the time of dose for typical subjects with between-subject variability from the final population pharmacokinetics model; CL=clearance; GA=gestational age

Source: Applicant's Population PK Report, Figure 23.

Reviewer's Comments: *The Applicant's population PK model is acceptable for describing exposures at studied and proposed dose levels, in Trials 03, 04, and 05, to directly support the extrapolation of efficacy from the population in Trials 03 & 04 to the populations in Trial 05. In particular the exposures at the proposed doses in Trial 05 appear to be comparable or at least as high as those in Trial 04 and for most subjects are higher than the target AUCbaselineCL value (See Table 83 through Table 85 below and Figure 24 and Figure 25 for these results below). As the therapeutic concentrations are believed to be similar between the trials 03 & 04 populations compared to the Trial 05 population, matching exposures at the proposed dosing regimen is necessary to bridge efficacy from one population to the other. The Applicant's model appears to capture the central tendency of the data and shrinkage values are low supporting the estimation of individual PK values. Thus, the model adequately supports the exposure matching approach to bridge efficacy to the Trial 05 population.*

The Applicant's population PK model is also acceptable for describing the PK of nirsevimab in the label at a population level and for covariate assessment as well as generating individual exposures for exposure-response analyses. The database was robust with over 9000 samples and a range of doses from 10 mg to 3000 mg. The RSE estimates, bootstrap 95% CI and shrinkage values for CL were reasonable and suggest that both the central tendency of the data and the between subject variability are reliably estimated.

Race was evaluated as a covariate on CL and included in the final model as a statistically significant variable with about a 9 percent reduction in CL for pacific ethnicities and regions versus a 13 percent increase for black or African Americans. Table 82 indicates the robustness of data across each of these groups. (b) (4)

Nirsevimab Exposure Comparisons to Support Extrapolation of Efficacy to High-Risk Pediatric Subjects

The Applicant's final PPK model was utilized to summarize the exposures observed in pediatric subjects across different trials at the studied doses. These exposure comparisons are essential to establish that the proposed dosing regimen achieves similar exposures in the Trial 05 population as those in the Trials 03 & 04 populations from which efficacy is being extrapolated. Details of this extrapolation are discussed further in Sections 1, 2.2, 3.2 and 6. Exposures for these comparisons are shown below in Table 83, Table 84, Table 85 and Figure 24 and Figure 25.

Table 83. Summary of Post Hoc Predicted PK Parameters for the Final popPK Model

Parameter		D5290C00003 (N = 542)	MELODY (N = 954)	MEDLEY Season 1 (N = 590)	MEDLEY Season 2 (N = 189)	Total (N = 2275)
Clearance at dosing (mL/day)	Mean (SD)	2.52 (0.781)	4.03 (1.80)	3.40 (1.61)	7.46 (2.64)	3.79 (2.08)
	Median [min, max]	2.43 [1.21, 6.05]	3.65 [1.03, 19.4]	3.09 [1.19, 14.3]	6.68 [3.31, 20.0]	3.27 [1.03, 20.0]
	Geo. mean (Geo. CV%, Geo. SD)	2.41 (30.8%, 1.35)	3.71 (41.3%, 1.49)	3.11 (43.4%, 1.51)	7.11 (30.4%, 1.35)	3.38 (49.4%, 1.60)
Total volume at dosing (mL)	Mean (SD)	332 (91.6)	519 (187)	450 (190)	860 (278)	485 (225)
	Median [min, max]	320 [170, 789]	492 [198, 1740]	425 [198, 2670]	794 [507, 2480]	439 [170, 2670]
	Geo. mean (Geo. CV%, Geo. SD)	320 (27.4%, 1.31)	491 (34.1%, 1.39)	421 (36.4%, 1.42)	830 (25.5%, 1.28)	445 (42.2%, 1.50)
Predicted terminal half-life (days)	Mean (SD)	71.0 (10.4)	70.7 (11.4)	72.9 (12.3)	71.2 (10.9)	71.4 (11.4)
	Median [min, max]	70.5 [34.6, 108]	70.7 [28.3, 148]	73.0 [31.2, 153]	73.2 [41.2, 103]	71.5 [28.3, 153]
	Geo. mean (Geo. CV%, Geo. SD)	70.2 (15.0%, 1.16)	69.8 (16.7%, 1.18)	71.9 (17.0%, 1.18)	70.3 (16.3%, 1.18)	70.5 (16.4%, 1.18)
Predicted C_{max} (µg/mL)	Mean (SD)	118 (29.2)	120 (28.0)	123 (27.1)	194 (42.2)	127 (35.9)
	Median [min, max]	115 [48.5, 205]	118 [40.9, 193]	123 [29.2, 190]	198 [64.1, 315]	122 [29.2, 315]
	Geo. mean (Geo. CV%, Geo. SD)	115 (25.5%, 1.29)	116 (25.0%, 1.28)	120 (24.2%, 1.27)	189 (25.5%, 1.28)	122 (28.5%, 1.32)
Predicted AUC_{0-365} (day•mg/mL)	Mean (SD)	10.4 (2.17)	12.2 (3.55)	12.3 (3.34)	21.5 (5.52)	12.6 (4.44)
	Median [min, max]	10.2 [4.87, 17.2]	11.8 [3.31, 24.9]	11.8 [4.14, 23.4]	21.8 [7.45, 41.9]	11.6 [3.31, 41.9]
	Geo. mean (Geo. CV%, Geo. SD)	10.1 (21.7%, 1.24)	11.7 (30.6%, 1.35)	11.8 (28.4%, 1.32)	20.8 (28.9%, 1.33)	11.9 (33.7%, 1.39)

Parameter		D5290C00003 (N = 542)	MELODY (N = 954)	MEDLEY Season 1 (N = 590)	MEDLEY Season 2 (N = 189)	Total (N = 2275)
$AUC_{baseline\ CL}$ (day•mg/mL)	Mean (SD)	21.7 (6.47)	21.3 (6.50)	22.6 (6.22)	29.5 (8.19)	22.4 (6.93)
	Median [min, max]	20.5 [8.27, 41.4]	20.4 [5.16, 48.7]	22.3 [6.98, 43.8]	30.0 [9.75, 60.4]	21.6 [5.16, 60.4]
	Geo. mean (Geo. CV%, Geo. SD)	20.7 (30.8%, 1.35)	20.4 (32.0%, 1.37)	21.7 (29.7%, 1.34)	28.3 (30.5%, 1.35)	21.4 (32.3%, 1.37)

Source: ASTR-NIRSE-run-plots-bla-Feb2023.R (run443)
Notes: Sixteen samples with |CWRES| >5 were removed in the final model. Only subjects pediatric subjects with weight <5kg receiving 50mg, weight ≥5kg receiving 100mg in Season 1, or subjects receiving 200mg in Season 2 are included.
Abbreviations: AUC0-365=predicted area under the serum concentration-time curve from Days 0 to 365 derived using densely predicted concentration-time curves from the final model; AUCbaseline CL =area under the serum concentration-time curve derived from post hoc clearance values at dosing from the final popPK model; C_{max}=maximum plasma concentration; CV=coefficient of variation; |CWRES| >5=the absolute value of the associated conditional weighted residual is greater than 5; geo.=geometric; max=maximum; min=minimum; N=number of subjects with available information; PK=pharmacokinetic; popPK=population PK; SD=standard deviation

Source: Applicant's Population PK report erratum, Table 10

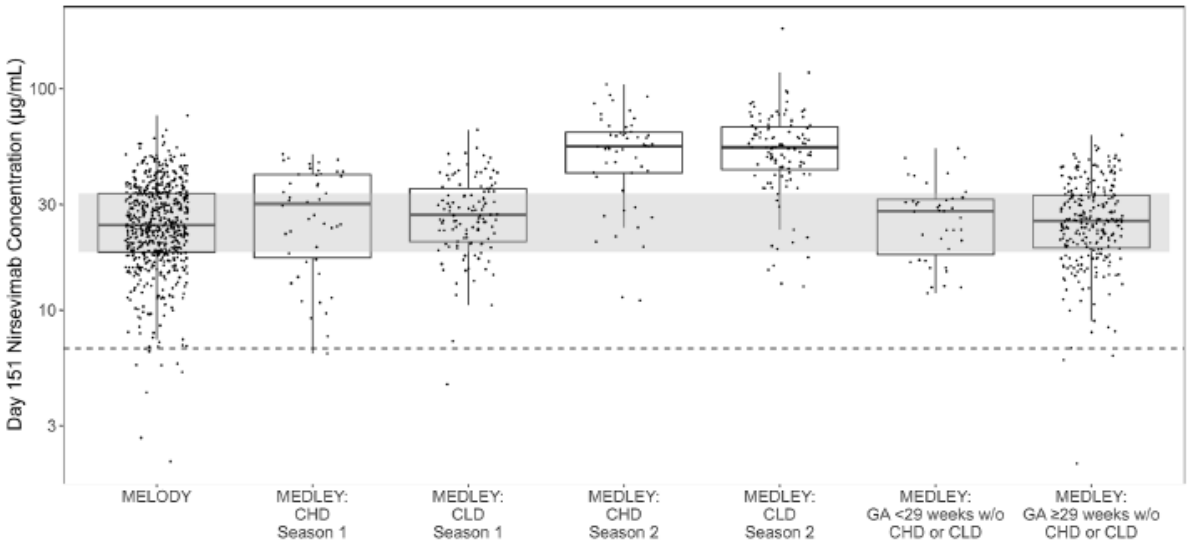
Table 84. Trial 05 Subgroups: Percent of Subjects With $AUC_{baselineCL}$ Above Target in Season 2

	CHD (N=58)	CLD (N=132)	Total (N=190)
$AUC_{baseline\ CL}$ ≥ Target	58 (100%)	129 (97.7%)	187 (98.4%)
$AUC_{baseline\ CL}$ < Target	0 (0%)	3 (2.3%)	3 (1.6%)

Source: ASTR-NIRSE-run-plots-bla-Feb2023.R (run443)
Notes: The target exposure 12.8 day·mg/mL, CHD group includes 9 subjects with CHD/CLD. One MEDLEY Season 2 subject ([redacted] (b) (6)) was not CHD or CLD (Down's Syndrome) and was excluded for extrapolation.
Abbreviations: $AUC_{baseline\ CL}$ =area under the serum concentration-time curve derived from post hoc clearance values at dosing from the final population pharmacokinetics model; CHD=congenital heart disease; CL=clearance; CLD=chronic lung disease; N=number of subjects with available information

Source: Applicant's Population PK Report erratum, Table 13

Figure 24. Boxplots of Model-Derived Day 151 Serum Concentrations in Trial 04 (MELODY) Subjects Compared to Trial 05 (MEDLEY)



Source: ASTR-NIRSE-run-plots-bla-Aug2022.R (run443)
Notes: Day 151 concentrations are Visit day 151 ± 14 days. Grey band is the reference inter-quartile range for MELODY Day 151. The horizontal dashed black line is the *preclinical* EC90 (6.8 µg/mL). Data presented are pediatric subjects who are <5kg receiving 50mg, ≥5kg receiving 100mg in Season 1, and/or receiving 200mg in Season 2. One MEDLEY Season 2 subject ([redacted] (b) (6)) was not CHD or CLD (Down's syndrome) and was excluded for extrapolation. The 2 groups on the right, MEDLEY: GA <29 weeks w/o CHD or CLD and MEDLEY: GA ≥29 weeks w/o CHD or CLD, contain only Season 1 subjects.
Abbreviations: CHD=congenital heart disease; CLD=chronic lung disease; EC90=90% effective concentration; GA=gestational age

Source: Applicant's Population PK report, Figure 28

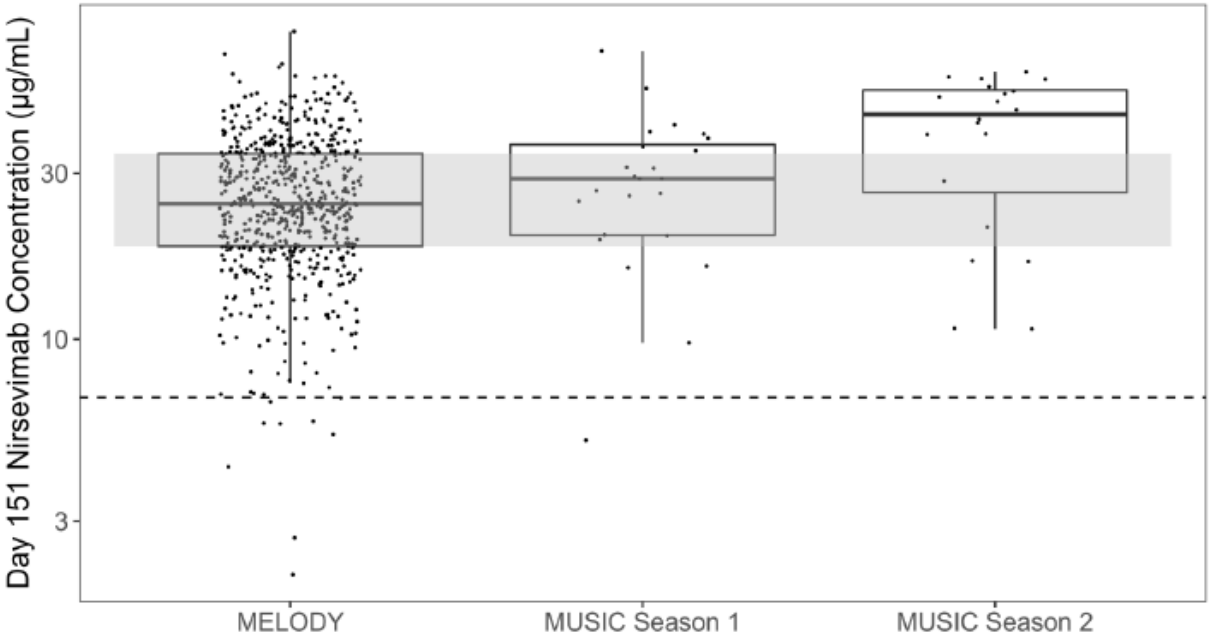
Table 85. Percentage of Pediatric Subjects in Trial 08 with AUC $_{baselineCL}$ values greater than the AUC target in Season 1 and Season 2.

	Season 1 (N = 35)	Season 2 (N = 24)	Total (N = 59)
$AUC_{baseline\ CL}$ ≥ Target	28 (80.0%)	20 (83.3%)	48 (81.4%)
$AUC_{baseline\ CL}$ < Target	7 (20.0%)	4 (16.7%)	11 (18.6%)

Source: ASTR-NIRSE-run-plots-bla-MUSIC.R
Notes: The target exposure 12.8 day·mg/mL. Subject [redacted] (b) (6) (MUSIC) was dosed 100mg at age 12.2 months (flagged as Season 2) but was included in Season 1 for extrapolation.
Abbreviations: $AUC_{baseline\ CL}$ =area under the serum concentration-time curve derived from post hoc clearance values at dosing from the final population pharmacokinetics model; N=number of subjects with available information

Source: Applicant's Population PK report, Table 15

Figure 25. Boxplots of Model-Derived Day 151 Serum Concentrations in Trial 08 Subjects Compared to Trial 04



Source: ASTR-NIRSE-run-plots-bla-MUSIC.R
Notes: Day 151 concentrations are Visit day 151 ± 14 days. Grey band is the reference inter-quartile range for MELODY Day 151 concentrations. The horizontal dashed black line is the *preclinical* EC90 (6.8 µg/mL). Subject (b) (6) (MUSIC) was dosed 100mg at age 12.2 months (flagged as Season 2) but was included in Season 1 for extrapolation. Data presented are pediatric subjects who are <5kg receiving 50mg or ≥5kg receiving 100mg in Season 1, or receiving 200mg in Season 2.
Abbreviations: EC90=90% effective concentration
Source: Applicant's Population PK report, Figure 31

Reviewer's Comments:

The Applicant's plots of exposure comparison suggest the following:

- *Day 151 concentrations at the proposed doses in Trials 04 and 05 are consistently higher than the EC90 value of 6.8 µg/mL identified from the cotton rate model of RSV in preclinical development.*
- *AUCbaselineCL values at the proposed doses in Trials 04 and 05 are predominantly above the AUCbaselineCL target identified from the exposure response analyses (12.8 mg*day/mL, see Section 2 for further details on this target).*
- *Exposures in Trial 05 appear to be similar to those in Trial 04 with the exception of those subjects in Season 2 who received the 200 mg dose.*
- *Nirsevimab exposures in subjects who received the 200 mg dose are higher than those evaluated in Trial 04 and also higher than those subjects who received lower doses (50 and 100 mg) in the first RSV season of Trial 05.*

Reviewer's Assessment of Dose Proportionality:

The reviewer also sought to evaluate the dose-proportionality claim proposed by the Applicant in the label: "The PK of nirsevimab-alip is dose-proportional following a single IM administration of doses ranging from 25 mg (0.5 times the lowest approved recommended dosage) to 200 mg in pediatric subjects." As the Applicant's final model is a linear model, the reviewer tested nonlinear a covariate on each of the following covariates sequentially: bioavailability (F1), CL, and Vd. The model was rerun with dose as a covariate, as defined below, to see if the parameter would be estimated differently from 1.0. If it was estimated to be 1 it suggests it is linear, if it's different than 1.0 it suggests nonlinearity.

F1 = TVF1(DOSE/100)**(1-Theta(18))*EXP(ETA())*

OR

CL = TVCL(DOSE/100)**(1-Theta(18))*EXP(ETA())*

Where TVCL indicates the typical value of CL defined in the population PK model and TVF1 is the typical value of F1 defined in the population PK model.

The Theta18 for F1 was estimated to be 0.955. The theta18 for CL was estimated to be 1.03. The objective function value improved in both cases compared to the final model. This gave a ΔOBJF of -11 for F1 and ΔOBJF of about -6 for CL. RSE of estimates for Theta18 were low.

The model included data from Trials 01 to 05. From the phase three data PK was available from 50 mg and 100 mg. From Trial 01 there were 6 adults with rich PK that received 300 mg, 6 that received 1000 mg and 6 that received 3000 mg. The 200 mg PK information came from season two in Trial 05 and also in Trial 08 (separately).

Based on the range of dose information included in the PK model, and that parameters evaluating dose proportionality suggested linearity, the Applicant's proposed labeling statement for the dose range of 25 mg to 200 mg is acceptable.

14.5.2. Exposure Response Analysis

The Applicant's exposure response analyses were utilized to inform dose selection and justify the proposed dosing regimen. Their analyses evaluated the relationship between the primary endpoint of MA RSV LRTI and nirsevimab exposure ($AUCbaselineCL$) with data from Trials 03 and 04. Early exposure response analyses identified their target exposure of 12.8 mg*day/mL for the $AUC_{baselineCL}$ based on Trial 03 data alone. This review focuses on the results of the combined analysis for Trials 03 and 04. Understanding the range of the relationship and the nature of relationship around the target AUC value helps to inform whether the proposed dosing regimen produces sufficient therapeutic exposures for the populations in Trials 03, 04, and 05.

Table 86 summarizes the variables evaluated as predictors of MA RSV LRTI. The Applicant applied a cox-proportional hazards model that was stratified by postmenstrual age and trial. $AUC_{baselineCL}$ for subjects in the E-R analysis dataset were derived from individual Bayesian CL values at baseline from Trial 04 final popPK model. AUC quartiles were defined based on

$AUC_{baselineCL}$ for subjects in Trial 03 (Q1 [4.4, 12.8], Q2 [>12.8, 18], Q3 [>18, 26.1], and Q4 [>26.1, 50.1]). $AUC_{baselineCL}$ for Trial 04 subjects were then mapped into the AUC quartiles.

Table 86. Summary of Variables Tested in the Exposure Response Analysis for Trials 03 and 04

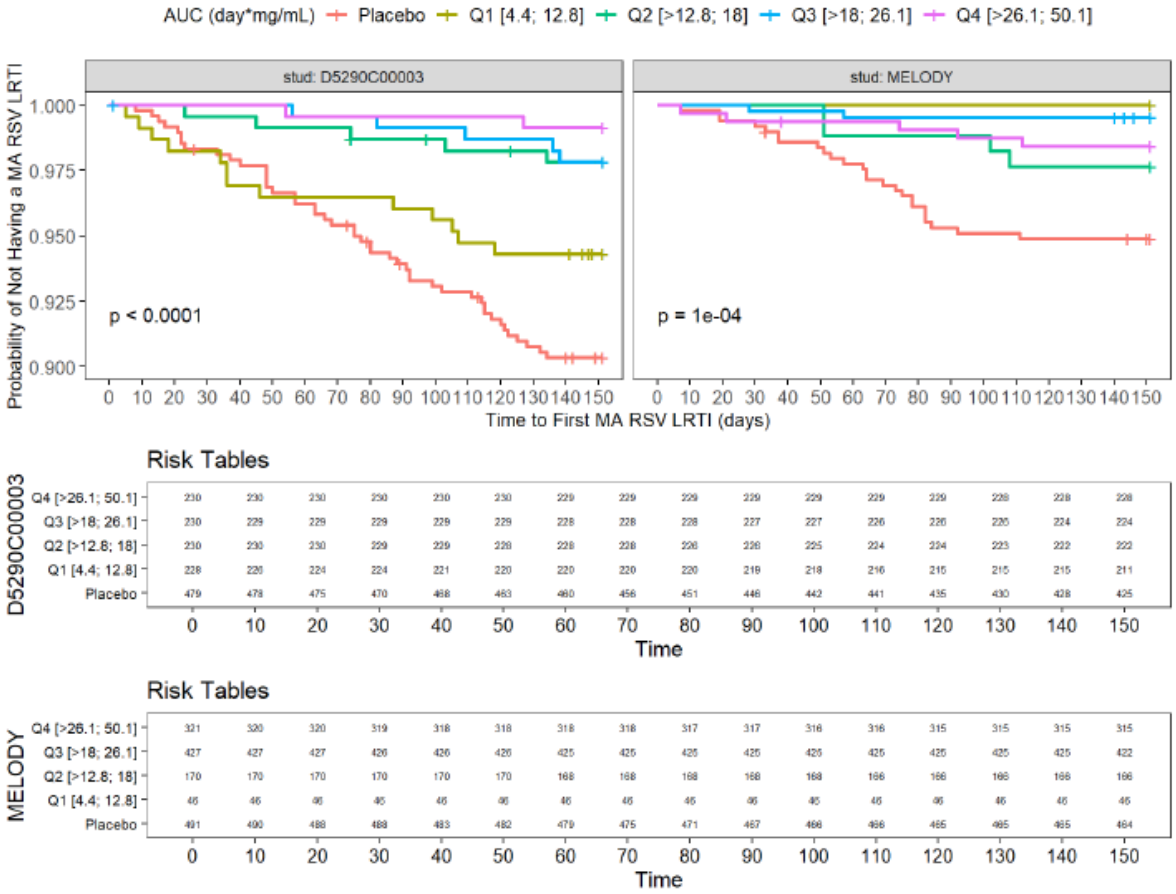
		D5290C00003		MELODY			Overall		
		Placebo (N = 479)	50 mg (N = 918)	Placebo (N = 491)	50 mg (N = 392)	100 mg (N = 572)	Placebo (N = 970)	50 mg (N = 1310)	100 mg (N = 572)
$AUC_{baseline\ CL}$ quartiles (day•mg/mL)	Placebo	479 (100%)	0 (0%)	491 (100%)	0 (0%)	0 (0%)	970 (100%)	0 (0%)	0 (0%)
	Q1 [4.4,12.8]	0 (0%)	228 (24.8%)	0 (0%)	19 (4.8%)	27 (4.7%)	0 (0%)	247 (18.9%)	27 (4.7%)
	Q2 [>12.8,18]	0 (0%)	230 (25.1%)	0 (0%)	91 (23.2%)	79 (13.8%)	0 (0%)	321 (24.5%)	79 (13.8%)
	Q3 [>18,26.1]	0 (0%)	230 (25.1%)	0 (0%)	202 (51.5%)	225 (39.3%)	0 (0%)	432 (33.0%)	225 (39.3%)
	Q4 [>26.1, 50.1]	0 (0%)	230 (25.1%)	0 (0%)	80 (20.4%)	241 (42.1%)	0 (0%)	310 (23.7%)	241 (42.1%)
Age group	≤3.0 months	255 (53.2%)	491 (53.5%)	283 (57.6%)	377 (96.2%)	183 (32.0%)	538 (55.5%)	868 (66.3%)	183 (32.0%)
	3.0-6.0 months	151 (31.5%)	307 (33.4%)	160 (32.6%)	13 (3.3%)	299 (52.3%)	311 (32.1%)	320 (24.4%)	299 (52.3%)
	>6.0 months	73 (15.2%)	120 (13.1%)	48 (9.8%)	2 (0.5%)	90 (15.7%)	121 (12.5%)	122 (9.3%)	90 (15.7%)
Baseline weight (kg)	<5 kg	289 (60.3%)	543 (59.2%)	190 (38.7%)	384 (98.0%)	1 (0.2%)	479 (49.4%)	927 (70.8%)	1 (0.2%)
	≥5 kg	190 (39.7%)	375 (40.8%)	301 (61.3%)	8 (2.0%)	571 (99.8%)	491 (50.6%)	383 (29.2%)	571 (99.8%)
Region	Northern	325 (67.8%)	614 (66.9%)	338 (68.8%)	263 (67.1%)	403 (70.5%)	663 (68.4%)	877 (66.9%)	403 (70.5%)
	Southern	154 (32.2%)	304 (33.1%)	153 (31.2%)	129 (32.9%)	169 (29.5%)	307 (31.6%)	433 (33.1%)	169 (29.5%)
Japanese	Japanese	0 (0%)	0 (0%)	17 (3.5%)	11 (2.8%)	21 (3.7%)	17 (1.8%)	11 (0.8%)	21 (3.7%)
	Not Japanese	479 (100%)	918 (100%)	474 (96.5%)	381 (97.2%)	551 (96.3%)	953 (98.2%)	1299 (99.2%)	551 (96.3%)
Race	White	351 (73.3%)	650 (70.8%)	268 (54.6%)	200 (51.0%)	311 (54.4%)	619 (63.8%)	850 (64.9%)	311 (54.4%)
	Black or Afr.Am.	67 (14.0%)	184 (20.0%)	136 (27.7%)	114 (29.1%)	158 (27.6%)	203 (20.9%)	298 (22.7%)	158 (27.6%)
	Japanese	0 (0%)	0 (0%)	17 (3.5%)	11 (2.8%)	21 (3.7%)	17 (1.8%)	11 (0.8%)	21 (3.7%)
	Asian	10 (2.1%)	4 (0.4%)	1 (0.2%)	3 (0.8%)	0 (0%)	11 (1.1%)	7 (0.5%)	0 (0%)
	Am.Ind. or Al.Nat.	1 (0.2%)	0 (0%)	26 (5.3%)	18 (4.6%)	38 (6.6%)	27 (2.8%)	18 (1.4%)	38 (6.6%)
	Nat.Haw. or Pac.Isl.	3 (0.6%)	8 (0.9%)	5 (1.0%)	3 (0.8%)	3 (0.5%)	8 (0.8%)	11 (0.8%)	3 (0.5%)
	Multiple	5 (1.0%)	12 (1.3%)	1 (0.2%)	5 (1.3%)	7 (1.2%)	6 (0.6%)	17 (1.3%)	7 (1.2%)
	Other	42 (8.8%)	59 (6.4%)	37 (7.5%)	37 (9.4%)	32 (5.6%)	79 (8.1%)	96 (7.3%)	32 (5.6%)
	Missing	0 (0%)	1 (0.1%)	0 (0%)	1 (0.3%)	2 (0.3%)	0 (0%)	2 (0.2%)	2 (0.3%)

Source: ASTR-CP-2207-MEDI8897-EDA-plots-27Jul2021.Rmd
Note: Values in the table are presented as number (% of total). AUC quartiles are based on $AUC_{baseline\ CL}$ derived from post hoc CL values at baseline from the MELODY final popPK model for Study D5290C00003 subjects.
Abbreviations: Afr.Am.=African American; Al.Nat.=Alaskan Native; Am.Ind.=American Indian; AUC=area under the concentration-time curve; CL=clearance; E-R=exposure-response; N=number of subjects with available information; Nat.Haw.=Native Hawaiian; Pac.Isl.=Pacific Islander; popPK=population pharmacokinetic; Q1=first quartile; Q2=second quartile; Q3=third quartile; Q4=fourth quartile; SD=standard deviation
Source: Applicant's Population PK Report, Table 11

The Applicant first performed exploratory graphical analysis with univariate Kaplan-Meier curves (Figure 26). The Asian subgroup was comprised mostly of Japanese subjects. Excluding all Japanese subjects from the analysis gave a p-value of 0.38 for race. The Applicant concluded based on this that Japanese status and not race was significant. The final model identified the Trial 03- defined AUC quartiles and Japanese status as key predictors to include in the parametric model. The Applicant chose to stratify the Cox model based on trial and age owing to differences between the placebo risk across trials differing risk dependent on age.

Reference ID: 5208810

Figure 26. Kaplan-Meier Plot of MA RSV LRTI Outcome in Trial 03 and Trial 04 (MELODY) Stratified by Trial 03 Quartiles



Source: MEDI8897-MALRTI-ER-22Sep2021.Rmd
Note: p-value method is log rank.
Abbreviations: $AUC_{baseline\ CL}$ =area under the serum concentration-time curve derived from post hoc clearance values at baseline from the MELODY final population pharmacokinetics model for study D5290C00003 subjects; CL=clearance; MA RSV LRTI=medically attended respiratory syncytial virus-confirmed lower respiratory tract infection; Q1=first quartile; Q2=second quartile; Q3=third quartile; Q4=fourth quartile
Source: Applicant's Population PK Report, Figure 32

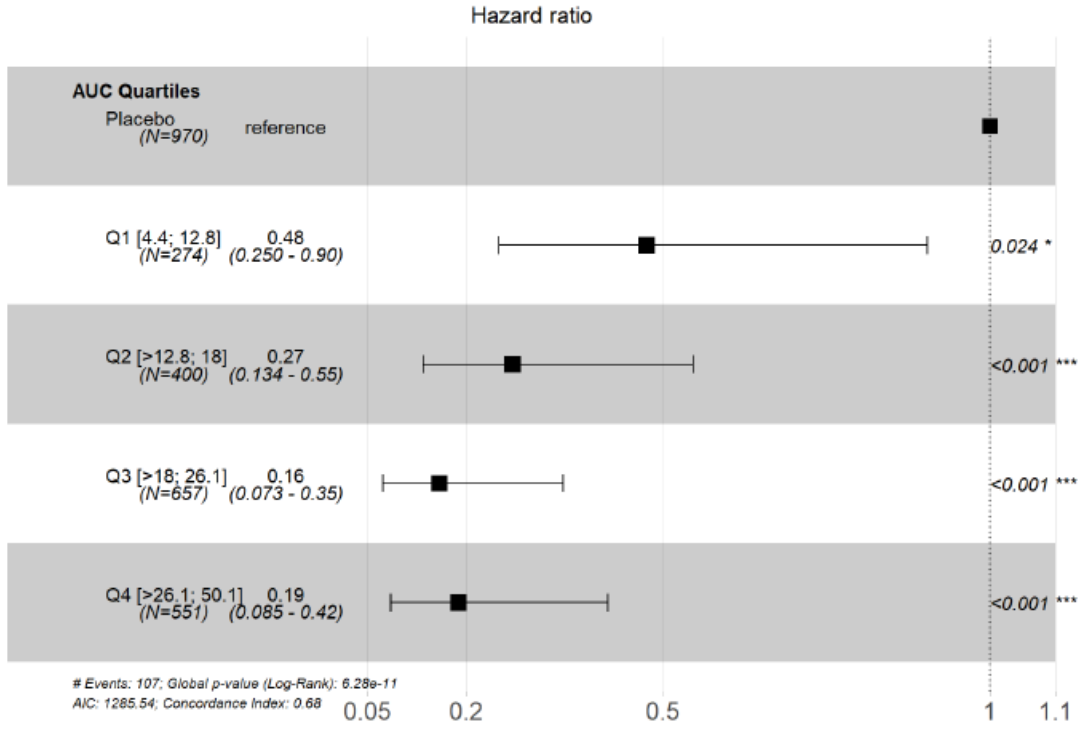
Per the Applicant:

> "The potential predictors identified in the Kaplan-Meier curves (AUC quartiles and Japanese) were included and tested for significance through a backward elimination process via a stratified Cox proportional hazards regression model (R survival package). The stratification variables included study and age group at dosing (<3.0 months, ≥3.0–6.0 months, and >6.0 months).
>
> "The likelihood ratio test was used to evaluate the significance of removing predictors from the model based at a significance level of 0.001. During the backward elimination process, predictors were removed from the model one at a time if their deletion leads to insignificant model deterioration. The likelihood ratio test indicates that the full model including AUC quartile and Japanese has the lowest loglikelihood. However, the high p-value (0.03616) suggests that inclusion of Japanese population is not significant. Exposure was also tested as

a continuous variable using log-transformed AUC to approximate an E_{max} function. The model with log-transformed AUC resulted in a decrease in AIC of only 1.8 point, indicating that the 2 models are equivalent. Consequently, the simpler AUC quartile model was chosen as the final model. The final E-R model includes AUC quartiles (defined based on Trial 03 data) as the predictor."

The Applicant's Final Exposure Response Model included $AUC_{baselineCL}$ as the only predictor. This relationship is shown in Figure 27. The lowest exposures in quartile 1 (Q1) appear to exhibit numerically less efficacy compared to quartiles 2 to 4. This supports the Applicants target of 12.8 identified from Trial 03 data. Above this value exposures appear to fall in the plateau of maximal response.

Figure 27. Forest Plot of Predictors in the Final E-R Model, Hazard Ratio (95% CI)



Source: MEDI8897-MALRTI-ER-22Sep2021.Rmd
Abbreviations: AUC=area under the serum concentration-time curve derived from post hoc clearance values at baseline from the MELODY final population pharmacokinetics model for study D5290C00003 subjects; CI=confidence interval; CL=clearance; E-R=exposure-response; N=number of subjects; Q1=first quartile; Q2=second quartile; Q3=third quartile; Q4=fourth quartile
Source: Applicant's Population PK Report, Figure 34

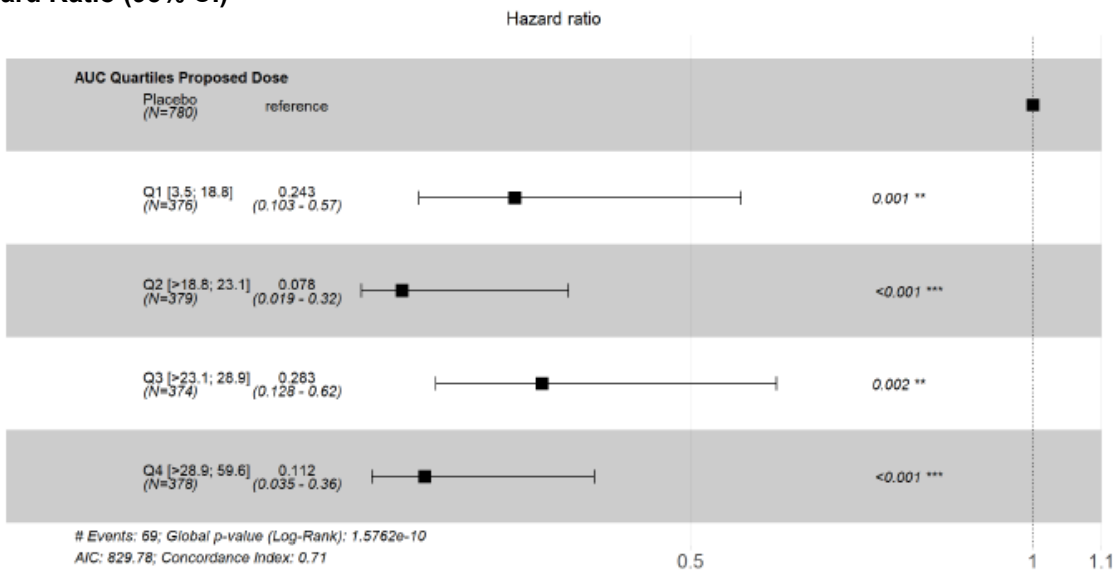
The Applicant also evaluated exposure response in subjects who only received the proposed dose. Any trend across these exposures might suggest that the doses were not in the plateau of maximal response.

A Cox proportional hazards model was estimated based on the data from the proposed dose only (i.e., 50 mg if <5 kg and 100 mg if ≥5 kg). This analysis included data from Trial 03 for infants <5 kg and all data from Trial 04 and evaluated exposure quartiles defined based on nirsevimab

Reference ID: 5208810

treated subjects in this subset. No slope was identified in the exposure-response relationship, only difference from placebo, ultimately supporting the proposed dosing regimen.

Figure 28. Exposure Response Based on Data From Subjects With the Proposed Dose Only, Hazard Ratio (95% CI)



Source: MEDI8897-MALRTI-ER-22Sep2021.Rmd
Abbreviations: AUC=area under the serum concentration-time curve derived from post hoc clearance values at baseline from the MELODY final population pharmacokinetics model for study D5290C00003 subjects; CI=confidence interval; E-R=exposure-response; N=number of subjects; Q1=first quartile; Q2=second quartile; Q3=third quartile; Q4=fourth quartile
Source: Applicant's Population PK Report, Appendix 4.2.6

Reviewer's Comments:

*The reviewer was able to rerun the Applicant's analysis and confirmed the results of the exposure-response assessment. Given the low number of RSV infections in these trials and the width of the confidence interval for the Hazard ratios of each quartile, the threshold value of 12.8 mg*day/mL appears to be a soft target and ensuring that concentrations are well above this point will support the probability that exposures are in the plateau of maximal response for nirsevimab. The population PK section above noted that in the majority of subjects the exposures at the proposed dosing regimen exceeds this target. This is further supported by the lack of events in the 614 subjects that received nirsevimab at the proposed doses in Trial 05.*

14.6. Pharmacogenetics

Not applicable.

15. Trial Design

15.1. Protocol Synopsis, Trial 03

Title

A phase 2b randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of nirsevimab, a monoclonal antibody with an extended half-life against Respiratory Syncytial Virus, in healthy preterm infants

Hypotheses

Primary Hypothesis

Compared to placebo, a single 50 mg intramuscular (IM) dose of nirsevimab will be efficacious in reducing MA LRTI caused by real-time reverse transcriptase polymerase chain reaction (RT-PCR)-confirmed respiratory syncytial virus (RSV) in healthy preterm infants born between 29 weeks 0 days and 34 weeks 6 days gestational age (GA) and entering their first RSV season, and the safety profile will be acceptable.

Secondary Hypotheses

1) There will be a reduction in the incidence of hospitalizations attributable to RSV
2) The predicted extended terminal half-life ($t_{1/2}$) will be adequate for the duration of the RSV season
3) Anti-drug antibody (ADA) to nirsevimab will not significantly impact the serum concentrations or safety of nirsevimab over the 5-month RSV season

Objectives

Primary Objective

To assess the efficacy of nirsevimab when administered as a single 50 mg IM dose to healthy preterm infants born between 29 weeks 0 days and 34 weeks 6 days GA and entering their first RSV season for the reduction of MA LRTI due to RT-PCR-confirmed RSV, compared to placebo

Secondary Objectives

1) To assess the efficacy of nirsevimab for the reduction of hospitalizations due to RT-PCR-confirmed RSV, compared to placebo
2) To evaluate the safety and tolerability of nirsevimab when administered as a single fixed IM dose, compared to placebo
3) To evaluate single-dose serum concentrations of nirsevimab
4) To evaluate ADA responses to nirsevimab in serum

Exploratory Objective

To assess healthcare resource utilization (HRU) and caregiver burden for nirsevimab recipients compared to placebo recipients

Study Endpoints

Primary Endpoint

Incidence of MA LRTI (inpatient and outpatient) due to RT-PCR-confirmed RSV over the duration of the 5-month RSV season

Secondary Endpoints

1) Incidence of hospitalizations due to RT-PCR-confirmed RSV over the duration of the 5-month RSV season
2) Safety and tolerability of nirsevimab as assessed by the occurrence of all treatment-emergent adverse events (TEAEs), treatment-emergent serious adverse events (TESAEs), adverse events of special interest (AESIs), and new onset chronic diseases (NOCDs)
3) Single dose nirsevimab serum concentrations
4) Incidence of ADA to nirsevimab in serum

Exploratory Endpoints

1) Magnitude of HRU (e.g., number of admissions to hospitals and intensive care units [ICUs] and duration of stay; number of subjects who require respiratory support and supplemental oxygen and the duration of use; number and type of outpatient visits, e.g., emergency room [ER], urgent care, outpatient clinic; and number of prescription and over-the-counter [OTC] medications and duration of use) for nirsevimab recipients compared to placebo recipients.
2) Caregiver burden (e.g., caregiver missed workdays, subject absence from day care) for subjects with MA LRTI caused by RT-PCR-confirmed RSV

Study Design

This pivotal phase 2b study is a randomized, double-blind, placebo-controlled, single-dose study to determine if nirsevimab will be efficacious in reducing MA RSV-confirmed LRTI in healthy preterm infants entering their first RSV season. The population to be enrolled is healthy preterm infants born between 29 weeks 0 days and 34 weeks 6 days GA who would not receive RSV prophylaxis based on the American Academy of Pediatrics (AAP) or other local or national guidelines. These infants will not be receiving palivizumab, allowing for a placebo comparator group for the determination of efficacy and the safety profile. A total of 1,500 infants will be randomized 2:1 to receive a 50 mg IM dose of nirsevimab (N=1000) or placebo (N=500). Randomization will be stratified by temperate zones in the northern and southern hemisphere and by subject age at the time of randomization (i.e., ≤3 months, >3 – ≤6 months, >6 months). Enrollment of infants >6 months of age will be limited to approximately 500. All infants will be followed for approximately 360 days after dosing.

Subjects will be monitored throughout the study for LRTI. All subjects seeking medical attention for a respiratory illness (inpatient or outpatient setting) will be evaluated for the occurrence of LRTI. Subjects who have a primary hospitalization for a respiratory illness, a respiratory deterioration during a hospitalization, or who seek outpatient medical attention, including ER visits for a respiratory illness, will be assessed for RSV by diagnostic testing of respiratory secretions and clinical assessment for the presence of LRTI. Testing for RSV will be performed centrally using the (U.S. FDA-approved and Conformité Européenne or European Conformity (CE) - marked in vitro diagnostic real-time RT-PCR assay (Lyra RSV+human metapneumovirus [hMPV] Assay, Quidel, San Diego, CA www.quidel.com). A diagnosis of RSV LRTI requires having a respiratory sample positive for RSV by RT-PCR.

Subjects with signs of LRTI must have documented physical exam findings of rhonchi, rales, crackles, or wheeze AND any of the following:

- Increased respiratory rate at rest (age <2 months, ≥60 breaths/min; age 2 to 6 months, ≥50 breaths/min; age >6 months – 2 years, ≥40 breaths/min) OR
- Hypoxemia (in room air: oxygen saturation <95% at altitudes ≤1800 meters or <92% at altitudes >1800 meters), OR
- Clinical signs of severe respiratory disease (e.g., acute hypoxic or ventilatory failure, new onset apnea, nasal flaring, intercostal, subcostal or supraclavicular retractions, grunting) or dehydration secondary to inadequate oral intake due to respiratory distress (need for intravenous fluid).

Target Subject Population

This study will be conducted in healthy preterm infants born between 29 weeks 0 days and 34 weeks 6 days GA who would not be recommended to receive palivizumab per AAP or local or national guidelines, and who are entering their first RSV season at the time of screening.

Investigational Product, Dosage, And Mode Of Administration

Subjects will be randomly assigned to receive a single dose of nirsevimab (50 mg IM) or placebo.

Statistical Analysis Plan

General Considerations

Tabular summaries will be presented by treatment group. Categorical data will be summarized by the number and percentage of subjects in each category. Continuous variables will be summarized by descriptive statistics. Additional details of statistical analyses will be described in the statistical analysis plan.

The intent-to-treat (ITT) Population is defined as all subjects who are randomized. Subjects will be included in the treatment group corresponding to their randomized treatment. All analyses, with the exception of safety, will be performed on the ITT Population.

The As-treated Population will include all subjects who are randomized into the study and who receive any amount of investigational product. Subjects will be included in the treatment group corresponding to the treatment actually received. All safety analyses will be performed on the As-treated Population.

Sample Size

The sample size of 1,500 is necessary based on advice from the U.S. FDA requesting that 1,000 preterm infants be exposed to nirsevimab in this phase 2b study. This sample size has approximately 99% power to detect 70% relative risk reduction, assuming a placebo group MA RSV LRTI incidence of 8%. Power calculations are based on Poisson regression model with robust variance (Zou 2004) comparing nirsevimab 50 mg versus placebo, with 2-sided significance level of $\alpha = 0.05$.

- The 70% relative risk reduction assumption is based on a placebo-controlled study in Native American infants in which there was 87% relative reduction in the incidence of RSV hospitalization (11.3% placebo; 1.5% motavizumab; $p<0.001$) and 71% relative reduction in the incidence of outpatient RSV LRTI (10.0% placebo; 2.9% motavizumab; $p<0.001$) in infants who received motavizumab prophylaxis (O'Brien et al. 2015).

In order to evaluate risk, a sample size of 1,000 subjects exposed to nirsevimab will provide a 90% probability of observing at least one adverse event (AE) if the true event rate is 0.2%; if no AEs are observed, this study provides 95% confidence that the true event rate is <0.3%.

Statistical Analyses

There are two planned analyses for this study: the primary analysis and the final analysis. The primary analysis will be conducted after all randomized subjects have completed follow-up through the 5-month RSV season (i.e., Day 151 visit) and will be the primary analysis for which the study is designed to assess efficacy. For the primary analysis, all efficacy, pharmacokinetics (PK), ADA, and safety data collected through at least Day 151 will be analyzed. The final analysis for safety follow-up will be conducted when all subjects have completed the last visit of the study (i.e., Day 361). Since efficacy endpoints are collected in the time interval from randomization up to Day 151, the efficacy analyses performed in the primary analysis will serve the purpose of evaluating the efficacy of nirsevimab in the study population.

The primary and secondary efficacy hypotheses will be assessed in the primary analysis by a hierarchical order. That is, the secondary hypothesis will be tested at a significance level of 0.05 only if the treatment effect on the primary efficacy endpoint is demonstrated at the significance level of 2-sided 0.05. With that, the overall Type I error is controlled at 0.05. Therefore, no further multiplicity adjustment is necessary.

Primary Endpoint Analysis

<u>Primary Efficacy Analysis</u>

The incidence of RSV LRTI (inpatient and outpatient) during 5 months of the RSV season will be based on RSV test results (performed centrally via RT-PCR) and objective clinical LRTI

criteria will be summarized by treatment group. For subjects with multiple MA RSV LRTI events, only the first occurrence will be used in the primary analysis.

The primary efficacy analysis will be conducted on the ITT Population. LRTI caused by RSV that occurs prior to discontinuation from participation in the study will contribute to the primary efficacy analysis. For subjects who do not have an RSV LRTI prior to discontinuation from participation, their event status will be imputed assuming the observed placebo RSV LRTI rate. This will be implemented by imputations based on repeated simulations and will be described in the Statistical Analysis Plan. A Poisson regression model with robust variance will be used as the primary efficacy analysis model to compare the incidence of medically attended RSV LRTI between nirsevimab and placebo, including treatment group, age at the time of randomization (i.e., ≤3 months, >3 – ≤6 months, >6 months), and dichotomous temperate (northern and southern) hemispheres as covariates. In addition, the 2-sided p-value and corresponding 2-sided 95% CI on the relative risk will be provided from the model. Relative risk reduction is defined as (1 - Pn/Ps) where Pn is the incidence of RSV LRTI during 5 months of the RSV season in the nirsevimab group and Ps is the incidence of RSV LRTI during 5 months of the RSV season in the placebo group generated by the model.

Statistical significance will be achieved if the 2-sided p-value is ≤0.05.

Additional Analyses of the Primary Endpoint

A CMH (Cochran-Mantel-Haenszel) approach stratified by hemisphere and age group at the time of randomization (i.e., ≤3 months, >3 – ≤6 months, >6 months) will be used to compare the incidence of RSV LRTI during 5 months of the RSV season between treatment groups as a sensitivity analysis for the primary endpoint. In addition, a time-to-event analysis assessing time to first RSV LRTI may be performed as a supplementary analysis.

An analysis may also include all RSV positive LRTI endpoints, using results from either the central lab or local lab.

Different approaches to handle missing data (i.e., early discontinuation and no RSV LRTI prior to discontinuation) may be considered for sensitivity analyses. Additional analyses or subgroup summaries may be performed to adjust duration of efficacy follow-up and/or other possible confounding factors. These analyses will be described in the Statistical Analysis Plan.

Secondary Endpoint Analyses

Efficacy

The incidence of RSV hospitalization during 5 months of the RSV season will be summarized by treatment group. The same methods described above for the primary efficacy endpoint will be used to assess treatment effect on RSV hospitalization. Following a hierarchical testing procedure, the secondary efficacy endpoint is tested at the significance level of 2-sided 0.05 if the primary analysis on the primary efficacy endpoint has achieved statistical significance at the level of 2-sided 0.05.

Safety

Safety of nirsevimab will primarily be assessed by the occurrence of treatment-emergent AEs and SAEs. Adverse events will be graded according to the current version of the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) where applicable for pediatric assessments. Adverse events will be coded by Medical Dictionary for Regulatory Activities (MedDRA) and the type, incidence, severity and relationship to study investigational product will be summarized by treatment group. Other safety assessments will include:

- Occurrence of AESIs to include targeted AEs of hypersensitivity (including anaphylaxis), thrombocytopenia, and immune complex disease (e.g., vasculitis, endocarditis, neuritis, glomerulonephritis) following investigational product administration.
- Occurrence of NOCDs following investigational product administration

Pharmacokinetics Analysis

Following a single dose of nirsevimab, individual nirsevimab serum concentration data will be tabulated by treatment group along with descriptive statistics. Terminal phase half-life ($t_{1/2}$) will be estimated using non- compartmental analysis, if data permit.

Antidrug Antibody Analysis

The incidence of ADA to nirsevimab will be assessed and summarized by number and percentage of subjects that are ADA positive by treatment group. The ADA titer will be listed by subject at different time points.

The impact of ADA on PK and association with TEAEs and TESAEs will be assessed.

Exploratory Endpoint Analyses

The magnitude of HRU (e.g., number of admissions to hospitals and ICUs and duration of stay; number of subjects who require respiratory support and supplemental oxygen and the duration of use; number and types of outpatient visits, e.g., ER, urgent care, outpatient clinic; and number of prescription and OTC medications and duration of use) will be summarized overall by treatment group, and for the following subgroups: subjects with at least one MA LRTI caused by RT-PCR-confirmed RSV, subjects with MA LRTI not caused by RSV, and subjects without MA LRTI.

Caregiver burden (e.g., caregiver missed workdays, subject absence from day care) for subjects with MA LRTI caused by RT-PCR-confirmed RSV will be summarized by treatment group

15.2. Protocol Synopsis, Trial 04

Title

A phase 3 Randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of nirsevimab, a monoclonal antibody with an extended half-life against respiratory syncytial virus, in healthy late preterm and term infants (MELODY)

Hypotheses

Primary Hypothesis

Compared to placebo, a single intramuscular (IM) nirsevimab dose, 50 mg if weight <5 kg or 100 mg if weight ≥5 kg, will be efficacious in reducing MA LRTI caused by real-time reverse transcriptase-polymerase chain reaction (RT-PCR)-confirmed respiratory syncytial virus (RSV) in healthy late preterm and term infants born ≥35 weeks 0 days gestational age (GA) and entering their first RSV season, and the safety profile will be acceptable.

Secondary Hypotheses

1) There will be a reduction in the incidence of hospitalizations attributable to RT-PCR-confirmed RSV
2) The predicted serum exposures of nirsevimab will be adequate for the duration of the RSV season

Anti-drug antibody (ADA) to nirsevimab will not impact the serum concentrations or safety of nirsevimab through 150 days postdosing (i.e., during a 5-month RSV season).

Objectives and Associated Endpoints

Table 87. Trial 04 Objectives and Endpoints

Type	Objective	Endpoint
Primary		
Efficacy	To assess the efficacy of nirsevimab when administered as a single fixed IM dose to infants ≥35 weeks 0 days GA and entering their first RSV season, in reducing MA LRTI due to RT-PCR-confirmed RSV, compared to placebo	Incidence of MA LRTI (inpatient and outpatient) due to RT-PCR-confirmed RSV through 150 days after dosing (i.e., during a typical 5-month RSV season)
Secondary		
Efficacy	To assess the efficacy of nirsevimab in reducing hospitalizations due to RT-PCR-confirmed RSV, compared to placebo	Incidence of hospitalizations due to RT-PCR- confirmed RSV through 150 days after dosing (i.e., during a typical 5-month RSV season)
Safety	To evaluate the safety and tolerability of nirsevimab when administered as a single fixed IM dose, compared to placebo	Safety and tolerability of nirsevimab as assessed by the occurrence of treatment-emergent adverse events (TEAEs), treatment-emergent serious adverse events (TESAEs), adverse events of special interest (AESIs), and new onset chronic diseases (NOCDs)
PK	To evaluate single-dose serum concentrations of nirsevimab	Summary of nirsevimab serum concentrations and estimated PK parameters: apparent clearance, AUC0-∞, if data permit
ADA	To evaluate ADA responses to nirsevimab in serum	Incidence of ADA to nirsevimab in serum
Exploratory		
Healthcare resource utilization and caregiver burden	To assess healthcare resource utilization and caregiver burden for nirsevimab recipients compared to placebo recipients	Magnitude of healthcare resource utilization (HRU; e.g., number of admissions to hospitals and intensive care units and duration of stay; number of subjects who require respiratory support and supplemental oxygen and the duration of use; number and type of outpatient visits, e.g., emergency room [ER], urgent care, outpatient clinic; and number of prescription and over-the- counter medications and duration of use). Caregiver burden (e.g., caregiver missed workdays, subject absence from day care) for subjects with MA LRTI caused by RT-PCR-confirmed RSV
RSV neutralizing antibody	To determine anti-RSV neutralizing antibody levels in serum afforded by a single dose of nirsevimab compared to maternally derived	Anti-RSV neutralizing antibody levels (IU/mL) in serum for nirsevimab recipients compared to placebo recipients

Type	Objective	Endpoint
	RSV neutralizing antibody levels and those elicited by RSV infection in the placebo group	
RSV serology	To evaluate exposure to RSV by measuring seroresponse to different RSV proteins	Antibody levels to RSV pre-F, post-F, Ga, Gb, and N at different time points Changes in antibody levels (seroresponse) indicating exposure to RSV
RSV resistance monitoring	To characterize resistance to nirsevimab through genotypic and phenotypic analyses	Genotypic analysis and susceptibility of RSV variants to neutralization by nirsevimab
RSV LRTI after Day 151	To assess the incidence of MA LRTI due to RT-PCR-confirmed RSV, compared to placebo after Day 151	Incidence of MA LRTI (inpatient and outpatient) due to RT-PCR-confirmed RSV from Day 152 to Day 361

Source: Applicant's protocol synopsis
Abbreviations: ADA, anti-drug ant body; AESI, adverse events of special interest; ER, emergency room; GA, gestational age; HRU, healthcare resource utilization; IM, intramuscular; LRTI, lower respiratory tract infection events; MA, medically attended; NOCD, new onset chronic diseases; PK, pharmacokinetic; RSV, respiratory syncytial virus; RT-PCR, reverse transcriptase-polymerase chain reaction; TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event

Trial Design

Trial 04 is a pivotal phase 3 randomized, double-blind, placebo-controlled, single-dose trial to determine if nirsevimab will prevent MA RSV-confirmed LRTI in healthy infants entering their first RSV season. The population to be enrolled is healthy late preterm and term infants born ≥35 weeks 0 days GA who would not receive RSV prophylaxis based on the American Academy of Pediatrics (AAP) or other local or national guidelines. A total of approximately 3,000 infants will be randomized 2:1 to receive a 50-mg (if weight <5 kg) or 100-mg (if weight ≥5 kg) IM dose of nirsevimab (N=2,000) or placebo (N=1,000). Randomization will be stratified by hemisphere (northern hemisphere [NH], southern hemisphere [SH]) and by subject age at the time of randomization (≤3 months, >3 to ≤6 months, >6 months). Enrollment of infants >6 months of age will be limited to approximately 500. The trial will comprise two cohorts: a primary cohort (N = ~1,500) and a complementary safety cohort (hereafter referred to as the safety cohort; N = ~1,500) for a total of approximately 3,000 subjects. The primary cohort will include subjects from the NH2019, SH2020, and NH2020 enrollment seasons. The safety cohort will include subjects enrolled after the NH2020 enrollment season. Given the largely reduced circulation of RSV due to the coronavirus disease 2019 (COVID-19) pandemic related measures, the efficacy analyses performed in the primary analysis for the primary cohort will serve the purpose of evaluating the efficacy of nirsevimab in the study population. Although efficacy data will also be collected for the safety cohort, only descriptive summaries will be provided and there is no intent to pool the efficacy data from the safety cohort with that from the primary cohort. Both the primary and safety cohorts, individually and combined, will serve the purpose of evaluating the safety of nirsevimab. All subjects will be followed for approximately 510 days after dosing. An independent data monitoring committee will review safety data regularly and make recommendations regarding further study conduct. Subjects will be monitored throughout the study for LRTI. All subjects seeking medical attention for a respiratory illness (in either the inpatient or outpatient setting) will be evaluated for the occurrence of LRTI.

All subjects found to have an LRTI and all subjects who require hospitalization for a respiratory infection, even if there is not a diagnosis of LRTI, should have respiratory samples obtained and respiratory assessment forms completed. Samples should be collected for all of these events (even those not meeting the protocol definition of LRTI). Subjects who have a primary hospitalization for a respiratory infection (i.e., upper or lower tract) or a respiratory deterioration during a hospitalization, or who seek outpatient medical attention (including ER visits) for a lower respiratory illness, will be assessed clinically for the presence of LRTI and for RSV by central laboratory diagnostic testing of respiratory secretions.

In addition to the clinical assessment of LRTI, there is a protocol definition using objective criteria for the determination of a MA protocol defined LRTI. To meet the protocol-defined endpoint of MA LRTI, subjects with signs of LRTI must have documented at least one physical exam finding of rhonchi, rales, crackles, or wheeze AND at least one of the following clinical signs:

- Increased respiratory rate at rest (age <2 months, ≥60 breaths/min; age 2 to 6 months, ≥50 breaths/min; age >6 months, ≥40 breaths/min) OR
- Hypoxemia (in room air: oxygen saturation <95% at altitudes ≤1,800 meters or <92% at altitudes >1,800 meters), OR

- Clinical signs of severe respiratory disease (e.g., acute hypoxic or ventilatory failure, new onset apnea, nasal flaring, intercostal, subcostal or supraclavicular retractions, grunting) or dehydration secondary to inadequate oral intake due to respiratory distress (need for intravenous fluid).

Testing for RSV will be performed centrally using the United States Food and Drug Administration-cleared and *Conformité Européenne* or European Conformity-marked in vitro diagnostic real-time RT-PCR assay (Lyra RSV+human metapneumovirus [hMPV] assay; Quidel Corporation, San Diego, CA). A diagnosis of RSV LRTI requires having a respiratory sample positive for RSV by the central laboratory RT-PCR.

Target Subject Population

Healthy late preterm and term infants >35 weeks 0 days GA entering their first RSV season.

Treatment Groups and Regimens

Subjects will be randomly assigned in a 2:1 ratio to receive a single IM dose of nirsevimab (N=2,000) or placebo (N=1,000). The nirsevimab dose level will be stratified by body weight at time of dosing: 50 mg nirsevimab for infants <5 kg or 100 mg nirsevimab for infants ≥5 kg.

Statistical Methods

General Considerations

Tabular summaries will be presented by treatment group. Categorical data will be summarized by the number and percentage of subjects in each category. Continuous variables will be summarized by descriptive statistics. Additional details of statistical analyses will be described in the Statistical Analysis Plan (SAP).

There will be two study cohorts: a primary cohort and a safety cohort. The primary cohort will include subjects from the NH2019, SH2020, and NH2020 enrollment seasons (enrollment was paused after one subject from NH2020 was enrolled due to the impact of the COVID-19 pandemic). The safety cohort will include subjects enrolled after the NH2020 enrollment season.

The Intent-to-treat (ITT) Population is defined as all subjects who are randomized. Subjects will be included in the treatment group corresponding to their randomized treatment. All analyses, with the exception of safety, will be performed on the ITT Population unless otherwise specified. Subjects in the ITT Population and from the primary cohort will be ITT Population 1 (ITT1). Subjects in the ITT Population and from the safety cohort will be ITT Population 2 (ITT2).

The As-treated Population will include all subjects who are randomized and who receive any amount of investigational product. Subjects will be included in the treatment group corresponding to the treatment actually received. All safety analyses will be performed on the As-treated Population. Subjects in the As- treated Population and from the primary cohort will be As-treated Population 1 (AT1). Subjects in the As- treated Population and from the safety cohort will be As-treated Population 2 (AT2).

Sample Size

This phase 3 study will enroll approximately 3,000 subjects of whom approximately 2,000 will receive nirsevimab and 1,000 will receive placebo. The 2,000 subjects to be dosed with nirsevimab in this study, together with the 968 subjects dosed with nirsevimab in the phase 2b Trial 03 and at least 600 subjects dosed with nirsevimab in the palivizumab-controlled phase 2/3 Trial 05, will contribute to a safety database of at least approximately 3,600 subjects exposed to nirsevimab.

For this phase 3 study, the original sample size of 3,000 was driven by the safety database requirement, and the study had at least 99% power for the primary efficacy endpoint. Reducing the sample size to 1,500 still allows the study to be sufficiently powered. More specifically, the sample size of approximately 1,500 subjects in the primary cohort has at least 99% power to detect 70% relative risk reduction (RRR), assuming a placebo group MA RSV LRTI incidence of 8% with a 2-sided $\alpha = 0.05$.

The 70% RRR assumption is based on the phase 2b Trial 03 in which there was 70% RRR in the incidence of MA RSV LRTI (9.5% placebo, 2.6% nirsevimab; $p<0.001$) and 79% RRR in the incidence of RSV hospitalization (4.1% placebo, 0.8% nirsevimab; $p<0.001$) in subjects who received nirsevimab prophylaxis. In addition, the assumption is supported by a placebo-controlled study in Native American term infants in which there was a 71% relative reduction in the incidence of outpatient RSV LRTI (10.0% placebo, 2.9% motavizumab; $p<0.001$) and 87% relative reduction in the incidence of RSV hospitalization (11.3% placebo, 1.5% motavizumab; $p<0.001$) in infants who received motavizumab prophylaxis. In the event that the incidence rate in the placebo group drops due to the impact of the COVID- 19 pandemic (e.g., social distancing), the sample size of 1,500 still provides at least 90% power to detect 70% RRR if the placebo incidence rate is 4% or higher.

To evaluate risk, a sample size of 2,000 subjects exposed to nirsevimab in this phase 3 study will provide a >99% probability of observing at least one adverse event (AE) if the true event rate is 0.3%; if no AEs are observed, this study provides 98% confidence that the true event rate is <0.2%.

Statistical Analyses

There are 3 planned analyses for this study: the primary analysis, safety analysis, and final analysis. The primary analysis will be conducted after all randomized subjects (except for one subject enrolled in the NH2020 season) from the primary cohort have been followed through Day 361 and will be the primary analysis for which the study is designed to assess efficacy. For the primary analysis, all efficacy, pharmacokinetics (PK), ADA, and safety data collected for the primary cohort through at least Day 361 will be analyzed. The safety analysis will be conducted when all subjects from the safety cohort have been followed through Day 151. For the safety analysis, in addition to the analyses conducted during the primary analysis based on the primary cohort, all available efficacy, PK, ADA, RSV neutralizing antibody, RSV serology, and safety data collected for the safety cohort will be analyzed (only descriptive summaries will be provided for the efficacy data collected for the safety cohort). The final analysis will be conducted when all subjects have completed the last visit of the study (i.e., Day 511). Given the largely reduced circulation of RSV due to the COVID-19 pandemic related measures, the

efficacy analyses performed in the primary analysis for the primary cohort will serve the purpose of evaluating the efficacy of nirsevimab in the study population.

Although efficacy data will also be collected for the safety cohort, only descriptive summaries will be provided and there is no intent to pool the efficacy data from the safety cohort with that from the primary cohort. Both the primary and the safety cohorts, individually and combined, will serve the purpose of evaluating the safety of nirsevimab.

The primary and secondary efficacy hypotheses will be assessed in the primary analysis for the primary cohort by a hierarchical order. That is, the secondary hypothesis will be tested at a significance level of 0.05 only if the treatment effect on the primary efficacy endpoint is demonstrated at the significance level of 2-sided 0.05. With that, the overall Type I error is controlled at 0.05. Therefore, no further multiplicity adjustment is necessary.

Primary Endpoint Analysis

Primary Efficacy Analysis

The incidence of RSV LRTI (inpatient and outpatient) during 5 months of the RSV season will be based on RSV test results (performed centrally via RT-PCR) and objective clinical LRTI criteria and will be presented by treatment group. For subjects with multiple MA RSV LRTI events, only the first occurrence will be used in the primary analysis.

The primary efficacy analysis of the primary endpoint will be conducted on ITT1. RSV LRTI that occurs through 150 days postdose will contribute to the primary efficacy analysis. For subjects who do not have a MA RSV LRTI and are not followed through 150 days postdose, their event status will be imputed assuming the observed placebo RSV LRTI rate conditional on stratification factors using multiple imputation techniques and will be described in the SAP. A Poisson regression model with robust variance will be used as the primary efficacy analysis model to compare the incidence of MA RSV LRTI between nirsevimab and placebo, including treatment group, age at the time of randomization (i.e., ≤3 months, >3 to ≤6 months, >6 months), and dichotomous temperate hemispheres (NH and SH) as covariates. In addition, the 2-sided p-value and corresponding 2-sided 95% confidence interval on the relative risk will be provided from the model. RRR is defined as (1 - Pn/Ps) where Pn is the incidence of RSV LRTI through 150 days postdose in the nirsevimab group and Ps is the incidence of RSV LRTI through 150 days postdose in the placebo group generated by the model. Statistical significance will be achieved if the 2-sided p-value is ≤0.05.

During blinded data review prior to database lock for the primary analysis, it was decided to drop the stratification factor hemisphere from the full model due to no incidence of MA RSV LRTI events through 150 days postdose for SH in the primary cohort, which would cause a known convergence or estimation issue. Similar consideration also applies to other analyses for the primary efficacy endpoint, where hemisphere will be dropped from the corresponding models.

Additional Analyses of the Primary Endpoint

A Cochran-Mantel-Haenszel approach stratified by age group at the time of randomization (i.e., ≤3 months, >3 to ≤6 months, >6 months) will be used to compare the incidence of RSV LRTI through 150 days postdose between treatment groups as a secondary analysis for the primary

endpoint. The additional analyses will be conducted on ITT1. In addition, a time-to-event analysis assessing time to first RSV LRTI may be performed as a supplementary analysis.

An analysis may also include all RSV positive LRTI endpoints, using results from either the central laboratory or local laboratory.

Different approaches to handle missing data (i.e., early discontinuation and no RSV LRTI prior to discontinuation) may be considered for supplementary analyses. Additional analyses may be performed to adjust duration of efficacy follow-up and to assess the efficacy within subgroups. These analyses will be described in the SAP.

The incidence of MA RSV LRTI through 150 days postdose will also be summarized by treatment group on ITT2.

Secondary Endpoint Analyses

Efficacy

The incidence of RSV LRTI hospitalization through 150 days postdose will be presented by treatment group. Similar methods as described above for the primary efficacy endpoint will be used to assess efficacy on RSV LRTI hospitalization on ITT1.

The incidence of RSV LRTI hospitalization through 150 days postdose will also be summarized by treatment group on ITT2.

Safety

The safety analyses will be conducted on the overall As-treated Population, AT1, and AT2. Safety of nirsevimab will primarily be assessed by the occurrence of TEAEs and TESAEs. Adverse events will be graded according to the current version of the National Cancer Institute Common Terminology Criteria for Adverse Events where applicable for pediatric assessments. Adverse events will be coded by Medical Dictionary for Regulatory Activities and the type, incidence, severity, and relationship to investigational product will be summarized by treatment group. Other safety assessments will include:

- Occurrence of AESIs to include targeted AEs of hypersensitivity (including anaphylaxis), thrombocytopenia, and immune complex disease (e.g., vasculitis, endocarditis, neuritis, glomerulonephritis) following investigational product administration.
- Occurrence of NOCDs following investigational product administration

Pharmacokinetics Analysis

Following a single dose of nirsevimab, individual nirsevimab serum concentration data will be tabulated by treatment group along with descriptive statistics. PK parameters, e.g., C_{max}, AUC, apparent clearance, and terminal half-life, will be estimated using noncompartmental analysis, if data permit.

Antidrug Antibody Analysis

The incidence of ADA to nirsevimab will be assessed and summarized by number and percentage of subjects that are ADA positive by treatment group. The ADA titer will be listed by subject at different time points. The impact of ADA on PK, efficacy, and association with TEAEs and TESAEs will be assessed. These summaries will be conducted on the overall As-treated Population, AT1, and AT2, unless specified otherwise.

Exploratory Endpoint Analyses

HRU and Caregiver Burden

The magnitude of HRU (e.g., number of admissions to hospitals and ICUs and duration of stay; number of subjects who require respiratory support and supplemental oxygen and the duration of use; number and types of outpatient visits, e.g., ER, urgent care, outpatient clinic; and number of prescription and over-the-counter medications and duration of use) will be summarized overall by treatment group, and for the following subgroups: subjects with at least one MA LRTI caused by RT-PCR-confirmed RSV, subjects with MA LRTI not caused by RSV, and subjects with nonprotocol defined LRTIs, which may be further broken down by RSV status. These summaries will be conducted on ITT1 and ITT2 (if data permit).

Caregiver burden (e.g., caregiver missed workdays, subject absence from day care) for subjects with MA LRTI caused by RT-PCR-confirmed RSV will be summarized by treatment group on ITT1 and ITT2 (if data permit).

RSV Neutralizing Antibody and RSV Serology

RSV neutralizing antibody levels afforded by nirsevimab will be compared to maternal RSV neutralizing antibody levels and those elicited following infection in the placebo group.

RSV seroresponses will be evaluated as a measure of RSV exposure in the placebo and nirsevimab groups.

Monitoring RSV Resistance to Nirsevimab

Genotypic analysis of the full-length mature F protein will be conducted on all RSV-positive isolates confirmed centrally using the Lyra RSV+hMPV real-time RT-PCR assay manufactured by Quidel Corporation. RSV genotypic analysis will report amino acid changes in the mature F protein sequence compared to contemporary RSV A and RSV B reference strains. Phenotypic analyses will report changes in susceptibility of engineered recombinant RSV variants to nirsevimab and palivizumab neutralization compared to laboratory-derived reference viruses.

RSV LRTI Occurring From Day 152 to Day 361

The incidence of MA RSV LRTI (inpatient and outpatient) from Day 152 to Day 361 will be based on RSV test results (performed centrally via RT-PCR) and objective clinical LRTI criteria and will be summarized by treatment group on ITT1 and ITT2.

15.3. Protocol Synopsis, Trial 05

Title

A phase 2/3 randomized, double-blind, palivizumab-controlled study to evaluate the safety of nirsevimab, a monoclonal antibody with an extended half-life against Respiratory Syncytial Virus, in high-risk children (MEDLEY).

Objectives and Associated Endpoints

Table 88. Trial 05 Objectives and Endpoints

Type	Objective	Endpoint
	Primary	
Safety	To evaluate the safety and tolerability of nirsevimab compared to palivizumab when administered to preterm infants entering their first RSV season and children with CLD or CHD entering their first and second RSV season	Safety and tolerability of nirsevimab as assessed by the occurrence of all TEAEs, TESAEs, AESIs, and NOCDs
	Secondary	
PK	To evaluate serum concentrations of nirsevimab and palivizumab	Nirsevimab and palivizumab serum concentrations Nirsevimab and palivizumab PK parameters: Summary of serum concentrations and estimated PK parameters (C_{max}, AUC, apparent clearance, and $t_{1/2}$, if data permit)
ADA	To evaluate ADA responses to nirsevimab and to palivizumab in serum	Incidence of ADA to nirsevimab and palivizumab in serum
Efficacy	To assess the descriptive efficacy of nirsevimab when administered as a single IM dose of 50 mg to infants <5 kg or 100 mg to infants ≥5 kg in the first RSV season or a single 200-mg IM dose administered in the second RSV season, in reducing MA LRTI (inpatient and outpatient) and hospitalization due to RT-PCR-confirmed RSV, compared to palivizumab	Incidence of MA LRTI (inpatient and outpatient) due to RT-PCR- confirmed RSV through 150 days after Dose 1 for Season 1 and Season 2 Incidence of hospitalizations due to RT-PCR-confirmed RSV through 150 days after Dose 1 for Season 1 and Season 2
	Exploratory	
RSV neutralizing antibody	To determine anti-RSV neutralizing antibody levels in serum afforded by a single dose of nirsevimab compared to 5 monthly doses of palivizumab	Anti-RSV neutralizing antibody levels (IU/mL) in serum for nirsevimab recipients compared to palivizumab recipients Summary of serum RSV neutralizing antibody levels (may include GMT, GMFR, C_{max}, apparent clearance, and $t_{1/2}$)
RSV serology	To evaluate exposure to RSV by measuring seroresponses to different RSV proteins in nirsevimab and palivizumab recipients	Antibody levels to RSV F, Ga, Gb, or N at different time points Changes in RSV antibody levels (seroresponse) indicating exposure to RSV
	To evaluate the levels of maternal RSV- specific antibody in nirsevimab and palivizumab recipients	RSV antigen antibody levels (AbU/mL) to multiple RSV antigens Summary of serum RSV antibody levels (may include GMT, GMFR, seroconversion rates, apparent clearance, and $t_{1/2}$)
HRU and caregiver burden	To assess HRU and caregiver burden for nirsevimab recipients compared to palivizumab recipients	Magnitude of HRU (e.g., number of admissions to hospitals and ICUs and duration of stay; number of subjects who require respiratory support and supplemental oxygen and the duration

Type	Objective	Endpoint
		of use; number and ty pe of outpatient visits [e.g., ER, urgent care, outpatient clinic]; and number of prescription and OTC medications and duration of use) for nirsevimab recipients compared to palivizumab recipients Caregiver burden (e.g., caregiver missed workdays, subject absence from day care) for subjects with MA LRTI caused by RT-PCR-confirmed RSV
RSV resistance monitoring	To characterize resistance to nirsevimab and palivizumab through genotypic and phenotypic analyses	Genotypic analysis and susceptibility of RSV variants to neutralization by nirsevimab and palivizumab
RSV LRTI after Day 151	To assess the incidence of MA LRTI due to RT-PCR-confirmed RSV, compared to palivizumab after Day 151	Incidence of MA LRTI (inpatient and outpatient) due to RT-PCR- confirmed RSV from Day 152 to Day 361 for Season 1 and Season 2

Source: Applicant's protocol synopsis
Abbreviations: AbU/mL, antibody unit per mL; ADA, anti-drug antibody; AESI, adverse event of special interest; AUC, area under the concentration-time curve ; CHD, congenital heart disease; CLD, chronic lung disease; C_{max}, maximum observed concentration; ER, emergency room; GMFR, geometric mean fold-rise; GMT, geometric mean titer; HRU, healthcare resource utilization; ICU, intensive care unit; IM, intramuscular; LRTI, lower respiratory tract infection; NOCD, new onset chronic disease; OTC, over- the-counter; PK, pharmacokinetic; RSV, respiratory syncytial virus; RT-PCR, reverse transcriptase-polymerase chain reaction; $t_{1/2}$, terminal half-life; TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event

Trial Design

Trial 05 is a pivotal phase 2/3 randomized, double-blind, palivizumab-controlled study to evaluate the safety, PK, ADA response, and descriptive efficacy for nirsevimab in high-risk infants eligible to receive palivizumab when entering their first or second RSV season (Season 1 or Season 2, respectively). Approximately 900 palivizumab-eligible infants entering their first RSV season will be enrolled into one of two cohorts: (1) preterm cohort, including approximately 600 preterm infants (≤35 weeks gestational age [GA]) without CLD/CHD, or (2) CLD/CHD cohort, including approximately 300 infants with CLD of prematurity or hemodynamically significant CHD. A minimum of 100 infants with hemodynamically significant CHD will be enrolled. Within each cohort, randomization will be stratified by hemisphere (northern, southern) and subject age at the time of Season 1 randomization (≤3 months, >3 to ≤6 months, >6 months).

Season 1, Preterm and CLD/CHD Cohorts

All subjects will be randomized 2:1 to either the nirsevimab group (approximately 600 subjects, including approximately 400 subjects in the preterm cohort and approximately 200 subjects in the CLD/CHD cohort) or palivizumab group (approximately 300 subjects, including approximately 200 subjects in the preterm cohort and approximately 100 subjects in the CLD/CHD cohort). Subjects in the nirsevimab group will receive a single fixed IM dose of nirsevimab followed by 4 once-monthly IM doses of placebo. The nirsevimab dose level will be stratified by weight band, i.e., 50 mg for infants weighing <5 kg or 100 mg for infants weighing ≥5 kg. Subjects in the palivizumab group will receive 5 once-monthly IM doses of 15 mg/kg palivizumab.

Season 2, CLD/CHD Cohort Only

- Subjects with CLD/CHD ≤24 months of age who were randomized to the nirsevimab group for Season 1 will receive a single fixed IM dose of 200 mg nirsevimab followed by 4 once-monthly IM doses of placebo (approximately 200 subjects).
- Subjects with CLD/CHD ≤24 months of age who were randomized to the palivizumab group for Season 1 will be re-randomized 1:1 to either the nirsevimab group or the palivizumab group. Subjects in the nirsevimab group will receive a single fixed IM dose of 200 mg nirsevimab followed by 4 once-monthly IM doses of placebo (approximately 50 subjects). Subjects in the palivizumab group will receive 5 once-monthly IM doses of 15 mg/kg palivizumab (approximately 50 subjects).

In Season 1 or Season 2, subjects in the CLD/CHD cohort who undergo cardiac surgery with cardiopulmonary bypass after receipt of Dose 1 but prior to receipt of Dose 5 will receive a replacement dose of the study drug they received for Dose 1 immediately following the surgery when determined by the physician to be medically stable for an IM injection. Any subsequent doses of study drug will continue to be given according to the protocol-specified dosing schedule.

Subjects in the preterm cohort will be followed through 1 year after Season 1/Dose 1, and subjects in the CLD/CHD cohort will be followed through 1 year after Season 2/Dose 1. Subjects

in the CLD/CHD cohort who receive a replacement dose in Season 2 will be followed through 1 year after the last replacement dose.

Subjects will be monitored throughout the study for LRTI. All subjects seeking medical attention for a respiratory illness (in either the inpatient or outpatient setting) will be evaluated for LRTI, including protocol- defined MA RSV LRTI. All subjects evaluated for LRTI will have respiratory samples obtained and tested centrally for RSV using the United States Food and Drug Administration-cleared and Conformité Européenne or European Conformity-marked in vitro diagnostic real-time RT-PCR assay.

Blood samples will be collected for PK, ADA, and RSV neutralizing antibody and RSV serology.

Target Subject Population

Preterm infants entering their first RSV season who are eligible to receive palivizumab, and children with CLD or CHD who are entering their first RSV season and the same children ≤24 months of age entering their second RSV season.

Treatment Groups and Regimens

Subjects will be randomly assigned to receive study drug as described in the Study Design section and presented in the table below.

Table 89. Trial 05 Dosing Regimens

Cohort	Season 1			Season 2		
Preterm (N=600)	Nirsevimab (N=400)	Dose 1	50 mg (if <5 kg) or 100 mg (if ≥5 kg) IM	Not applicable		
		Doses 2, 3, 4, 5	Placebo IM			
	Palivizumab (N=200)	Doses 1, 2, 3, 4, 5	15 mg/kg IM			
CLD/CHD (N=300)	Nirsevimab (N=200)	Dose 1	50 mg (if <5 kg) or 100 mg (if ≥5 kg) IM	MEDI8897 (N=200)	Dose 1	200 mg IM
		Doses 2, 3, 4, 5	Placebo IM		Doses 2, 3, 4, 5	Placebo IM
	Palivizumab (N=100)	Doses 1, 2, 3, 4, 5	15 mg/kg IM	MEDI8897 (N=50)	Dose 1	200 mg IM
					Doses 2, 3, 4, 5	Placebo IM
				Palivizumab (N=50)	Doses 1, 2, 3, 4, 5	15 mg/kg IM

Source: Applicant's protocol synopsis
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; IM, intramuscular; N, number of subjects

Statistical Methods

Sample Size

With respect to safety, 600 subjects exposed to nirsevimab in Season 1 will provide a 95% probability of observing at least 1 adverse event (AE) if the true event rate is 0.5%; if no AEs are

observed, this study provides 95% confidence that the true event rate is <0.5%. The sample size is for safety consideration.

With respect to efficacy, approximately 600 subjects will be exposed to nirsevimab and 300 subjects will be exposed to palivizumab in Season 1 to observe numerically similar efficacy for both monoclonal antibodies. Because of the reduced incidence of RSV disease in this population following the introduction of palivizumab, a superiority or noninferiority design is not practical. A valid noninferiority margin cannot be established due to the lack of historical efficacy data for the MA RSV LRTI endpoint for palivizumab.

Therefore, there is no hypothesis testing for efficacy. Using an assumption of a 6% RSV LRTI rate in palivizumab recipients, approximately 18 events will be observed in that group. The 6% RSV LRTI rate (1.9% RSV hospitalizations and 3.9% outpatient RSV illness) was based on a prior study in preterm infants with and without CLD who received palivizumab. Assuming a 6% rate of RSV LRTI in nirsevimab recipients, 600 nirsevimab subjects in Season 1 will provide approximately 36 events in that group. However, because of the largely reduced RSV circulation due to COVID-19 pandemic-related measures, the observed event rates could be much lower. Only summaries will be provided for efficacy unless specified otherwise.

Statistical Analyses

There are 3 planned analyses for this study: the primary analysis, Season 2 analysis, and the final analysis. The primary analysis will be conducted after all randomized subjects have completed follow-up through the first 5-month RSV season (i.e., Season 1 Day 151 visit) and include all available Season 1 safety, efficacy, PK, and ADA data at the time of the data cutoff. The Season 2 analysis will be conducted after all CLD/CHD subjects have completed follow-up through the second 5-month RSV season (i.e., Season 2 Day 151 visit) and include all available Season 1 data and Season 2 safety, efficacy, PK, and ADA data at the time of the data cutoff. The final analysis will be conducted when all subjects have completed the last visit of the study and include all data collected in the study.

Safety

Safety of nirsevimab will be summarized by treatment group based on the As-treated Population (defined as all subjects who receive any investigational product analyzed according to treatment received) for each season, as well as for the 2 consecutive RSV seasons (i.e., Season 1 and Season 2). For the Season 1 summary, the analysis dataset will include subjects from the preterm cohort and CLD/CHD cohort, presented by the treatment received in Season 1; for the Season 2 summary and the 2 consecutive-season summary, the analysis dataset will include subjects from the CLD/CHD cohort, presented by the treatment received through the 2 seasons.

AEs will be graded according to the current version of the National Cancer Institute Common Terminology Criteria for Adverse Events where applicable for pediatric assessments. AEs will be coded by Medical Dictionary for Regulatory Activities and the type, incidence, severity, and relationship to study drug will be summarized by treatment group. Other safety assessments will include the occurrence of AESIs, defined as AEs of hypersensitivity to study drug (including anaphylaxis), thrombocytopenia, and immune complex disease (e.g., vasculitis, endocarditis, neuritis, glomerulonephritis), and NOCDs following study drug administration.

Efficacy

All efficacy summaries will be based on the Intent-to-treat (ITT) Population (defined as all randomized subjects analyzed according to randomized treatment assignment). The incidence of MA RSV LRTI (inpatient and outpatient) through 150 days post Dose 1 (i.e., during a typical 5-month RSV season) in Season 1, based on RSV test results (performed centrally using real-time RT-PCR) and objective protocol-defined LRTI criteria, is the primary efficacy endpoint and will be summarized by Season 1 treatment group for all 900 subjects. The 95% confidence interval (CI) of the percentage of subjects meeting the primary efficacy endpoint will be presented by treatment group. In addition, the incidence of RSV LRTI through 150 days post Dose 1 in Season 2 will be summarized for the 300 subjects in the CLD/CHD cohort based on the treatment assignments through Season 1 and Season 2: i.e., (a) nirsevimab (Season 1)/nirsevimab (Season 2), (b) palivizumab (Season 1)/nirsevimab (Season 2), and (c) palivizumab (Season 1)/palivizumab (Season 2).

The incidence of RSV hospitalization through 150 days after dosing (i.e., during the 5-month RSV season) will be summarized by treatment group using a similar strategy as described for RSV LRTI.

PK

Individual nirsevimab and palivizumab serum concentration data will be tabulated by treatment group along with descriptive statistics. PK parameters will be estimated using noncompartmental analysis, if data permit.

ADA

The incidence of ADA to nirsevimab and to palivizumab will be assessed and summarized by number and percentage of subjects who are ADA positive by treatment group. The impact of ADA on PK, and association with TEAEs and TESAEs, will be assessed.

RSV Neutralizing Antibody

Individual nirsevimab and palivizumab serum anti-RSV neutralizing antibody levels will be tabulated by treatment group along with descriptive statistics. Anti-RSV neutralizing antibody levels in serum will be summarized by geometric mean titer and geometric mean-fold rise and corresponding 95% CI for each treatment group at each visit. Anti-RSV neutralizing antibody level $t_{1/2}$ will be estimated using noncompartmental analysis, if data permit.

RSV Serology

Analysis of anti-RSV antigens antibody levels in serum in nirsevimab and palivizumab recipients will be summarized by geometric mean titer and geometric mean-fold rise and corresponding

95% CI for each treatment group at each visit. Seroresponses in nirsevimab and palivizumab recipients will be determined by examining the fold-rise in antibodies to Ga, Gb, and N antigens.

HRU and Caregiver Burden

The HRU and caregiver burden summaries will be performed on the ITT Population. The magnitude of HRU (e.g., number of admissions to hospitals and ICUs and duration of stay; number of subjects who require respiratory support and supplemental oxygen and the duration of use; number and types of outpatient visits, e.g., ER, urgent care, outpatient clinic; and number of prescription and OTC medications and duration of use) will be summarized overall by treatment group and for subjects with at least one MA LRTI (protocol defined) caused by RT-PCR-confirmed RSV.

Caregiver burden (e.g., caregiver missed workdays, subject absence from day care) for subjects with MA LRTI (protocol defined) caused by RT-PCR-confirmed RSV will be summarized by treatment group.

Monitoring RSV Resistance to Nirsevimab and Palivizumab

Genotypic analysis of the full-length mature F protein will be conducted on all RSV-positive isolates confirmed centrally using the Lyra RSV+human metapneumovirus real-time RT-PCR assay manufactured by Quidel Corporation. RSV genotypic analysis will report the sequence changes in the mature F protein from all RSV positive isolates compared to contemporary RSV A and RSV B reference strains. Susceptibility of novel RSV variants to nirsevimab and palivizumab will be tested and compared to control viruses.

RSV LRTI Occurring From Day 152 to Day 361

The incidence of MA RSV LRTI (inpatient and outpatient) from Day 152 to Day 361 for Season 1 and Season 2 will be based on RSV test results (performed centrally via RT-PCR) and objective clinical LRTI criteria and will be summarized by treatment group.

16. Efficacy

Trial 03

Demographics

This section summarizes demographics by country of recruitment based on data submitted by the Applicant. Please see additional demographic information in Table 14.

Table 90. Baseline Demographic Characteristics, Trial 03 (Continued)

Demographic	Nirsevimab 50 mg N=969	Placebo N=484	Total N=1453
Country, n (%)			
Argentina	7 (<1)	0	7 (<1)
Australia	10 (1.0)	3 (<1)	13 (<1)
Belgium	0	2 (<1)	2 (<1)
Brazil	18 (1.9)	8 (1.7)	26 (1.8)
Bulgaria	86 (8.9)	55 (11.4)	141 (9.7)
Canada	3 (<1)	0	3 (<1)
Chile	104 (10.7)	52 (10.7)	156 (10.7)

Demographic	Nirsevimab 50 mg N=969	Placebo N=484	Total N=1453
Czech Republic	17 (1.8)	7 (1.4)	24 (1.7)
Estonia	14 (1.4)	5 (1.0)	19 (1.3)
Finland	4 (<1)	3 (<1)	7 (<1)
France	27 (2.8)	11 (2.3)	38 (2.6)
Hungary	83 (8.6)	37 (7.6)	120 (8.3)
Italy	48 (5.0)	24 (5.0)	72 (5.0)
Latvia	6 (<1)	6 (1.2)	12 (<1)
Lithuania	1 (<1)	4 (<1)	5 (<1)
New Zealand	6 (<1)	7 (1.4)	13 (<1)
Poland	23 (2.4)	15 (3.1)	38 (2.6)
South Africa	165 (17.0)	85 (17.6)	250 (17.2)
Spain	84 (8.7)	44 (9.1)	128 (8.8)
Sweden	7 (<1)	10 (2.1)	17 (1.2)
Turkey	37 (3.8)	23 (4.8)	60 (4.1)
United Kingdom	12 (1.2)	3 (<1)	15 (1.0)
United States	207 (21.4)	80 (16.5)	287 (19.8)

Source: adsl.xpt; Tool: SAS
Abbreviations: N, number of subjects; n, number of subjects with specific demographic

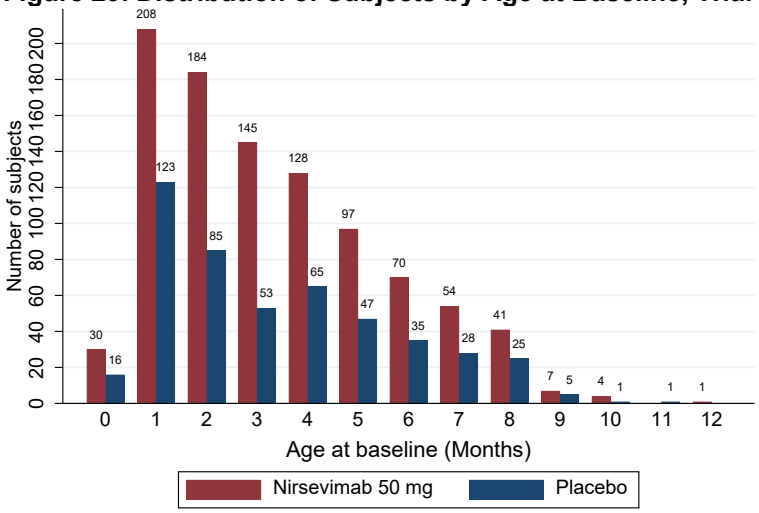
Graphical Exploration

The goal of this section is to examine the impact of baseline age, weight, and gestational age on rates of MA RSV LRTIs (infection rates).

The bar graph below (Figure 29) shows that there was only a small number of subjects who were enrolled in the trial at 8 months of age or older. Of note, since the randomization was 2:1 for nirsevimab versus placebo, the number of subjects on nirsevimab (red bars) was larger in each age category.

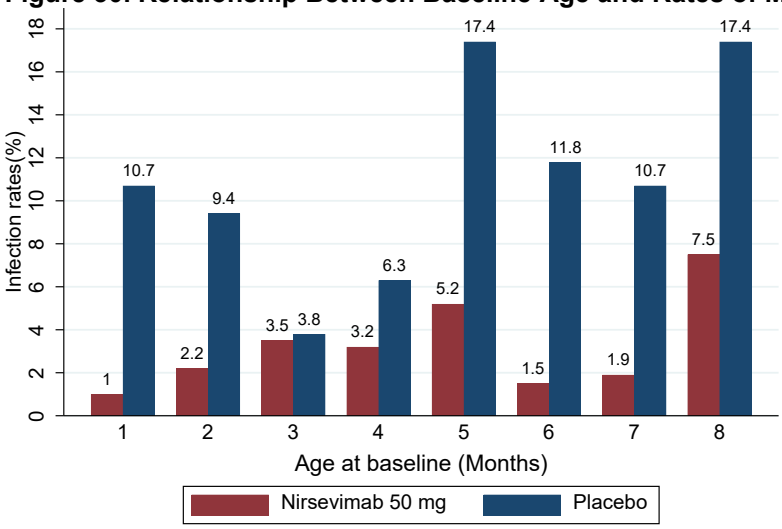
A careful examination of infection rates (Figure 30) suggest that event rates were numerically higher for subjects on placebo in each age category.

Figure 29. Distribution of Subjects by Age at Baseline, Trial 03



Source: FDA statistical reviewer
Legend: The bar graph shows the number of subjects (y-axis) by age at baseline (x-axis). The numbers on the top of the bars indicate the number of subjects in each category. Age at baseline was rounded to a whole number.

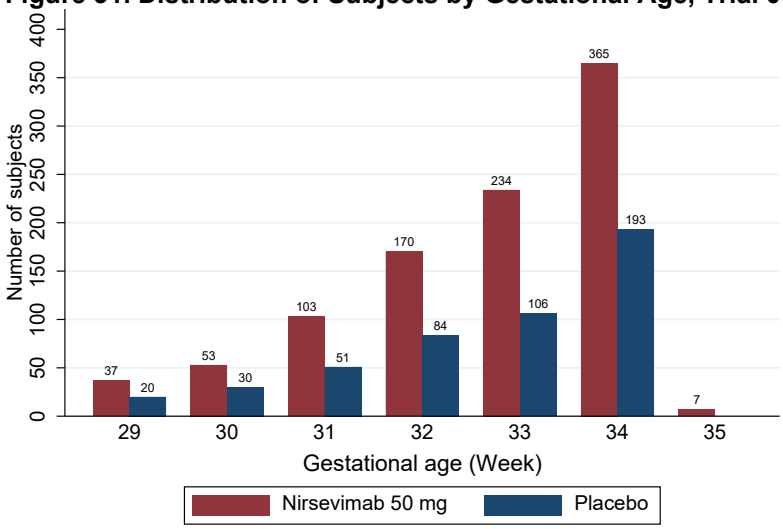
Figure 30. Relationship Between Baseline Age and Rates of MA RSV LRTI (Infection Rates)



Source: FDA statistical reviewer
Legend: The bar graph shows the percent of subjects who experienced an MA RSV LRTI (y-axis) by age at baseline (x-axis). The numbers on the top of the bars indicate the percentage of subjects in each category. Age at baseline was rounded to a whole number.
Abbreviation: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection events

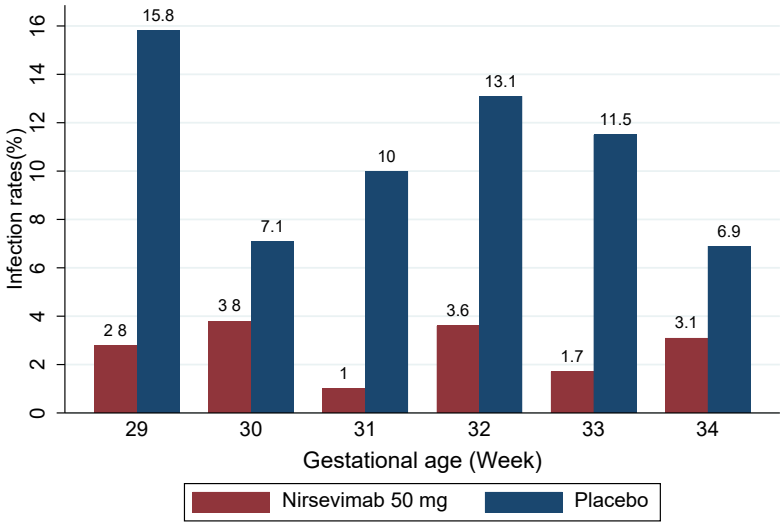
The bar graph below (Figure 31) suggest that a larger fraction of trial participants was born at 34 weeks GA. Similar to the exploration by age, placebo subjects had higher infection rates in each gestational age category (Figure 32).

Figure 31. Distribution of Subjects by Gestational Age, Trial 03



Source: FDA statistical reviewer
Legend: The bar graph shows the number of subjects (y-axis) by gestational age at birth (x-axis). The numbers on the top of the bars indicate the number of subjects in each category.

Figure 32. Relationship Between Gestational Age and Rates of MA RSV LRTI (Infection Rates), Trial 03



Source: FDA statistical reviewer
Legend: The bar graph shows the percent of subjects who experienced an MA RSV LRTI (y-axis) by gestational age at birth (x-axis). The numbers on the top of the bars indicate the percentage of subjects in each category.
Abbreviation: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection events

Very Severe RSV

Very severe RSV was defined as a patient with RSV hospitalization, requiring oxygen or IV fluids. The estimated relative risk reduction is presented in Table 91. The estimated relative risk reduction (RRR) was 87.6%, with a 95% CI of (63.1% to 95.8%) in favor of nirsevimab.

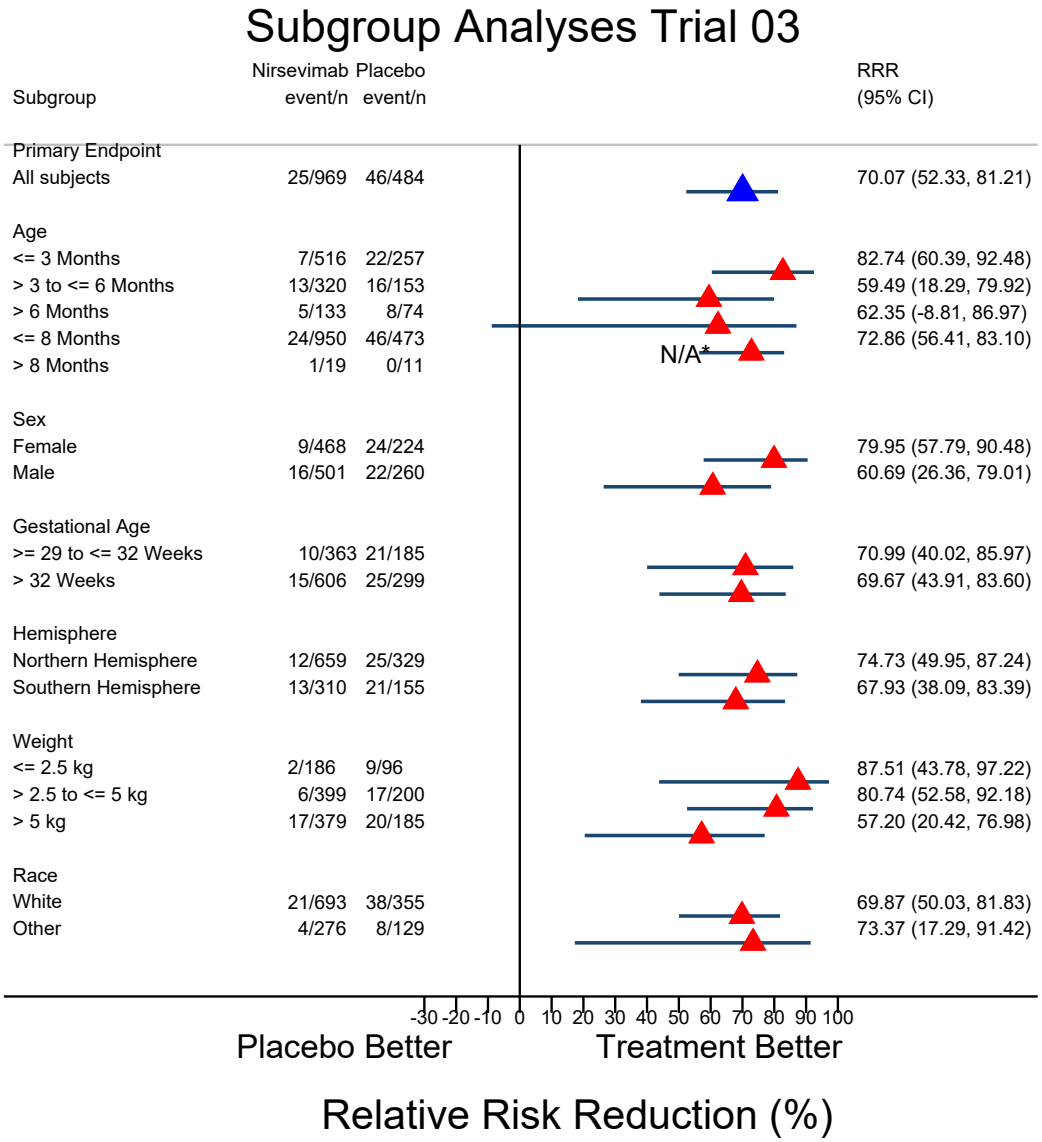
Table 91. Incidence of Very Severe RSV by Day 151 Postdose, Trial 03

	Trial 03	
Statistic	**Nirsevimab N=969**	**Placebo N=484**
Events (# of subjects, n (%))	4 (0.4)	16 (3.3)
RRR (95% CI)[§]	87.6% (63.1% to 95.8%)	

Source: FDA statistical reviewer
[§]Based on Poisson regression model with robust variance with of treatment group as a covariate. For this analysis no missing data were imputed.
Abbreviations: CI, confidence interval; N, number of subjects; n, number of subjects with specific incidence; RRR, relative risk reduction; RSV, respiratory syncytial virus

Reference ID: 5208810

Figure 33. Subgroup Analyses of the Primary Endpoint (MA RSV LRTI), Trial 03



Source: FDA statistical reviewer
Abbreviations: CI, confidence interval; n, number of subjects; N/A, not applicable; RRR, relative risk reduction; RSV, respiratory syncytial virus

Subgroup Analyses

The treatment effect based on the incidence of MA RSV LRTI (primary endpoint) was consistent across subgroups and with the overall treatment effect. Only in the subgroup of subjects older than 6 months, the lower bound of the 95% confidence interval for the estimated relative risk reduction (RRR) was below zero. This result could be due to the small subgroup size (5 events out of 133 subjects on nirsevimab and 8 events out of 734 on placebo). The detailed subgroup results are presented in Figure 33.

Trial 04

Demographics

This section summarizes demographics by country of recruitment based on data submitted by the Applicant. Please see additional demographic information in Table 20.

Table 92. Baseline Demographics Characteristics, Trial 04 (Continued)

	Primary Cohort			Safety Cohort		
Demographic	**Nirsevimab N=994**	**Placebo N=496**	**Total N=1490**	**Nirsevimab N=1015**	**Placebo N=507**	**Total N=1522**
Country, n (%)						
Argentina	0	0	0	119 (11.7)	59 (11.6)	178 (11.7)
Australia	0	0	0	19 (1.9)	8 (1.6)	27 (1.8)
Austria	3 (<1)	2 (<1)	5 (<1)	3 (<1)	0	3 (<1)
Belgium	5 (<1)	6 (1.2)	11 (<1)	2 (<1)	1 (<1)	3 (<1)
Bulgaria	51 (5.1)	23 (4.6)	74 (5.0)	36 (3.5)	21 (4.1)	57 (3.7)
Canada	5 (<1)	3 (<1)	8 (<1)	4 (<1)	1 (<1)	5 (<1)
Chile	0	0	0	26 (2.6)	19 (3.7)	45 (3.0)
Colombia	0	0	0	32 (3.2)	22 (4.3)	54 (3.5)
Czech Republic	9 (<1)	6 (1.2)	15 (1.0)	6 (<1)	8 (1.6)	14 (<1)
Estonia	28 (2.8)	20 (4.0)	48 (3.2)	21 (2.1)	4 (<1)	25 (1.6)
Finland	24 (2.4)	13 (2.6)	37 (2.5)	5 (<1)	2 (<1)	7 (<1)
France	9 (<1)	5 (1.0)	14 (<1)	12 (1.2)	4 (<1)	16 (1.1)
Germany	8 (<1)	5 (1.0)	13 (<1)	27 (2.7)	18 (3.6)	45 (3.0)
Israel	15 (1.5)	10 (2.0)	25 (1.7)	13 (1.3)	6 (1.2)	19 (1.2)
Italy	0	0	0	4 (<1)	3 (<1)	7 (<1)
Japan	33 (3.3)	17 (3.4)	50 (3.4)	67 (6.6)	30 (5.9)	97 (6.4)
Korea (The Republic of)	1 (<1)	1 (<1)	2 (<1)	0	0	0
Latvia	23 (2.3)	11 (2.2)	34 (2.3)	11 (1.1)	5 (1.0)	16 (1.1)
Lithuania	27 (2.7)	12 (2.4)	39 (2.6)	11 (1.1)	7 (1.4)	18 (1.2)
Mexico	0	0	0	1 (<1)	0	1 (<1)
New Zealand	0	0	0	15 (1.5)	6 (1.2)	21 (1.4)
Panama	0	0	0	342 (33.7)	163 (32.1)	505 (33.2)
Poland	19 (1.9)	7 (1.4)	26 (1.7)	29 (2.9)	3 (<1)	32 (2.1)
Russian Federation	33 (3.3)	20 (4.0)	53 (3.6)	25 (2.5)	15 (3.0)	40 (2.6)
South Africa	308 (31.0)	154 (31.0)	462 (31.0)	0	0	0
Spain	82 (8.2)	39 (7.9)	121 (8.1)	68 (6.7)	24 (4.7)	92 (6.0)

	Primary Cohort			Safety Cohort		
Demographic	**Nirsevimab N=994**	**Placebo N=496**	**Total N=1490**	**Nirsevimab N=1015**	**Placebo N=507**	**Total N=1522**
Sweden	6 (<1)	5 (1.0)	11 (<1)	15 (1.5)	6 (1.2)	21 (1.4)
Turkey	0	0	0	9 (<1)	7 (1.4)	16 (1.1)
Ukraine	0	0	0	26 (2.6)	8 (1.6)	34 (2.2)
United Kingdom	4 (<1)	3 (<1)	7 (<1)	1 (<1)	0	1 (<1)
United States	301 (30.3)	134 (27.0)	435 (29.2)	66 (6.5)	57 (11.2)	123 (8.1)

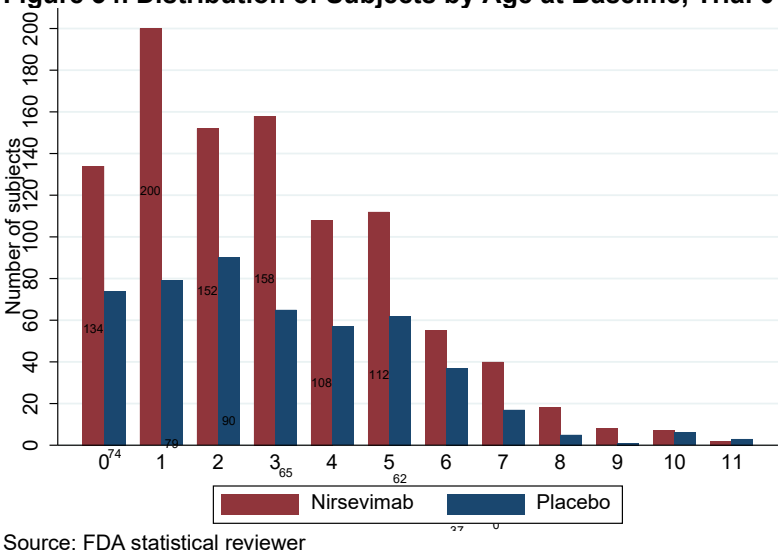
Source: adsl.xpt
Abbreviations: N, number of subjects; n, number of subjects with specific demographic

Graphical Exploration

Similar to Trial 03, the bar graph below (Figure 34) shows that there was only a small number of subjects who were enrolled in the trial at 8 months of age or older. Of note, since the randomization was 2:1 for nirsevimab versus placebo, the number of subjects on nirsevimab (red bars) was larger in each age category.

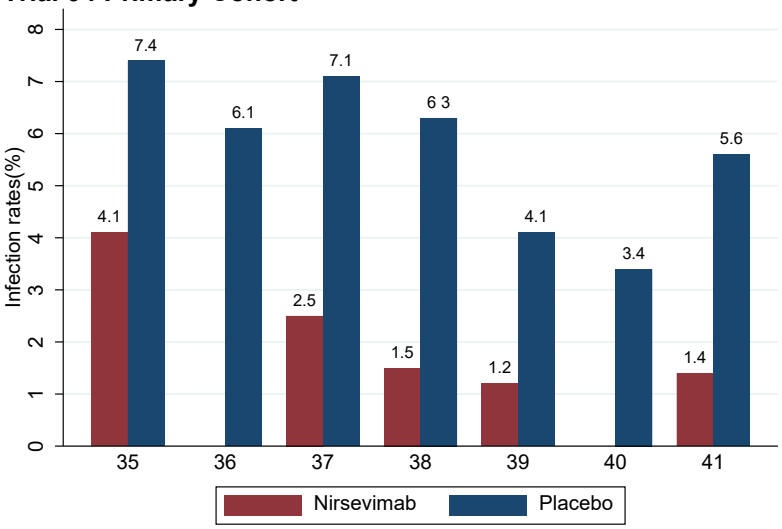
A careful examination of infection rates (Figure 35) suggests that event rates were numerically higher for subjects on placebo in each age category.

Figure 34. Distribution of Subjects by Age at Baseline, Trial 04 Primary Cohort



Source: FDA statistical reviewer

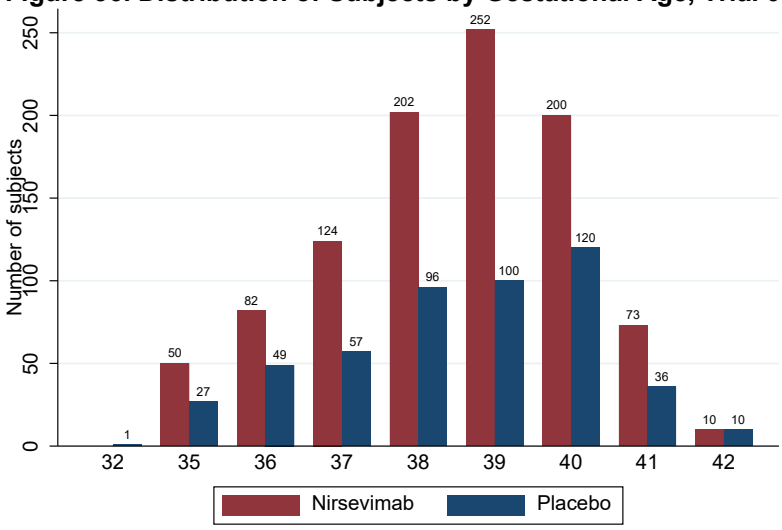
Figure 35. Relationship Between Baseline Age and Rates of MA RSV LRTI (Infection Rates), Trial 04 Primary Cohort



Source: FDA statistical reviewer
Abbreviation: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection events

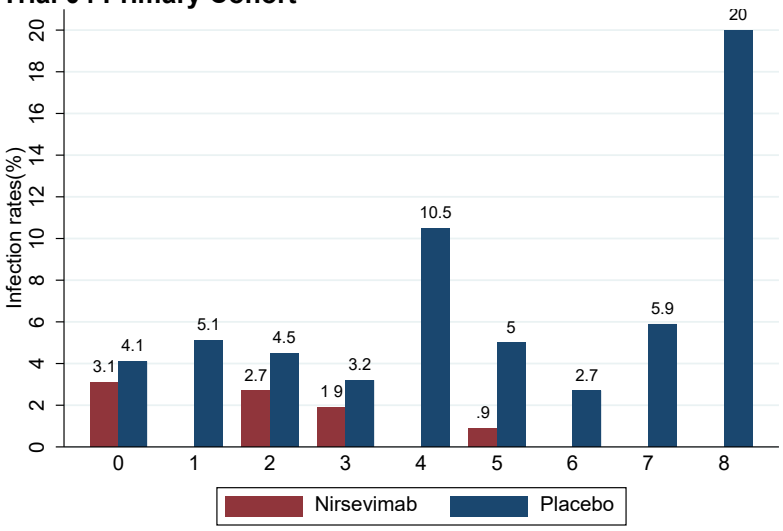
The bar graph below (Figure 36) suggest that a larger fraction of trial participants was born at 39 weeks GA. Similar to the exploration by age, placebo subjects had higher infection rates in each gestational age category (Figure 37).

Figure 36. Distribution of Subjects by Gestational Age, Trial 04 Primary Cohort



Source: FDA statistical reviewer

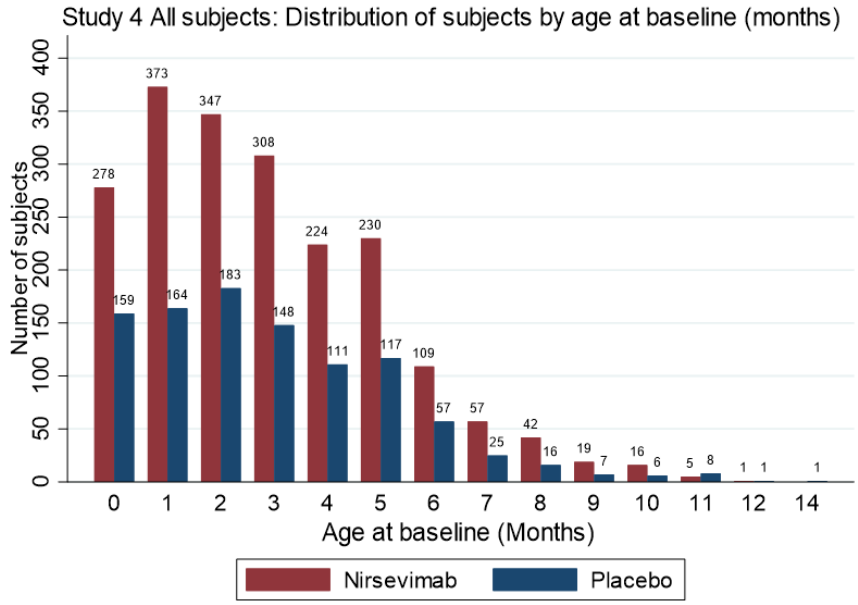
Figure 37. Relationship Between Gestational Age and Rates of MA RSV LRTI (Infection Rates), Trial 04 Primary Cohort

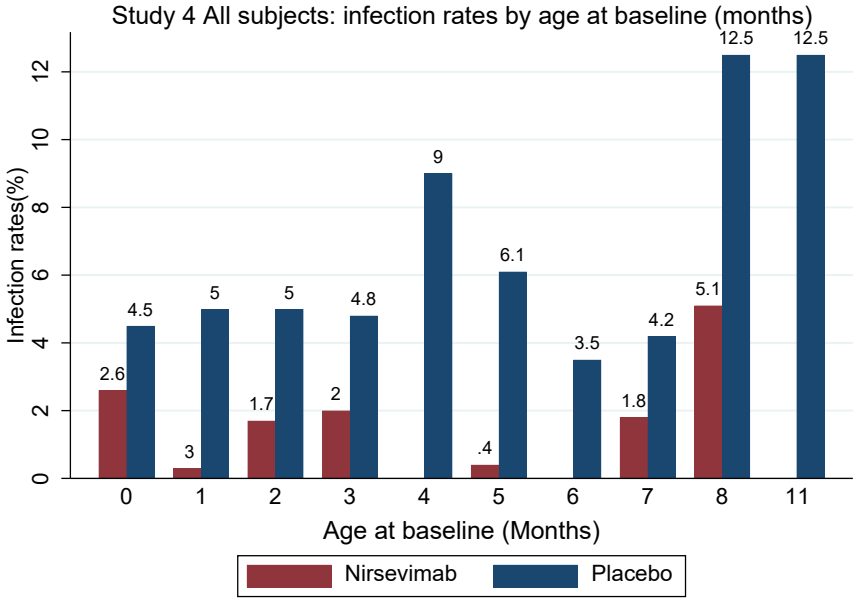


Source: FDA statistical reviewer
Abbreviation: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection events

After both Primary and Safety Cohorts were combined, the patterns in distribution of MA RSV LRTI events were similar to the pattern of events in the Primary Cohort alone (Figure 38 and Figure 39).

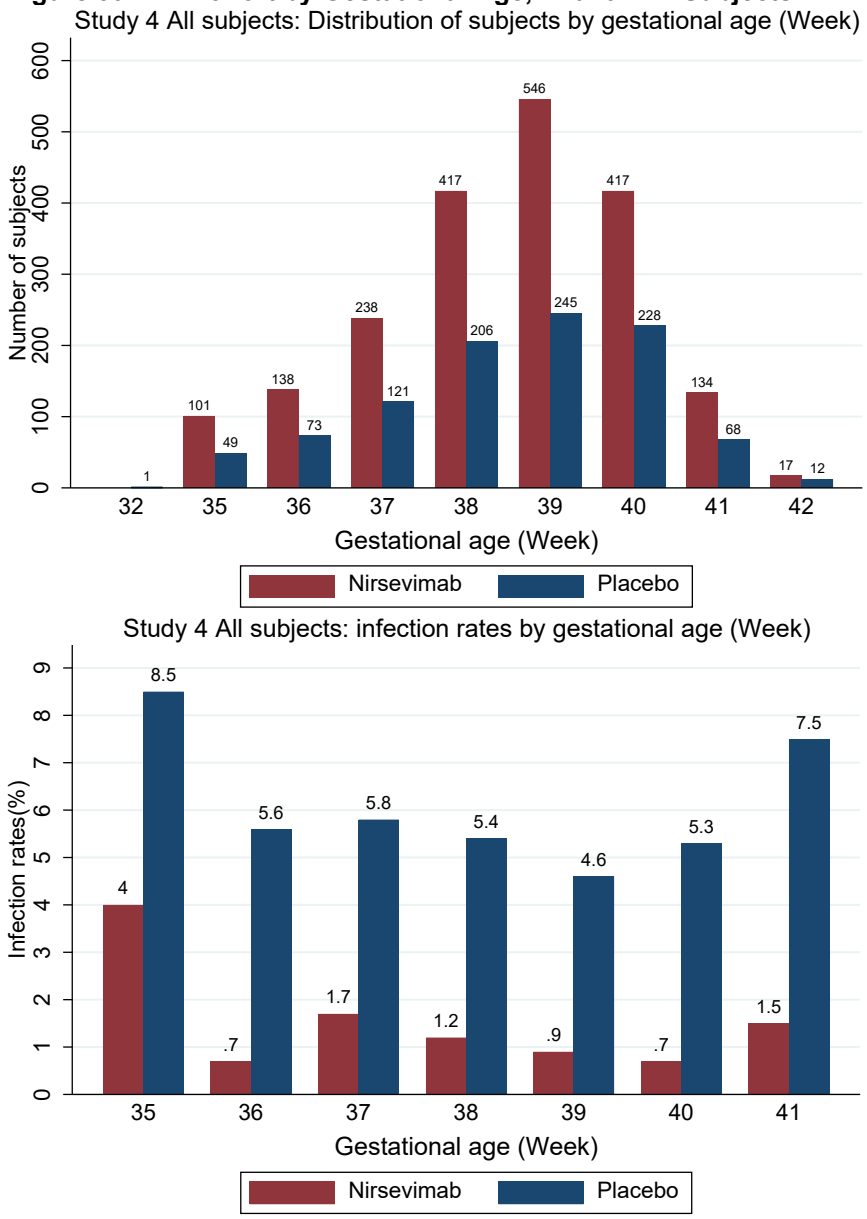
Figure 38. ITT Cohort by Age, Trial 04 All Subjects





Source: FDA statistical reviewer
Abbreviations: ITT, intent-to-treat

Figure 39. ITT Cohort by Gestational Age, Trial 04 All Subjects



Source: FDA statistical reviewer
Abbreviations: ITT, intent-to-treat

Very Severe RSV (Primary Cohort)

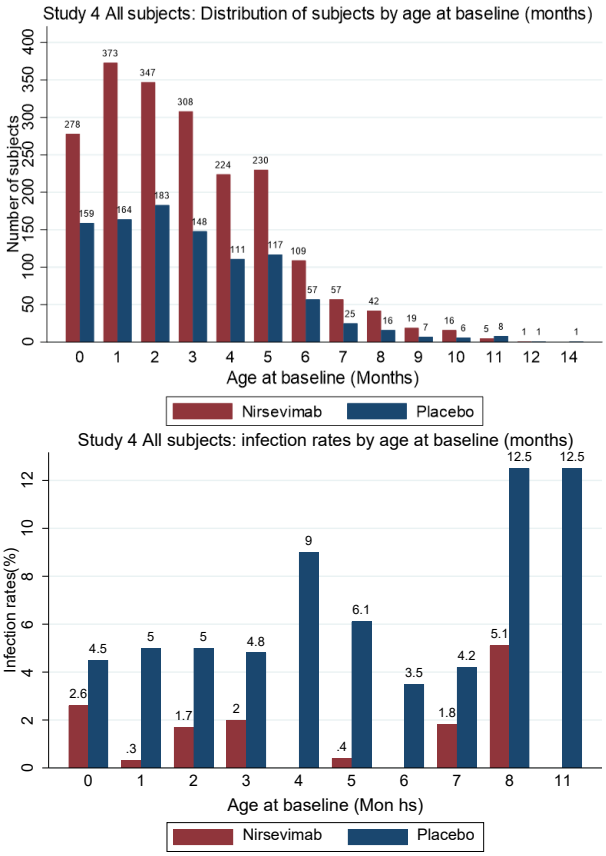
Very severe RSV was defined as a patient with RSV hospitalization events, requiring oxygen or IV fluids. The estimated relative risk reduction is presented in Table 93.

Table 93. Incidence of Very Severe RSV by Day 151 Postdose, Trial 04 Primary Cohort

	Trial 04 Primary Cohort	
Statistic	**Nirsevimab N=994**	**Placebo N=496**
Events (# of subjects, n (%))	5 (0.5)	7(1.4)
RRR (95% CI)§	64.3% (-11.9% to 88.6%)	

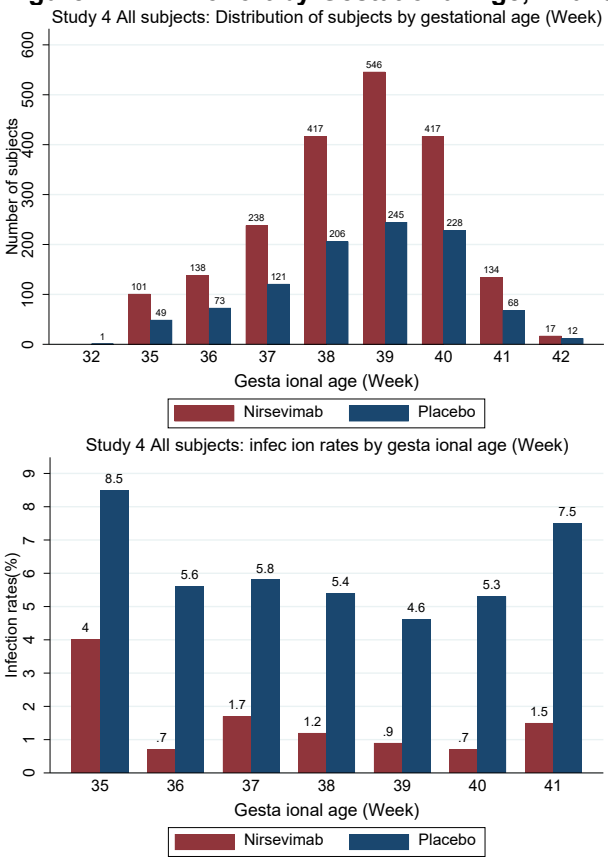
Source: FDA statistical reviewer; adeff3.xpt; tool: SAS
§Based on Poisson regression model with robust variance with of treatment group as a covariate. For this analysis no missing data were imputed.
Abbreviations: CI, confidence interval; N, number of subjects; n, number of subjects with specific incidence; RRR, relative risk reduction; RSV, respiratory syncytial virus

Figure 40. ITT Cohort by Age, Trial 04 All Subjects



Source: FDA statistical reviewer
Abbreviation: ITT, intent-to-treat

Figure 41. ITT Cohort by Gestational Age, Trial 04 All Subjects



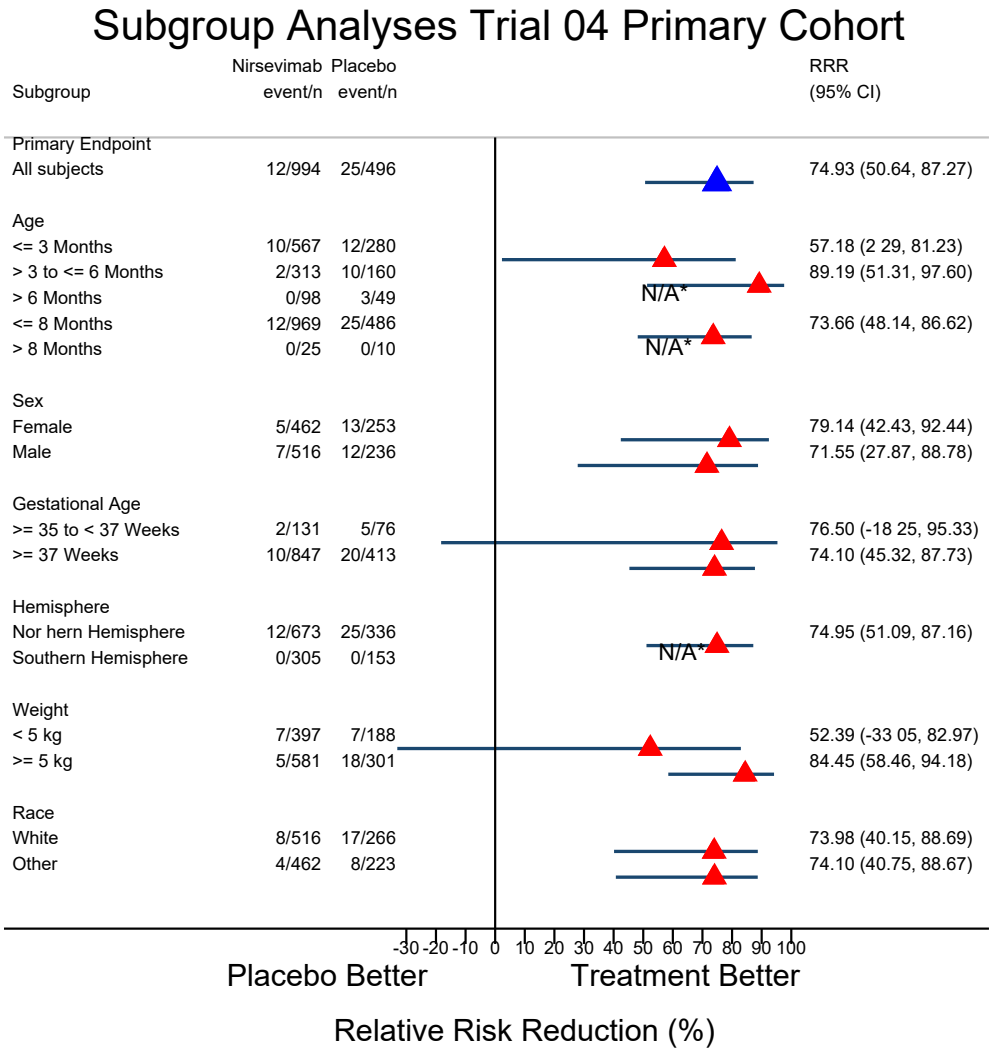
Source: FDA statistical reviewer
Abbreviation: ITT, intent-to-treat

Subgroup Analyses (for the Primary Cohort)

The treatment effects based on the incidence of MA RSV LRTI (primary endpoint) were consistent across subgroups and with the overall treatment effect. There were no events in the subgroup of subjects older than 6 months among participants treated with nirsevimab. Similarly, there were no events in the Southern Hemisphere. Because of it, the outcomes in those subgroups could not be evaluated.

For the subgroups with subjects born between 35 and 37 weeks of gestation and subjects weighing less than 5 kg, the lower bound of the 95% confidence interval for the estimated relative risk reduction (RRR) was below zero. This result could be due to the small subgroup size (2 events out of 131 subjects on nirsevimab and 5 events out of 76 on placebo for subjects who were born 35 to 37 weeks of gestation; 7 events out of 397 and 7 events out of 188 for subjects who weigh less than 5 kg at baseline) in subjects on nirsevimab and placebo, respectively. The detailed subgroup results are presented in Figure 42.

Figure 42. Primary Cohort Subgroup Analyses, Trial 04



Source: FDA statistical reviewer
*The RRR cannot be calculated because the number of events was not sufficient
Abbreviations: CI, confidence interval; n, number of subjects; N/A, not applicable; RRR, relative risk reduction

Appropriateness of Pooling (Primary and Safety Cohorts)

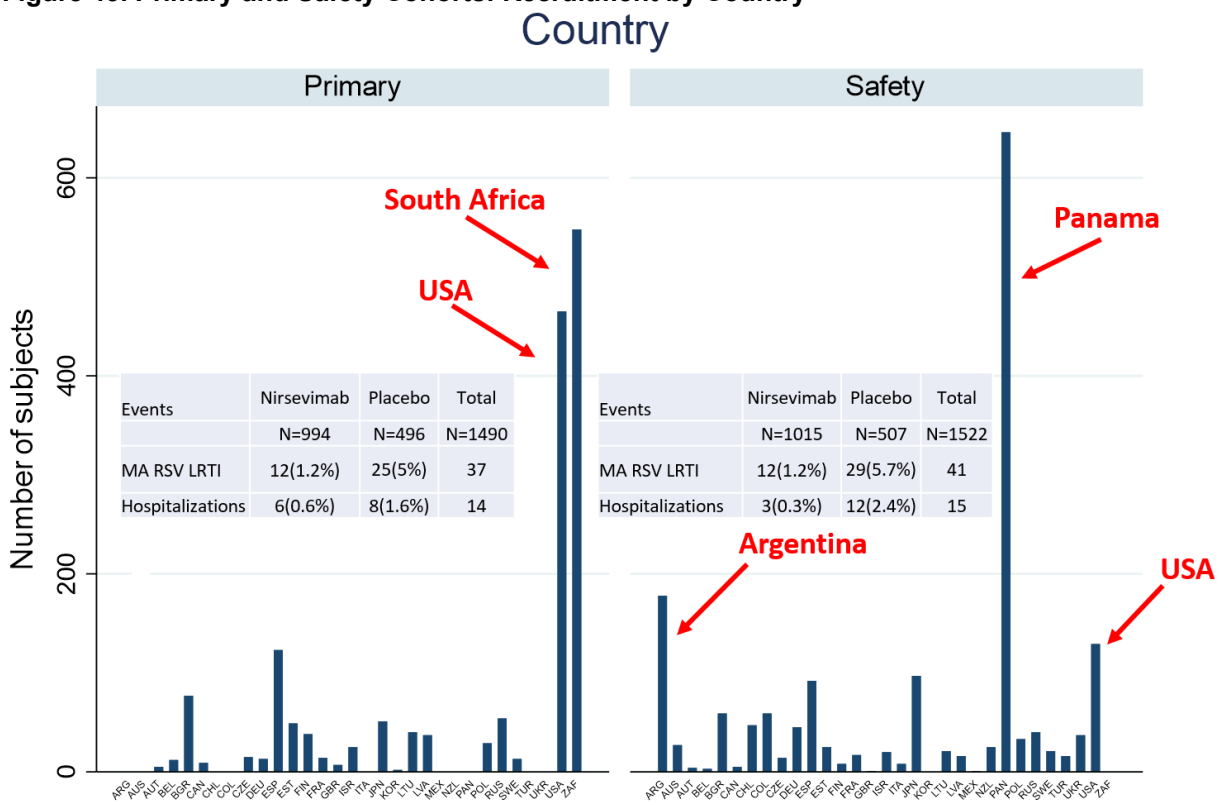
The purpose of this section is to examine appropriateness of pooling of Primary and Safety Cohorts together. This pooled analysis was not prespecified and therefore, will only be considered as exploratory for supportive evidence.

The demographic and baseline characteristics between the Primary and Safety Cohorts were rather similar (Table 88), except for race. The Primary Cohort included 422 (28%) of Black or African American subjects and Safety Cohort randomized only 15 (1%) Black or African American participants. The largest race group (518 subjects, 34%) in the Safety Cohort was described as 'Other', while only 108 (7%) of subjects from the Primary Cohort were categorized as 'Other'. These differences in racial composition could be explained by the country locations for the clinical trial sites that enrolled subjects in the two cohorts.

A graphical representation of trial enrollment by country is presented in Figure 43. The largest groups of subjects in the Primary Cohort were from the USA and South Africa; and the largest groups of subjects in the Safety Cohort were from Panama, Argentina, and the USA. A detailed list of recruitment by country is presented in Table 92.

Of note, the rates of MA RSV LRTI and RSV hospitalization events were similar in both cohorts (table inset, Figure 43).

Figure 43. Primary and Safety Cohorts: Recruitment by Country



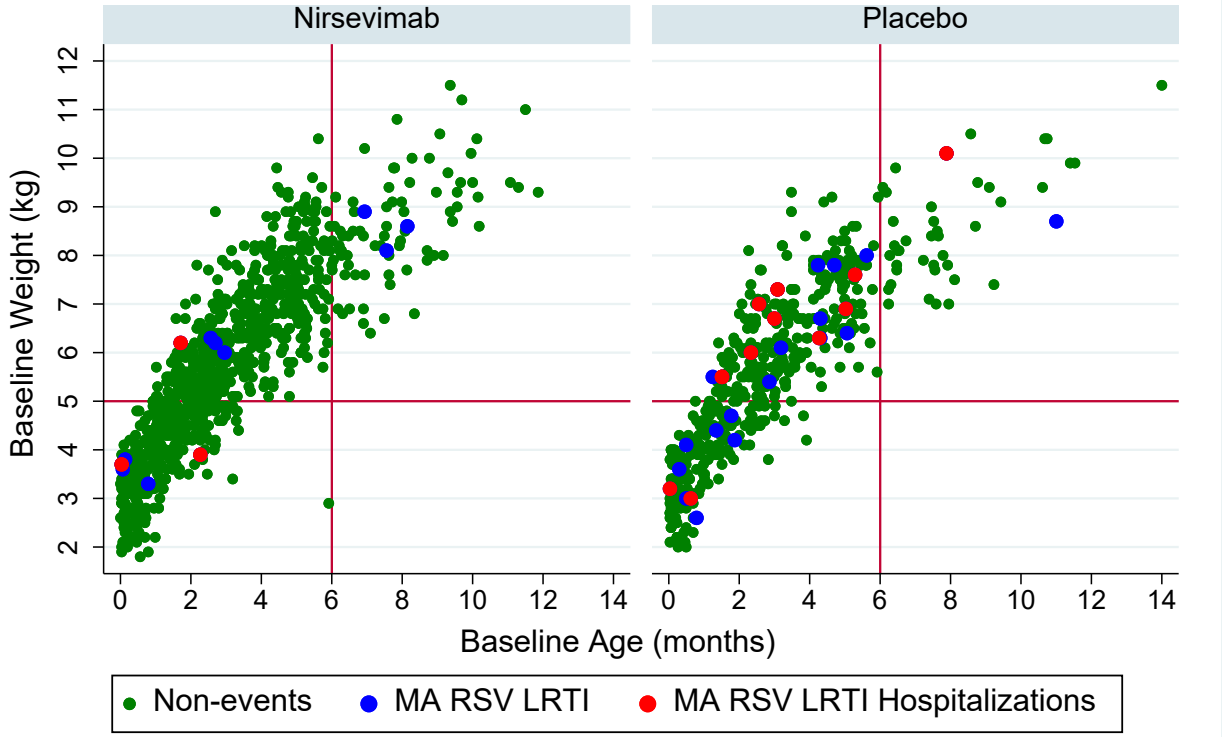
Events	Nirsevimab	Placebo	Total
	N=994	N=496	N=1490
MA RSV LRTI	12(1.2%)	25(5%)	37
Hospitalizations	6(0.6%)	8(1.6%)	14

Events	Nirsevimab	Placebo	Total
	N=1015	N=507	N=1522
MA RSV LRTI	12(1.2%)	29(5.7%)	41
Hospitalizations	3(0.3%)	12(2.4%)	15

Source: FDA statistical reviewer
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects

The distribution of baseline age and weight followed similar patterns in both Primary and Safety Cohorts (Figure 44 and Figure 45). In both cohorts, the majority of subjects were younger than 8 months old, and subjects older than 6 months received a 100 mg dose.

Figure 44. Relationship Between Baseline Weight, Baseline Age, and Outcome Status (Day 151), Trial 04 Safety Cohort



Source: FDA statistical reviewer
Legend: Relationship between baseline weight and baseline age for subjects who did not experience MA RSV LRTI (green circles), subjects who experienced MA RSV LRTI without hospitalization (blue circles), and those who experienced MA RSV LRTI and were hospitalized (red circles). Each circle represents age and weight at baseline so that each subject is represented only once.
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

Given similarity in demographic, baseline characteristics, event rates, and identical inclusion criteria, it is acceptable to pool the Primary and Safety Cohorts together for the purpose of exploratory analyses.

Although the enrollment into the Safety Cohort started after the unblinded data from the Primary Cohort was analyzed, there is no evidence to suggest any potential bias introduced in the enrollment for the Safety Cohort, and further, both of these cohorts had independent randomization processes.

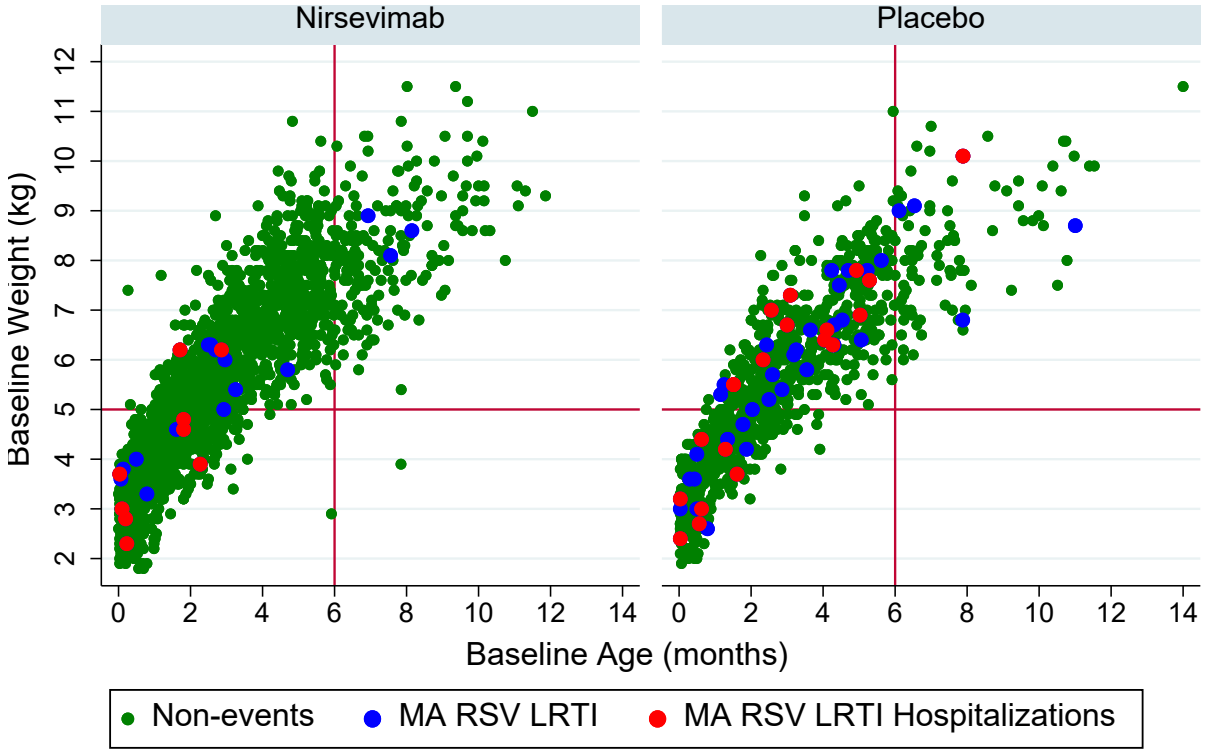
Overall Outcome and Visualization of the Data (Pooled)

After both Primary and Safety Cohorts were combined, despite the 2:1 randomization rate of nirsevimab to placebo, there were more subjects on placebo who experienced events than subjects who had events after being treated with nirsevimab. All subjects older than 6 months of age except for one participant in the nirsevimab treatment arm weighed more than 5 kg and therefore received a 100 mg dose of nirsevimab.

There were only three subjects older than 6 months of age who experienced MA RSV LRTI events in the nirsevimab group. None of those events included a hospitalization. Contrary, there were 5 events among subjects on placebo in the same age group who experienced MA RSV LRTI events (4 events without hospitalization and one event with hospitalization).

Given that there were generally more subjects on nirsevimab (randomization 2:1), the MA RSV LRTI event rates were higher among subjects on placebo in all age and weight groups.

Figure 45. Relationship Between Baseline Weight, Baseline Age, and Outcome Status (Day 151), Trial 04, All Subjects Cohort (Primary and Safety Together)



Source: FDA statistical reviewer
Legend: Relationship between baseline weight and baseline age for subjects who did not experience MA RSV LRTI (green circles), subjects who experienced MA RSV LRTI without hospitalization (blue circles), and those who experienced MA RSV LRTI and were hospitalized (red circles). Each circle represents age and weight at baseline so that each subject is represented only once.
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection

Pooled Exploratory Analyses Based on Primary and Safety Cohorts

Given similarity in demographic, baseline characteristics, event rates, and identical inclusion criteria, it was acceptable to pool the Primary and Safety Cohorts together for the purpose of exploratory analyses.

Although the enrollment into the Safety Cohort started after the unblinded data from the Primary Cohort was analyzed, there is no evidence to suggest any potential bias introduced in the enrollment for the Safety Cohort, and further, both of these cohorts had independent randomization processes.

Exploratory Analysis of MA RSV LRTI (Pooled)

The number of subjects who experienced MA RSV LRTI by Day 151 in each of the treatment arms and the results of the analysis of the primary endpoint are presented in Table 94. The estimated relative risk reduction (RRR) was 76.7% (95%CI: 62.4% to 85.6%) in favor of

nirsevimab. Further exploration of MA RSV LRTI incidence in subgroups for all-subjects cohort is presented in Figure 46.

Table 94. Incidence of MA RSV LRTI by Day 151 Postdose, Trial 04, All Subjects Cohort (Primary and Safety Together)

Statistic	Trial 04 Pooled Primary and Safety Cohorts	
	Nirsevimab N=2009	Placebo N=1003
Events (# of subjects, n (%))	24 (1.2)	54 (5.4)
Subjects requiring imputation*, n (%)	31(1.5)	17(1.7)
RRR (95% CI)§	76.7% (62.4% to 85.6%)	

Source: FDA statistical reviewer
*Subjects with missing outcomes on Day 151. The final status of those subjects was imputed based on the observed placebo rate conditional on stratification factors using multiple imputation approach.
§ Based on Poisson regression model with robust variance with of treatment group and age group at randomization (i.e., age ≤3 months, age >3 to ≤6 months, age >6 months) and dichotomous temperate (northern and southern) hemispheres as covariates
Abbreviations: CI, confidence interval; N, number of subjects; n, number of subjects with specific incidence; RRR, relative risk reduction; RSV, respiratory syncytial virus

Sensitivity Analysis

An additional analysis that assumed that all subjects on nirsevimab who had a missing outcome (i.e., subjects requiring imputation) had their outcomes imputed as events yielded a relative risk reduction of 50.9% with 95%CI (29.0%, 66.0%) in favor of nirsevimab. This result suggests that the outcome of the analysis of MA RSV LRTI was robust.

Exploratory Analysis of MA RSV LRTI With Hospitalization Events (Pooled)

In the pooled analysis (Primary and Safety Cohorts together), the number of subjects who experienced MA RSV LRTI and were hospitalized by Day 151 in each of the treatment arms and the results of the analysis of the RSV hospitalization endpoint are presented in Table 95. The estimated relative risk reduction (RRR) was 75% (95% CI: 45.7%, 88.5%) in favor of nirsevimab.

Table 95. Incidence of RSV Hospitalization Events by Day 151 Postdose, Trial 04, All Subjects Cohort (Primary and Safety Together)

Statistic	Trial 04 Pooled Primary and Safety Cohorts	
	Nirsevimab N=2009	Placebo N=1003
Events (# of subjects, n (%))	9(0.4)	20(2.0)
Subjects requiring imputation*, n (%)	31(1.5)	18(1.8)
RRR (95% CI)§	75.0% (45.7% to 88.5%)	

Source: FDA statistical reviewer
*Subjects with missing outcomes on Day 151. The final status of those subjects was imputed based on the observed placebo rate conditional on stratification factors using multiple imputation approach.
§Based on Poisson regression model with robust variance with of treatment group as a covariate.
Abbreviations: CI, confidence interval; N, number of subjects; n, number of subjects with specific incidence; RRR, relative risk reduction; RSV, respiratory syncytial virus

Reference ID: 5208810

Exploratory Analysis of Very Severe RSV Events (Pooled)

Very severe RSV was defined as in-patient, requiring oxygen or IV fluids. The estimated relative risk reduction is presented in Table 96. The estimated relative risk reduction (RRR) was 79.4% (50.7% to 91.5%) in favor of nirsevimab.

Table 96. Incidence of Very Severe RSV by Day 151 Postdose, Trial 04, All Subjects Cohort (Primary and Safety Together)

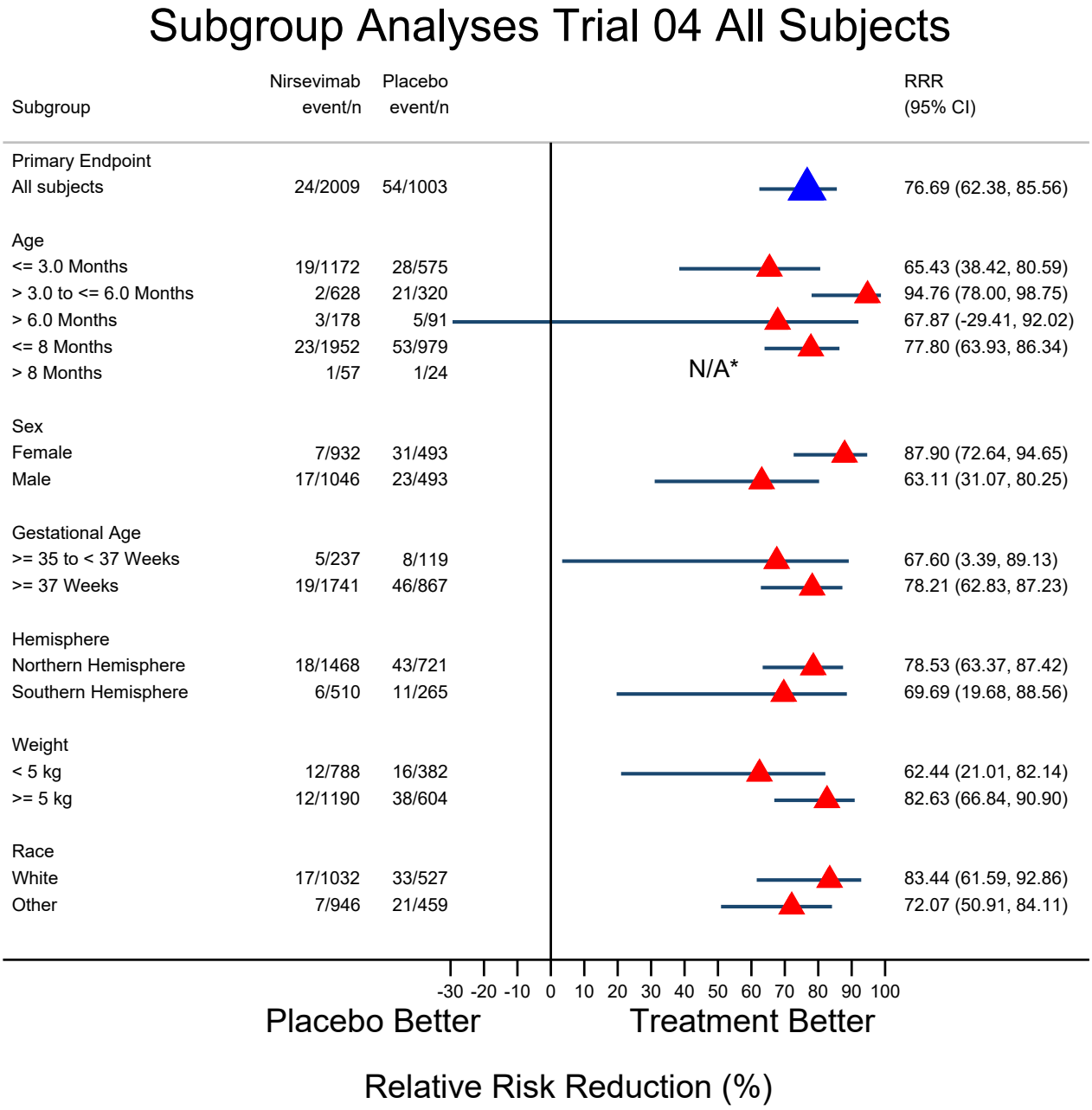
	Trial 04 Pooled Primary and Safety Cohorts	
Statistic	**Nirsevimab N=2009**	**Placebo N=1003**
Events (# of subjects, n (%))	7 (0.3)	17 (1.7)
RRR (95% CI)[§]	79.7% (50.7% to 91.5%)	

Source: FDA statistical reviewer
[§]Based on Poisson regression model with robust variance with of treatment group as a covariate. For this analysis no missing data were imputed.
Abbreviations: CI, confidence interval; N, number of subjects; n, number of subjects with specific incidence; RRR, relative risk reduction; RSV, respiratory syncytial virus

Subgroup Analyses

The treatment effect based on the incidence of MA RSV LRTI (primary endpoint) were consistent across subgroups and with the overall treatment effect. The 95% confidence interval for the estimated relative risk reduction (RRR) crossed the zero threshold only in the subgroup of subjects older than 6 months. This result could be due to the small subgroup size (3 events out of 178 subjects on nirsevimab and 5 events out of 91 on placebo). The detailed subgroup results are presented in Figure 46.

Figure 46. All Subjects Subgroup Analyses, Trial 04



Source: FDA statistical reviewer;
*The RRR cannot be calculated because the number of events was not sufficient
Abbreviations: CI, confidence interval; n, number of subjects; N/A, not applicable; RRR, relative risk reduction

RSV Subtypes

Most of the RSV cases in the Primary Cohort were RSV A (n=33) and most cases in the Safety Cohort were of RSV B subtype (n=35) as shown in Table 97.

Table 97. RSV Subtypes in Primary and Safety Cohorts, Trial 04

	Primary Cohort		Safety Cohort	
RSV subtype	Nirsevimab N=994	Placebo N=496	Nirsevimab N=1015	Placebo N=507
Total	12(1.2)	25(5.0)	12(1.2)	29(5.7)
RSV A	12(1.2)	21(4.2)	2(0.2)	4(0.8)
RSV B	0(0.0)	4(0.8)	10(1.0)	25(4.9)

Source: FDA statistical reviewer
Abbreviations: N, number of subjects RSV, respiratory syncytial virus

17. Clinical Safety

17.1. Safety Results, Trial 03

Trial 03 enrolled infants who were born at ≥29 to <35 weeks of gestational age and were born during or entering their first RSV season.

Please see Section 7.6 for a description of safety monitoring for Trial 03 and for the results of pooled safety data from Trials 03 and 04. Please refer to Section 7 for the definitions of treatment-emergent adverse events, serious adverse events, severity of adverse events, and assessment of treatment causality.

17.1.1. Overview of Treatment-Emergent Adverse Events Summary, Trial 03

Table 98 shows the results for SAEs and AEs by weight. All subjects in Trial 03 received a single 50 mg intramuscular dose of nirsevimab or placebo. Based on the results of Trial 03, dosing was optimized and adjusted for weight (single 50 mg IM dose for infants weighing <5 kg, and 100 mg IM dose for infants weighing ≥5 kg); this means that subjects in Trial 03 who weighed less than 5 kg received the recommended dose of 50 mg IM, and subjects who weighed 5 kg or more got a dose that is lower than the recommended nirsevimab dose.

As shown in Table 98, serious adverse events were observed more frequently in the placebo arm than in the nirsevimab arm. The percentage of subjects with any treatment-emergent AE was similar in the nirsevimab and placebo arms. In the overall population, there were more moderate and severe AEs in the placebo arm compared to the nirsevimab arm. Results for the two subgroups (according to body weight) were similar to the results for the overall population.

There were two deaths in the nirsevimab arm and 3 in the placebo arm; these are discussed in Section 17.1.2.

Table 98. Overview of Adverse Events, Trial 03

	Subjects <5 kg		Subjects ≥5 kg		Total Subjects	
Event Category	NIRS 50MG N=572 n (%)	PBO N=288 n (%)	NIRS 50MG N=392 n (%)	PBO N=190 n (%)	NIRS 50MG N=968 n (%)	PBO N=479 n (%)
SAE	70 (12.2)	61 (21.2)	37 (9.4)	20 (10.5)	108 (11.2)	81 (16.9)
SAEs with fatal outcome	2 (0.3)	3 (1.0)	0	0	2 (0.2)	3 (0.6)
Any AE	485 (84.8)	245 (85.1)	345 (88.0)	170 (89.5)	834 (86.2)	416 (86.8)
Severe and worse	41 (7.2)	43 (14.9)	36 (9.2)	17 (8.9)	77 (8.0)	60 (12.5)
Moderate	149 (26.0)	81 (28.1)	115 (29.3)	63 (33.2)	265 (27.4)	145 (30.3)
Mild	295 (51.6)	121 (42.0)	194 (49.5)	90 (47.4)	492 (50.8)	211 (44.1)

Source: adae.xpt; Software: R
Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with at least one event; NIRS, nirsevimab; PBO, placebo; SAE, serious adverse event

17.1.2. Deaths, Trial 03

There were two deaths in the nirsevimab arm (0.3% of subjects).

- 14-week-old Black female in South Africa received a single 50 mg dose of nirsevimab on Day 1. Her birth weight was 1.3 kg, and she was a twin. She had no significant past medical history. Her treatment-emergent adverse events included an upper respiratory tract infection 3 months before her death, and a Grade 1 rash two months prior to her death. She was healthy when she was put to bed and was found dead in her crib the next morning (Day 123). Her death was judged as not related to related to nirsevimab. Although an autopsy was done, the results are not available.
- A 192-day old White male was enrolled at a trial site in Estonia and received a single dose of nirsevimab on Day 1. He born at 32 weeks gestational age and was a twin. He had a history of respiratory distress syndrome, anemia, omphalitis, and colic. He was hospitalized for acute bronchitis, esophagitis, and dehydration 2.5 months after receiving nirsevimab. He was discharged but was readmitted in cardiac failure and was diagnosed with pulmonary vein stenosis. He had a long hospital course and died of a nosocomial *E. coli* pneumonia on Day 97. His autopsy showed bilateral bronchopneumonia, cardiac failure, and pulmonary vein stenosis. His death was judged as not related to nirsevimab.

There were three deaths in the placebo arm (1%) of subjects.

- 14-week-old Black female was received placebo. She was previously healthy and was being carried by mother on the mother's back on Day 343. After an unclear amount of time, the mother noticed that the infant was not breathing. The infant was dead when she reached health care providers. Mother told investigators that she was told that a virus had attacked the infant's heart.
- A 1-month-old Black male, enrolled in South Africa, was born at 30 weeks GA. His neonatal course was complicated by neonatal sepsis and respiratory distress syndrome. One week after receiving placebo, the infant was taken to the hospital with apnea. He was admitted and diagnosed with *E. coli* meningitis and sepsis. He developed pneumonia while in the hospital and died on Day 26.

Reference ID: 5208810

- A 2-week-old Black male, enrolled in South Africa, became sick at an unknown time and was taken to traditional healers. However, the infant's condition worsened on traditional medicine, and he was brought to the hospital, where he was pronounced dead on arrival (Day 109). His death was attributed to pneumonia.

One of the deaths in the nirsevimab arm was due to a previously undiagnosed cardiac defect and in the opinion of the review team, the death was not related to nirsevimab. The other death in the nirsevimab arm was likely due to SIDS. While this death does not appear to be related to nirsevimab temporally, it is hard to reach definitive conclusions regarding the cause of death. Causes of death in Trial 03 are shown in Table 99.

Table 99. Deaths, Trial 03

	Subjects <5 kg		Subjects ≥5 kg		Total Subjects	
Preferred Term	**NIRS 50MG N=572 n (%)**	**PBO N=288 n (%)**	**NIRS 50MG N=392 n (%)**	**PBO N=190 n (%)**	**NIRS 50MG N=968 n (%)**	**PBO N=479 n (%)**
Any AE leading to death	2 (0.3)	3 (1.0)	0	0	2 (0.2)	3 (0.6)
Death	1 (0.2)	0	0	0	1 (0.1)	0
Pulmonary vein stenosis	1 (0.2)	0	0	0	1 (0.1)	0
Pericardial effusion	0	1 (0.3)	0	0	0	1 (0.2)
Pneumonia	0	2 (0.7)	0	0	0	2 (0.4)

Source: CDS: adae.xpt; Software: R
Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo

17.1.3. Serious Treatment-Emergent Adverse Events, Trial 03

The percentage of subjects with serious adverse events was higher in the placebo arm for the overall trial population as well as in both weight-based subgroups (Table 100). SAEs were reported in less than 2% of nirsevimab subjects, except for bronchiolitis in the overall population and in subjects weighing 5 kg or greater. However, except for the subgroup of subjects weighing >5 kg in which bronchiolitis was reported at similar incidence in nirsevimab and placebo arms, bronchiolitis was reported more frequently in subjects who received placebo. Overall, the types of SAEs were observed in a similar percentage of subjects in the nirsevimab and placebo arms or were observed at a higher percentage in the placebo arm.

Table 100. Serious Adverse Event Reported in at Least 2 Subjects in the Nirsevimab Arm (Trial 03)

	Subjects <5 kg		Subjects ≥5 kg		Total Subjects	
System Organ Class Preferred Term	NIRS 50MG N=572 n (%)	PBO N=288 n (%)	NIRS 50MG N=392 n (%)	PBO N=190 n (%)	NIRS 50MG N=968 n (%)	PBO N=479 n (%)
Any SAE	70 (12.2)	61 (21.2)	37 (9.4)	20 (10.5)	108 (11.2)	81 (16.9)
Bronchiolitis	10 (1.7)	15 (5.2)	10 (2.6)	5 (2.6)	20 (2.1)	20 (4.2)
Lower respiratory tract infection	10 (1.7)	11 (3.8)	4 (1.0)	4 (2.1)	14 (1.4)	15 (3.1)
Bronchitis	10 (1.7)	10 (3.5)	4 (1.0)	1 (0.5)	14 (1.4)	11 (2.3)
Pneumonia	8 (1.4)	7 (2.4)	5 (1.3)	3 (1.6)	13 (1.3)	10 (2.1)
Gastroenteritis	5 (0.9)	2 (0.7)	4 (1.0)	2 (1.1)	9 (0.9)	4 (0.8)
Respiratory syncytial virus infection	4 (0.7)	0	0	1 (0.5)	4 (0.4)	1 (0.2)
Pyrexia	2 (0.3)	2 (0.7)	1 (0.3)	0	3 (0.3)	2 (0.4)
Dehydration	2 (0.3)	1 (0.3)	1 (0.3)	0	3 (0.3)	1 (0.2)
Sepsis	2 (0.3)	1 (0.3)	0	0	2 (0.2)	1 (0.2)
Pneumonia aspiration	2 (0.3)	0	0	0	2 (0.2)	0
Urinary tract infection	0	3 (1.0)	0	1 (0.5)	0	4 (0.8)

Source: CDS: adae.xpt; Software: R
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo; SAE, serious adverse event

17.1.4. Treatment-Emergent Adverse Events, Trial 03

The percentage of subjects with TEAEs was similar in the nirsevimab and the placebo arms for the overall population and for both weight subgroups (Table 101). In the nirsevimab-treated subjects, there were no TEAEs that were observed more frequently than a 5% difference between the nirsevimab arm and the placebo arm. Although URTI was reported more commonly in the nirsevimab arm than the placebo arm, nasopharyngitis was reported more commonly in the placebo arm. In addition, otitis media can occur with URTI, and otitis media was more commonly reported in the placebo arm. Overall, there were no increases in one type or class of TEAEs observed among subjects who received nirsevimab. In addition, there was no increase in TEAEs in subjects who weighed less than 5 kg and received the proposed dose of nirsevimab.

Table 101. Common Adverse Events Occurring at ≥5% Frequency in Subjects Who Received Nirsevimab, Trial 03

	Subjects <5 kg		Subjects ≥5 kg		Total Subjects	
System Organ Class Preferred Term	**NIRS 50MG N=572 n (%)**	**PBO N=288 n (%)**	**NIRS 50MG N=392 n (%)**	**PBO N=190 n (%)**	**NIRS 50MG N=968 n (%)**	**PBO N=479 n (%)**
Any TEAE	485 (84.8)	245 (85.1)	345 (88.0)	170 (89.5)	834 (86.2)	416 (86.8)
Upper respiratory tract infection	236 (41.3)	107 (37.2)	164 (41.8%)	77 (40.5%)	403 (41.6%)	184 (38.4)
Nasopharyngitis	81 (14.2)	55 (19.1)	80 (20.4)	39 (20.5)	161 (16.6)	94 (19.6)
Pyrexia	66 (11.5)	38 (13.2)	64 (16.3)	40 (21.1)	130 (13.4)	78 (16.3)
Gastroenteritis	71 (12.4)	25 (8.7)	51 (13)	21 (11.1)	122 (12.6)	46 (9.6)
Rhinitis	52 (9.1)	24 (8.3)	59 (15.1)	25 (13.2)	112 (11.6)	50 (10.4)
Diarrhea	46 (8.0)	28 (9.7)	52 (13.3)	22 (11.6)	100 (10.3)	50 (10.4)
Acute otitis media	41 (7.2)	29 (10.1)	57 (14.5)	29 (15.3)	98 (10.1)	58 (12.1)
Bronchitis	50 (8.7)	32 (11.1)	47 (12.0)	24 (12.6)	97 (10.0)	56 (11.7)
Bronchiolitis	51 (8.9)	33 (11.5)	44 (11.2)	21 (11.1)	96 (9.9)	54 (11.3)
Lower respiratory tract infection	51 (8.9)	33 (11.5)	37 (9.4)	22 (11.6)	88 (9.1)	55 (11.5)
Conjunctivitis	50 (8.7)	25 (8.7)	35 (8.9)	14 (7.4)	86 (8.9)	39 (8.1)
Diaper dermatitis	47 (8.2)	23 (8.0)	28 (7.1)	13 (6.8)	76 (7.9)	36 (7.5)
Nasal congestion	45 (7.9)	10 (3.5)	25 (6.4)	14 (17.4)	70 (7.2)	24 (5.0)
Rhinorrhea	38 (6.6)	16 (5.6)	25 (6.4)	12 (6.3)	63 (6.5)	28 (5.8)
Teething	30 (5.2)	11 (3.8)	32 (8.2)	21 (11.1)	62 (6.4)	32 (6.7)
Pharyngitis	27 (4.7)	13 (4.5)	31 (7.9)	12 (6.3)	63(6.5)	28 (5.8)
Cough	31 (5.4)	14 (4.9)	24 (6.1)	10 (5.3)	55 (5.7)	24 (5.0)

Source: CDS: adae.xpt; Software: R
Abbreviations: AE, adverse events; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo

The percentage of subjects with any treatment related TEAEs (adverse reactions) was low and was similar in the nirsevimab and placebo arms for the overall population and for both weight subgroups. Injection site reactions, including induration, edema and pain were reported in four subjects who received nirsevimab and were not reported in subjects who received placebo. Rashes were reported in seven subjects (0.7%) who received nirsevimab; the preferred terms included: rash, macular rash, maculopapular rash, and petechiae. Rashes were reported in three subjects (0.6%) who received placebo (preferred terms: rash and papular rash).

Table 102. Adverse Reactions, Trial 03

	Subjects <5 kg		Subjects ≥5 kg		Total Subjects	
Preferred Term	**NIRS 50MG N=572 n (%)**	**PBO N=288 n (%)**	**NIRS 50MG N=392 n (%)**	**PBO N=190 n (%)**	**NIRS 50MG N=968 n (%)**	**PBO N=479 n (%)**
Any treatment-related AE	8 (1.4)	3 (1.0)	13 (3.3)	7 (3.7)	22 (2.3)	10 (2.1)
Hypersomnia	1 (0.2)	0	3 (0.8)	0	4 (0.4)	0
Rash	1 (0.2)	0	2 (0.5)	2 (1.1)	4 (0.4)	2 (0.4)
Irritability	0	0	2 (0.5)	1 (0.5)	2 (0.2)	1 (0.2)
Decreased activity	1 (0.2)	0	0	0	1 (0.1)	0
Eczema	0	0	1 (0.3)	0	1 (0.1)	0
Injection site induration	0	0	1 (0.3)	0	1 (0.1)	0
Injection site oedema	0	0	1 (0.3)	0	1 (0.1)	0
Injection site pain	1 (0.2)	0	0	0	1 (0.1)	0
Injection site reaction	0	0	1 (0.3)	0	1 (0.1)	0
Petechiae	1 (0.2)	0	0	0	1 (0.1)	0
Rash macular	0	0	1 (0.3)	0	1 (0.1)	0
Rash maculo-papular	1 (0.2)	0	0	0	1 (0.1)	0
Decreased appetite	1 (0.2)	0	0	1 (0.5)	1 (0.1)	1 (0.2)
Pharyngitis	0	1 (0.3)	1 (0.3)	0	1 (0.1)	1 (0.2)
Pyrexia	1 (0.2)	1 (0.3)	0	0	1 (0.1)	1 (0.2)
Anemia	0	1 (0.3)	0	0	0	1 (0.2)
Erythema	0	1 (0.3)	0	0	0	1 (0.2)
Rash papular	0	0	0	1 (0.5)	0	1 (0.2)
Somnolence	0	0	0	1 (0.5)	0	1 (0.2)
Vomiting	0	0	0	2 (1.1)	0	2 (0.4)

Source: CDS: adae.xpt; Software: R
Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo

17.1.5. Subgroup Analyses, Trial 03

The percentage of subjects with adverse events by demographic subgroup is shown in Table 103. For both sexes, the percentage of subjects with AEs are similar in the nirsevimab and placebo arm. The percentage of males with AEs is slightly higher than the percentage of females with AEs, but this is observed in both the nirsevimab and control arms. The percentage of subjects in the three age strata with AEs was similar in each stratum and similar between the nirsevimab and placebo arms. It is difficult to definitively identify differences in AEs by race, because the majority of subjects in the trial were White. The percentage of White subjects with AEs was similar in the nirsevimab and placebo arms. The percentage of Black/African American subjects with AEs was higher than in White subjects both in the overall population and in each weight group. However, the percentage of Black/African American subjects with AEs was similar between the nirsevimab and placebo arm. The reason for the difference between Whites and Blacks/African Americans is unclear but may be due to smaller number of Black/African American subjects enrolled. In the overall trial population, AEs were reported in 80% of Asian subjects in the nirsevimab arm compared to 70% of Asian subjects in the placebo arm; however, this difference was likely related to the small number of Asian subjects (N=15) in the trial. There was a higher percentage of Hispanics or Latinos with AEs compared to non-Hispanic or Latino. However, the percentage of Hispanics or Latinos with AEs was similar in the nirsevimab and placebo arm. The reason for an increase in AEs in non-Hispanics or Latinos is unclear. It is possible that it is related to or affected by smaller number of subjects in that subgroup (N=316) compared to non-Hispanic or Latino (N=1,130). Finally, the percentage of U.S. subjects with AEs was similar in the nirsevimab and placebo arm, and the percentage of non-U.S. subjects with AEs was also similar in the nirsevimab and placebo arms. However, the percentage of subjects with AEs was higher in U.S. subjects than non-U.S. subjects. The reason for this is unclear but could be related to access to health care.

Table 103. Adverse Events by Demographic Subgroup, Trial 03

Demographic Characteristic	Subjects <5 kg		Subjects ≥5 kg		Total Subjects	
	NIRS 50MG N=572 n/N_s (%)	PBO N=288 n/N_s (%)	NIRS 50MG N=392 n/N_s (%)	PBO N=190 n/N_s (%)	NIRS 50MG N=968 n/N_s (%)	PBO N=479 n/N_s (%)
Sex, n (%)						
Female	228/273 (83.5)	116/139 (83.5)	166/191 (86.9)	73/83 (88.0)	397/467 (85.0)	190/223 (85.2)
Male	257/299 (86.0)	129/149 (86.6)	179/201 (89.1)	97/107 (90.7)	437/501 (87.2)	226/256 (88.3)
Age group, months, n (%)						
≤3 months	416/490 (84.9)	208/245 (84.9)	22/25 (88.0)	9/9 (100)	440/517 (85.1)	218/255 (85.5)
>3 months ≤6 months	69/82 (84.1)	35/41 (85.4)	208/237 (87.8)	96/109 (88.1)	278/320 (86.9)	131/150 (87.3)
>6 months	0/0 (NA)	2/2 (100)	115/130 (88.5)	65/72 (90.3)	116/131 (88.5)	67/74 (90.5)
Race, n (%)						
American Indian or Alaska Native	0/0 (NA)	0/0 (NA)	0/0 (NA)	1/1 (100)	0/0 (NA)	1/1 (100)
Asian	2/3 (66.7)	4/6 (66.7)	2/2 (100)	3/4 (75.0)	4/5 (80.0)	7/10 (70.0)
Black or African American	115/120 (95.8)	37/40 (92.5)	65/67 (97.0)	27/27 (100)	180/187 (96.3)	64/67 (95.5)
Multiple	4/6 (66.7)	2/3 (66.7)	6/6 (100)	2/2 (100)	10/12 (83.3)	4/5 (80.0)
Native Hawaiian or Other Pacific Islander	6/6 (100)	3/3 (100)	2/2 (100)	0/0 (NA)	8/8 (100)	3/3 (100)
Other	37/40 (92.5)	31/31 (100)	20/22 (90.9)	10/11 (90.9)	57/62 (91.9)	41/42 (97.6)
White	320/396 (80.8)	168/205 (82.0)	250/293 (85.3)	127/145 (87.6)	574/693 (82.8)	296/351 (84.3)
Missing	1/1 (100)	0/0 (NA)	0/0 (NA)	0/0 (NA)	1/1 (100)	0/0 (NA)
Ethnicity, n (%)						
Hispanic or Latino	110/118 (93.2)	41/44 (93.2)	99/106 (93.4)	43/47 (91.5)	210/225 (93.3)	84/91 (92.3)
Not Hispanic or Latino	374/453 (82.6)	204/244 (83.6)	246/286 (86.0)	127/143 (88.8)	623/742 (84.0)	332/388 (85.6)
Missing	1/1 (100)	0/0 (NA)	0/0 (NA)	0/0 (NA)	1/1 (100)	0/0 (NA)
Is in United States, n (%)						
United States	117/129 (90.7)	40/46 (87.0)	70/75 (93.3)	32/33 (97.0)	188/205 (91.7)	72/79 (91.1)
Non-United States	368/443 (83.1)	205/242 (84.7)	275/317 (86.8)	138/157 (87.9)	646/763 (84.7)	344/400 (86.0)

Source: adae.xpt; Software: R
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; NA, not applicable; NIRS, nirsevimab; N_s, total number of subjects for each specific subgroup and were assigned to that specific arm; PBO, placebo

17.1.6. Laboratory Studies, Trial 03

Laboratory monitoring was not obtained for Trial 03.

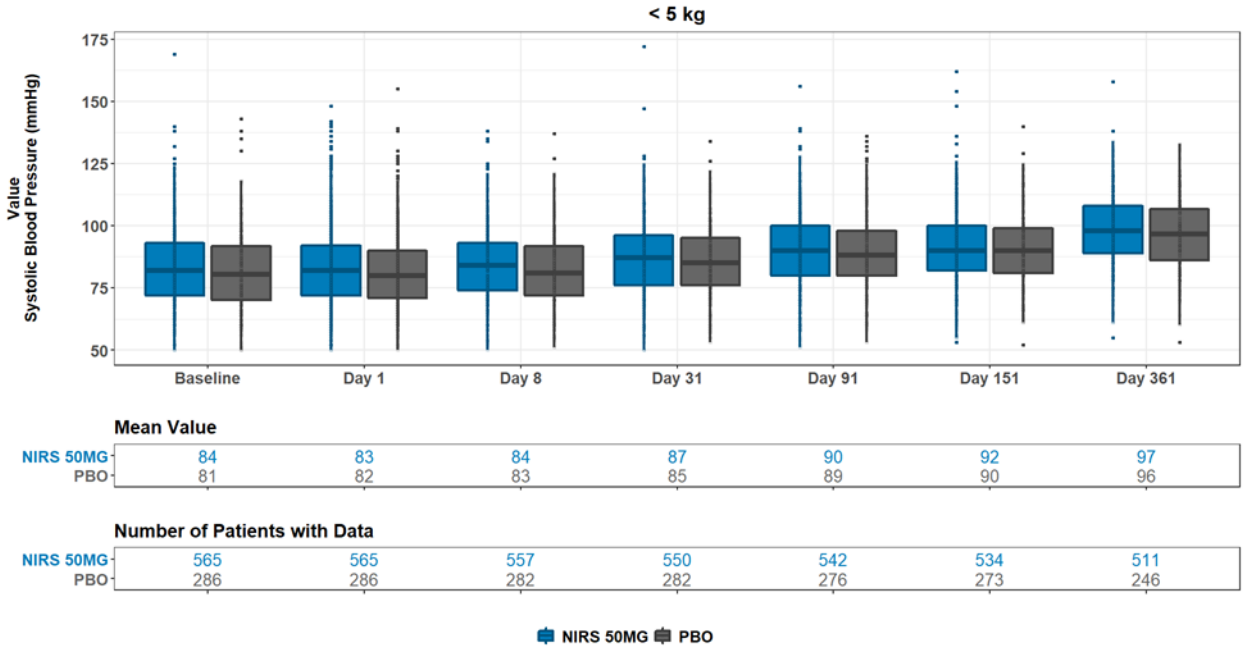
17.1.7. Vital Signs

Vital signs are shown for the subgroup of infants weighing <5 kg. These infants received the proposed dose of nirsevimab, while infants weighing ≥5 kg received a lower dose than proposed. The results shown are consistent with the results for the total subject population.

17.1.7.1. Blood Pressure

Systolic blood pressure at each visit is shown in Figure 47. Blood pressure decreases with decreasing weight and blood pressure is also lower in younger infants, so blood pressures are lower in this population than in older infants and children. At each visit the mean systolic blood pressure is similar in the nirsevimab and placebo arms, and no differences between the two arms were observed. While there appears to be a few more outliers in the nirsevimab arm compared to the placebo arm, this is probably due to the 2:1 randomization scheme.

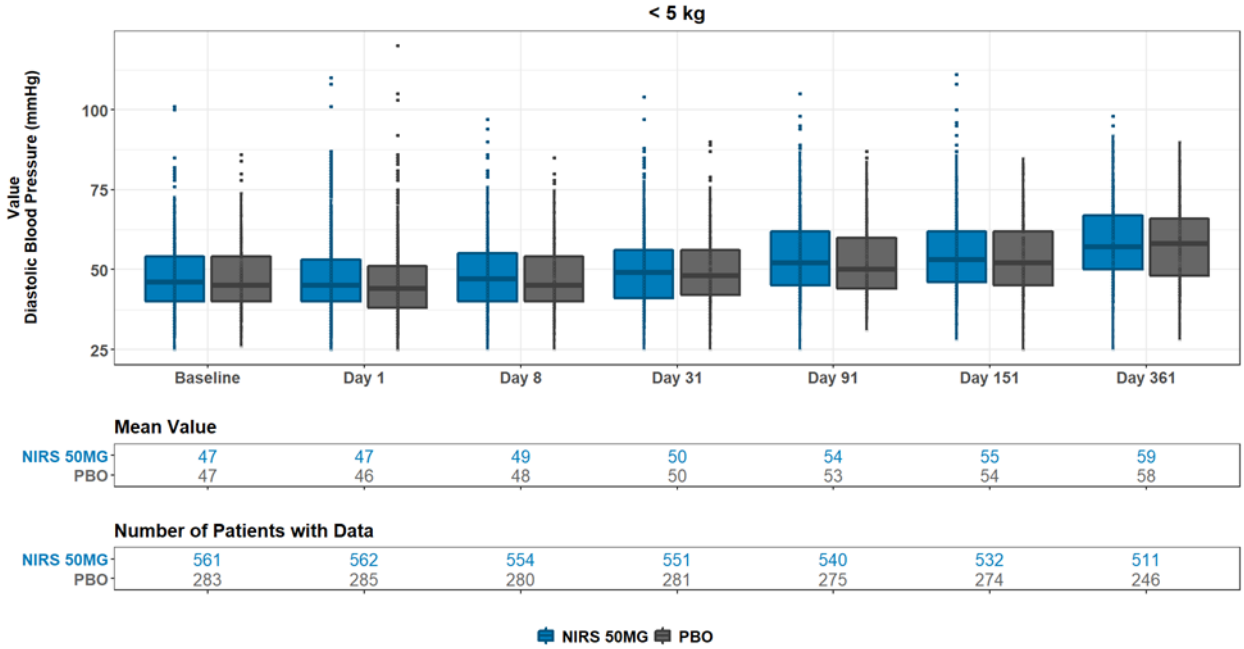
Figure 47. Median and Interquartile Range of Systolic Blood Pressure Over Time in Infants Weighing <5 kg by Treatment Arm, Trial 03



Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

The mean values for diastolic blood pressure are similar for the two treatment arms in Trial 03 (Figure 48). As with systolic blood pressure, there were outliers. However, the overall results for diastolic blood pressure are similar in the nirsevimab and placebo arms.

Figure 48. Median and Interquartile Range of Diastolic Blood Pressure Over Time in Infants Weighing <5 kg by Treatment Arm, Trial 03

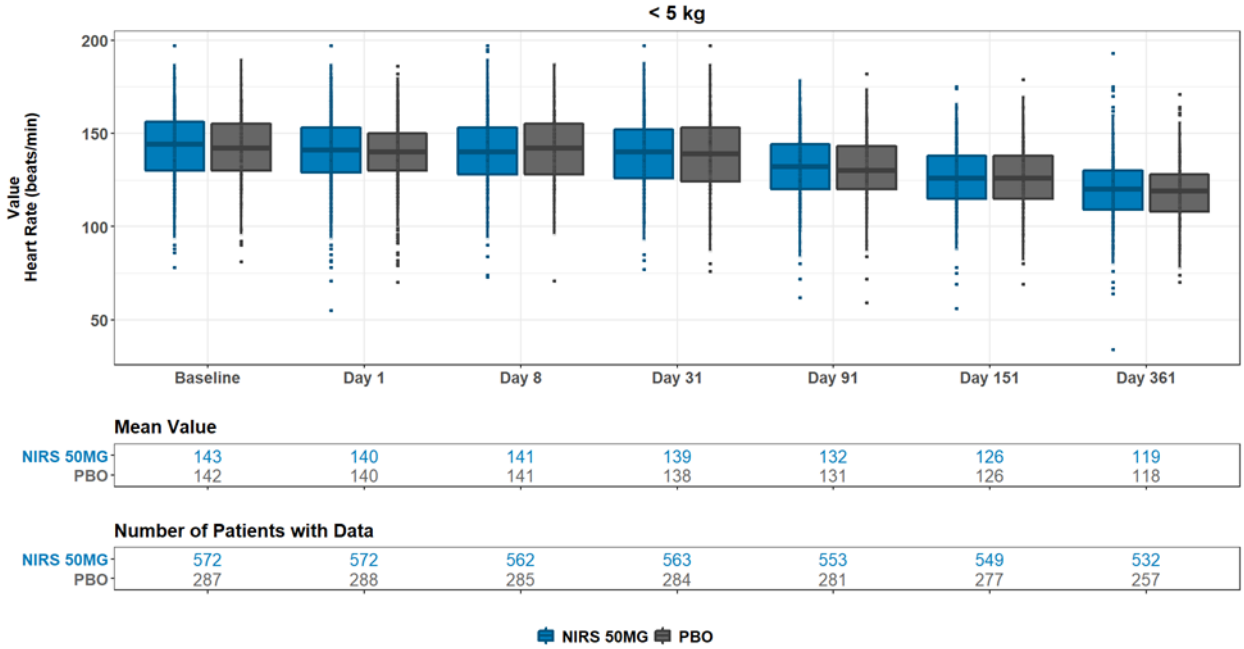


Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.1.7.2. Heart Rate

The mean and median heart rates are similar in the nirsevimab and placebo arms at each trial visit. The heart rates are higher in infants than in older children and decrease over time. This is shown in Figure 49, where the mean heart rate is 142 to 143 beats per minute at baseline and decreases to 118 to 199 beats per minute at Day 361, after trial subjects had aged. The results are similar between the nirsevimab and placebo arms, and no safety concerns were observed in heart rate.

Figure 49. Median and Interquartile Range of Heart Rate Over Time in Infants Weighing <5 kg by Treatment Arm, Trial 03

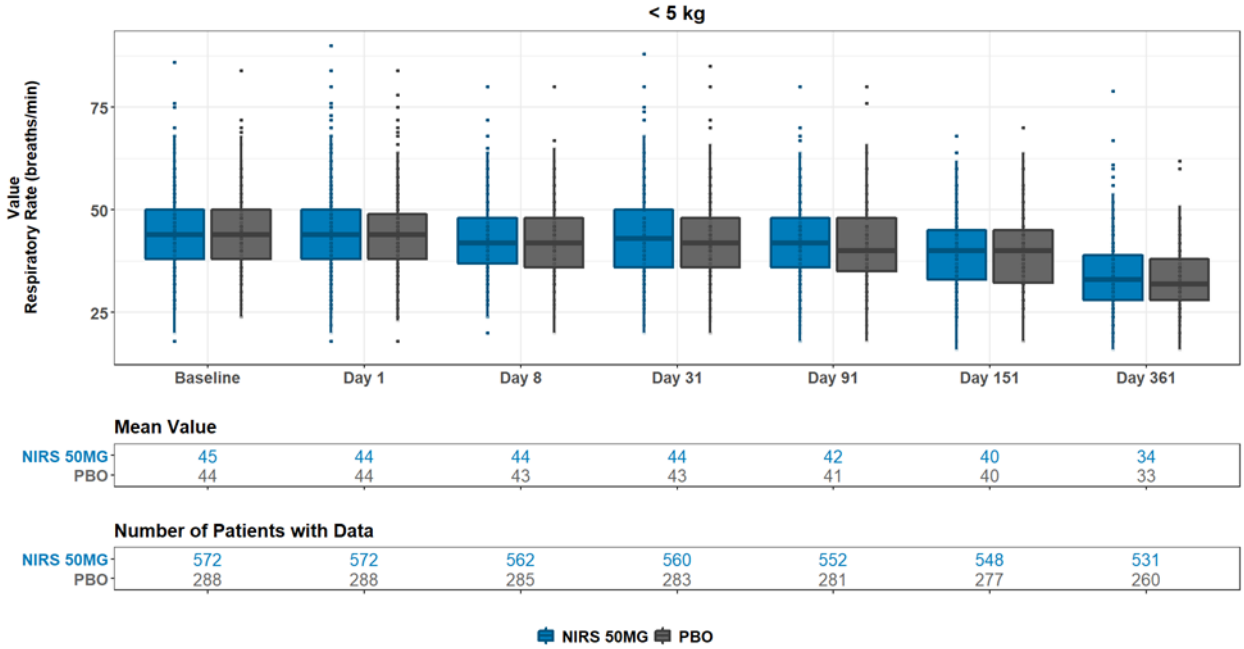


Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.1.7.3. Respiratory Rate

Respiratory rate at each visit is shown in Figure 50. The mean and median respiratory rate is similar in the two treatment arms. Infants typically have slightly higher respiratory rates early in the first year of life that decrease with age. The respiratory rates in both arms are consistent with the trial population, and no safety concerns were identified with respiratory rate.

Figure 50. Median and Interquartile Range of Respiratory Rate Over Time in Infants Weighing <5 kg by Treatment Arm, Trial 03

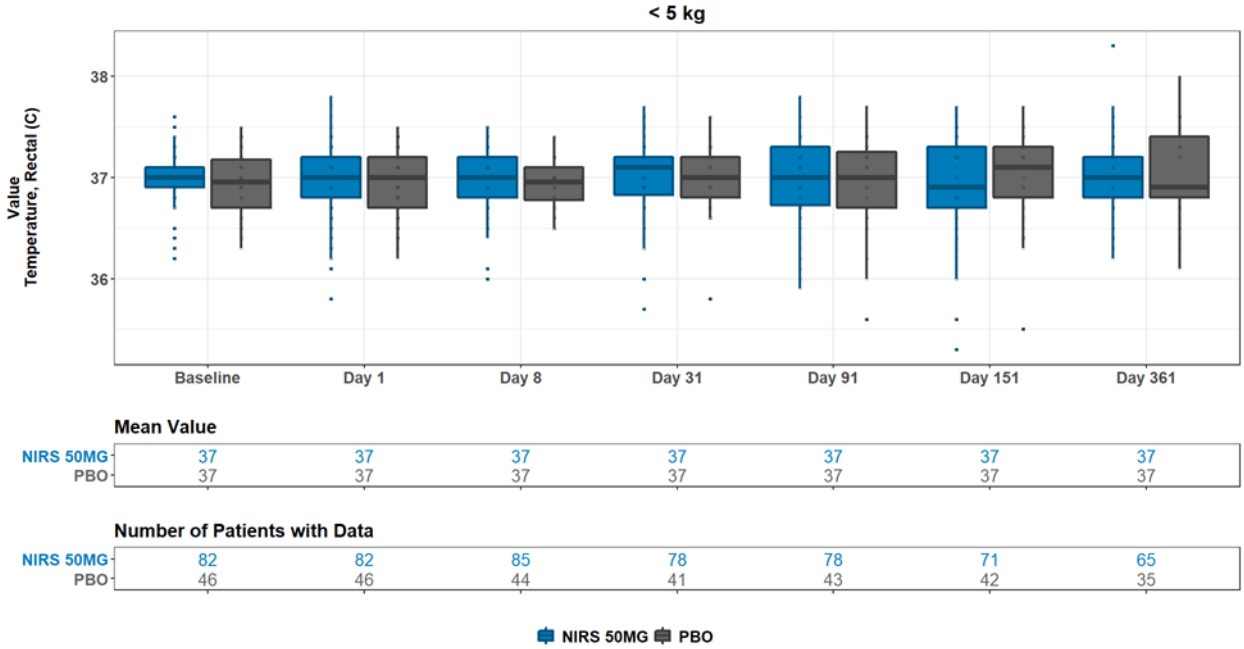


Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.1.7.4. Temperature

Temperature at each trial visit is shown in Figure 51. The number of subjects who had their temperature taken is much smaller than the total population; the reason for this was not provided. This was not observed with other vital signs. It is unclear why temperature was not recorded for all subjects. The mean temperature at each trial visit was 37° C at each visit. There is more variation around the median temperatures, but the median temperatures are similar and cluster around 37° C (as shown on the y axis). No abnormalities in temperature are demonstrated in this figure.

Figure 51. Median and Interquartile Range of Body Temperature Over Time in Infants Weighing <5 kg, Trial 03



Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.1.8. Conclusion

Trial 03 enrolled infants who were born at ≥29 to <35 weeks of gestational age and were born during or entering their first RSV season. The percentage of subjects with adverse events, adverse reactions, and serious adverse events was similar between the nirsevimab and the placebo arms. There was a slight increase in the percentage of subjects with rashes in the nirsevimab arm that were considered adverse reactions, but the overall percentage of subjects with rashes was low. In addition, injection site reactions were observed in the nirsevimab arm but not the placebo arm. However, the percentage of subjects with injection site reactions was low. There were two deaths in subjects who received nirsevimab, but neither death appeared to be related to nirsevimab. There was no difference in adverse events or serious adverse events by weight subgroup. While there were no major safety concerns identified in this trial, it is possible that rash and injection site reactions will be observed after administration of nirsevimab postmarketing.

17.2. Safety Results, Trial 04

Please see Section 7 for a description of safety monitoring for Trial 04 and for the results of pooled safety data from Trials 03 and 04. Please refer to Section 7 for the definitions of treatment-emergent adverse events, serious adverse events, severity of adverse events, and assessment of treatment causality.

Reference ID: 5208810

The safety analyses for Trial 04 are based on the pooled data from the Primary Cohort which included the first 1,478 subjects who were randomized and dosed, and the subsequently enrolled additional 1,516 subjects (Safety Cohort) to supplement the overall safety assessment. Therefore, the safety database (overall safety population) for Trial 04 includes 2,994 subjects (1998 in the nirsevimab arm, 996 in the placebo arm).

17.2.1. Overview of Treatment-Emergent Adverse Events Summary, Trial 04

In Trial 04, the percentage of subjects with a SAE or with a severe adverse event was similar in the nirsevimab and the placebo arms (Table 104). The overall percentage of subjects with any adverse event was also similar between the nirsevimab and placebo arm.

Table 104. Overview of Adverse Events, Trial 04

Event Category	NIRS N=1998 n (%)	PBO N=996 n (%)
SAE	125 (6.3)	74 (7.4)
SAEs with fatal outcome	4 (0.2)	0
Any AE[1]	1673 (83.7)	815 (81.8)
Severe and worse	61 (3.1)	38 (3.8)
Moderate	375 (18.8)	208 (20.9)
Mild	1237 (61.9)	569 (57.1)

Source: CDS: adae.xpt; Software: R
[1]Severity as assessed by the investigator.
Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with at least one event; NIRS, nirsevimab; PBO, placebo; SAE, serious adverse event

17.2.2. Deaths, Trial 04

There were four deaths in the nirsevimab arm and no deaths in the placebo arm (Table 105).

- A mixed-race male from Panama was enrolled in the trial at 5 months of age. He died on Day 97 due to a skull fracture after being hit by a car.
- A Black male from South Africa was enrolled in Trial 04 at 3 months of age. He received a single 100 mg dose of nirsevimab on Day 1. He had one adverse event of Grade 1 gastroenteritis one week after dosing, and a serious adverse event of gastroenteritis 3 months after enrollment. At his Day 91 visit, he was noted to have poor weight gain. On Day 141, he had two episodes of vomiting and diarrhea and was then noted to have trouble breathing. He was taken that day to the local fire department, where he died.
- A Black female from South Africa was enrolled in Trial 04 at 7 months of age. She had no adverse events during trial participation until 10 months after enrollment, when she developed vomiting and diarrhea. She was given an oral rehydration solution at home. After 3 days of vomiting and diarrhea, the infant was found to be listless and was taken to the local fire station where she was pronounced dead on Day 338.
- A white male from Israel was enrolled in Trial 04 on day of life 1. His birth weight was 2.9 kg, and he had no neonatal complications. However, he had two hospitalizations

during the trial for failure to thrive. He also had two adverse events of vomiting, recurrent hypoglycemia, anemia, and a history of an elevated alanine aminotransferase (ALT). His primary physician hypothesized that the infant had an underlying congenital or genetic disorder. The infant was doing well and was put to bed on Day 139. He was found dead in the crib the next morning.

Although there were four deaths in the nirsevimab arm and none in the placebo arm, the four deaths represent a small percentage of the trial population (0.2%). All four deaths were 140 days or more after receiving nirsevimab, which is well past the peak plasma concentration of nirsevimab. In addition, there were clear causes for three deaths: trauma and gastroenteritis. The fourth death was in an infant with a probable underlying disease. In the opinion of the review team, none of the deaths were related to nirsevimab.

Table 105. Deaths, Trial 04

Preferred Term	NIRS N=1998 n (%)	PBO N=996 n (%)
Any AE leading to death	4 (0.2)	0
Gastroenteritis	2 (0.1)	0
Death	1 (0.05)	0
Skull fractured base	1 (0.05)	0

Source: CDS: adae.xpt; Software: R
Abbreviations: AE, adverse event N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo

17.2.3. Serious, Treatment-Emergent Adverse Events, Trial 04

The overall percentage of subjects with serious adverse events was slightly higher in the placebo arm compared to the nirsevimab arm, see Table 106. The only SAE reported in more than 1% of subjects was bronchiolitis, which was reported more frequently in the placebo arm. Although there were some SAEs that were observed more often in the nirsevimab arm than in the placebo arm, the differences in incidence between SAEs in the treatment arms were very small. Overall, the serious adverse events reported were common illnesses observed in childhood, and there were no major differences observed between the nirsevimab and placebo arms.

Table 106. Serious Adverse Event Reported in at Least Two Subjects in the Nirsevimab Arm, Trial 04

System Organ Class Preferred Term	NIRS N=1998 n (%)	PBO N=996 n (%)
Any SAE	125 (6.3)	74 (7.4)
Bronchiolitis	24 (1.2)	17 (1.7)
Gastroenteritis	11 (0.6)	3 (0.3)
Pneumonia	9 (0.5)	5 (0.5)
Urinary tract infection	7 (0.4)	4 (0.4)
Nervous system disorders (SOC)	7 (0.4)	3 (0.3)
Lower respiratory tract infection	6 (0.3)	0
Metabolism and nutrition disorders (SOC)	5 (0.3)	2 (0.2)

System Organ Class Preferred Term	NIRS N=1998 n (%)	PBO N=996 n (%)
Pyrexia	5 (0.3)	2 (0.2)
Respiratory syncytial virus bronchiolitis	4 (0.2)	9 (0.9)
Upper respiratory tract infection	4 (0.2)	2 (0.2)
Febrile convulsion	3 (0.2)	0
Viral upper respiratory tract infection	3 (0.2)	0
Pneumonia respiratory syncytial viral	3 (0.2)	1 (0.1)
Diarrhea	3 (0.2)	0
Laryngitis	3 (0.2)	2 (0.2)
Kawasaki's disease	2 (0.1)	1 (0.1)
Seizure	2 (0.1)	1 (0.1)
Dehydration	2 (0.1)	1 (0.1)
Bronchitis	2 (0.1)	4 (0.4)
Adenoviral upper respiratory infection	2 (0.1)	0
COVID-19	2 (0.1)	0
Escherichia urinary tract infection	2 (0.1)	0
Influenza	2 (0.1)	0
Nasopharyngitis	2 (0.1)	0
Viral infection	2 (0.1)	0
Lower respiratory tract infection viral	2 (0.1)	1 (0.1)
Gastroesophageal reflux disease	2 (0.1)	0
Vomiting	2 (0.1)	0

Source: CDS: adae.xpt; Software: R
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo; SOC, system organ class

17.2.4. Treatment-Emergent Adverse Events, Trial 04

The percentage of subjects with treatment-emergent AEs was slightly higher in the nirsevimab arm than the placebo arm (Table 107). The types of TEAEs observed were consistent with common childhood illnesses. The differences in frequency in TEAEs that were reported more commonly in the nirsevimab arm were ≤1% except for fever, which was reported in 14.3% of subjects who received nirsevimab and 12.3% who received placebo. While the overall incidence of TEAEs were similar between the two arms, it is possible that fever may be observed more frequently after administration of nirsevimab.

Table 107. Common Treatment-Emergent Adverse Events Occurring at ≥2.5% Frequency in the Nirsevimab Arm, Trial 04

Preferred Term	NIRS N=1998 n (%)	PBO N=996 n (%)
Any AE	1673 (83.7)	815 (81.8)
Upper respiratory tract infection	590 (29.5)	287 (28.8)
Nasopharyngitis	407 (20.4)	214 (21.5)
Pyrexia	286 (14.3)	123 (12.3)
Dermatitis diaper	206 (10.3)	92 (9.2)
Rhinitis	178 (8.9)	94 (9.4)
Gastroenteritis	173 (8.7)	85 (8.5)

Preferred Term	NIRS N=1998 n (%)	PBO N=996 n (%)
Nasal congestion	162 (8.1)	84 (8.4)
Diarrhea	147 (7.4)	75 (7.5)
Rhinorrhea	133 (6.7)	58 (5.8)
Teething	129 (6.5)	69 (6.9)
Otitis media	121 (6.1)	64 (6.4)
Viral upper respiratory tract infection	122 (6.1)	51 (5.1)
Bronchiolitis	120 (6.0)	81 (8.1)
Conjunctivitis	106 (5.3)	49 (4.9)
Cough	100 (5.0)	49 (4.9)
Rash	89 (4.5)	45 (4.5)
Eczema	86 (4.3)	48 (4.8)
Constipation	84 (4.2)	34 (3.4)
Viral rash	76 (3.8)	29 (2.9)
Vaccination complication	71 (3.6)	32 (3.2)
Otitis media acute	67 (3.4)	33 (3.3)
Miliaria	59 (3.0)	35 (3.5)
COVID-19	55 (2.8)	33 (3.3)
Vomiting	53 (2.7)	25 (2.5)
Bronchitis	53 (2.7)	33 (3.3)
Oral candidiasis	50 (2.5)	22 (2.2)
Pharyngitis	50 (2.5)	22 (2.2)

Source: CDS: adae.xpt; Software: R
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo

As shown in Table 108, adverse reactions, i.e., adverse events considered treatment-related by the investigator, were uncommon in both treatment arms. Of note, in the nirsevimab arm, maculo-papular rash was reported as an adverse reaction in six subjects, drug eruption in two subjects, rash in one subject, and dermatitis in one subject, compared to one subject in the placebo arm with macular rash. Overall rash was more common in the nirsevimab arm; however, the percentage of subjects with any rash in the nirsevimab arm was low (0.5%). While fever was reported more often as an adverse event in subjects who received nirsevimab, it was not reported as an adverse reaction, i.e., was not consider drug-related. Two subjects who received nirsevimab had adverse reactions related to the injection site; no injection site reactions were observed in subjects who received placebo. In conclusion, the percentages of subjects with adverse drug reactions were low, but rashes and injection site reactions were observed more commonly in subjects who received nirsevimab.

Table 108. Adverse Reactions, Trial 04

Preferred Term	NIRS N=1998 n (%)	PBO N=996 n (%)
Any treatment-related AE	25 (1.3)	15 (1.5)
Rash maculo-papular	6 (0.3)	0
Irritability	4 (0.2)	3 (0.3)
Pyrexia	2 (0.1)	2 (0.2)
Diarrhea	2 (0.1)	0
Drug eruption	2 (0.1)	0
Dermatitis	1 (0.05)	0
Gastroenteritis	1 (0.05)	0
Injection site pain	1 (0.05)	0

Preferred Term	NIRS N=1998 n (%)	PBO N=996 n (%)
Injection site swelling	1 (0.05)	0
Rash	1 (0.05)	0
Skin hypopigmentation	1 (0.05)	0
Nasal congestion	1 (0.05)	1 (0.1)
Rash papular	1 (0.05)	1 (0.1)
Somnolence	1 (0.05)	1 (0.1)
Constipation	0	1 (0.1)
Eczema	0	1 (0.1)
Fever neonatal	0	1 (0.1)
Neutropenia	0	1 (0.1)
Rash macular	0	1 (0.1)
Upper respiratory tract infection	0	1 (0.1)
Vaccination complication	0	1 (0.1)
Vomiting	0	1 (0.1)

Source: CDS: adae.xpt; Software: R
Abbreviations: AE, adverse event N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PBO, placebo

17.2.5. Subgroup Analyses, Trial 04

Adverse events by demographic subgroup are shown in Table 109. The percentages of subjects with AEs were similar in females and males in both the nirsevimab and placebo arms. There was no difference in adverse events by treatment arm for the weight subgroups. There were more adverse events in the >3.0 months to ≤6-month age group who received nirsevimab compared to the other two weight groups. However, there was no correlation of age and AE, and the differences between the age groups were relatively small. There was a lower percentage of AEs in White subjects compared to the other racial subgroups. The reason for this is unclear. There was a higher percentage of subjects with adverse events in the nirsevimab arm than in the control arm for American Indians or Alaskan Natives, for Asians, and for Native Hawaiians or Other Pacific Islanders; differences noted in these subgroups are likely due to the small size of each of the subgroups. There were no differences in adverse events by ethnicity. There was a small increase in AEs in U.S. subjects in the nirsevimab arm compared to non-U.S. subjects in the nirsevimab arm. The reason for this difference is unclear.

Table 109. Adverse Events by Demographic Subgroup, Trial 04

Characteristic	NIRS N=1998 n/N_s (%)	PBO N=996 n/N_s (%)
Sex, n (%)		
Female	779/935 (83.3)	403/498 (80.9)
Male	894/1063 (84.1)	412/498 (82.7)
Age group, months, n (%)		
≤3.0 months	988/1185 (83.4)	480/583 (82.3)
>3.0 months ≤6.0 months	547/634 (86.3)	265/321 (82.6)
>6.0 months	138/179 (77.1)	70/92 (76.1)

Characteristic	NIRS N=1998 n/N_s (%)	PBO N=996 n/N_s (%)
Race, n (%)		
American Indian or Alaska Native	87/92 (94.6)	43/52 (82.7)
Asian	96/108 (88.9)	40/50 (80.0)
Black or African American	272/296 (91.9)	129/138 (93.5)
Multiple	17/19 (89.5)	7/8 (87.5)
Native Hawaiian or Other Pacific Islander	14/15 (93.3)	5/8 (62.5)
Other	407/420 (96.9)	195/205 (95.1)
White	777/1045 (74.4)	396/535 (74.0)
Missing	3/3 (100)	0/0 (NA)
Ethnicity, n (%)		
Hispanic or Latino	567/675 (84.0)	268/331 (81.0)
Not Hispanic or Latino	1102/1319 (83.5)	545/663 (82.2)
Missing	4/4 (100)	2/2 (100)
Is in United States, n (%)		
United States	329/364 (90.4)	166/188 (88.3)
Non-United States	1344/1634 (82.3)	649/808 (80.3)

Source: adae.xpt; Software: R
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; NA, not applicable; NIRS, nirsevimab; N_s, total number of subjects for each specific subgroup and were assigned to that specific arm; PBO, placebo

17.2.6. Laboratory Monitoring, Trial 04

17.2.6.1. Abnormal Laboratory Results

Laboratory safety tests (complete blood count with differential and platelet count, aspartate aminotransferase (AST), ALT, total bilirubin, and creatinine) were obtained at Japanese trial sites only. Blood for laboratory testing was collected at baseline and at Days 8, 31, and 151. Laboratory toxicity was graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Grade 3 and four adverse events are shown in Table 110.

Two infants in the nirsevimab arm had Grade 3 decreases in hemoglobin. One infant had a normal hemoglobin at baseline, and the other had a Grade 1 decrease in hemoglobin at baseline. Both infants had a gradual decrease in hemoglobin during the trial with the hemoglobin value reaching Grade 3 at Day 151 (7.7 g/L in one infant and 7.8 g/L in the second infant). Both infants were diagnosed with iron deficiency anemia and started on iron.

Increased bilirubin levels (≥ Grade 3) were reported in 18.2% of infants (N=18) in the nirsevimab arm and in 14.9% of infants in the placebo arm (N=7). In the nirsevimab arm, 14 subjects had increased bilirubin levels at baseline, and 11 of these subjects had Grade 3 or 4 bilirubin levels at baseline. Of the four infants with normal bilirubin levels at baseline, one infant had an increased bilirubin at Day 8 and the other 3 had increased bilirubin at Day 31. Seven of the infants in the nirsevimab arm were enrolled during the first week of life, an additional five infants were younger than 6 weeks of age, and the remaining 5 infants were 45 to 63 days of age at enrollment. Grade 4 bilirubin levels ranged from 104.31 to 174.42 umol/L. Upper limit of normal for the testing laboratory was 20.52 umol/L. All abnormal bilirubin levels resolved. There were no associated increases in ALT or AST.

Eight subjects in the nirsevimab arm (0.4%) and one (0.1%) in the placebo arm had jaundice reported as an adverse event. One subject (0.1%) had an AE of hyperbilirubinemia. One infant in the nirsevimab arm was hospitalized for 3 days with jaundice and received 2 days of phototherapy.

There was a high percentage of infants in both arms with Grade 3 and 4 increases in bilirubin. All infants were enrolled at a young age and developed hyperbilirubinemia early in the trial. None of the cases of hyperbilirubinemia were associated with increased liver enzymes, and all cases resolved. The increases in bilirubin are consistent with neonatal hyperbilirubinemia, which is reported in up to 60% of infants in the first week of life and continues to be common until 12 weeks of life (Porter and Dennis 2002).

There were 2 infants with anemia, both were diagnosed with iron deficiency anemia and treated with iron. These increases in anemia are consistent with iron deficiency anemia, which is reported in approximately 20% of infants in the first year of life (Moscheo et al. 2022).

Both anemia and hyperbilirubinemia are consistent with conditions observed in infants.

Table 110. Number of Subjects With Grade 3 and 4 Laboratory Abnormalities, Trial 04 (Japanese Trial Sites)

Laboratory Abnormalities	Nirsevimab N=99	Placebo N=47
Hemoglobin		
Grade 3	2	0
Bilirubin		
Grade 3	12	6
Grade 4	0	7

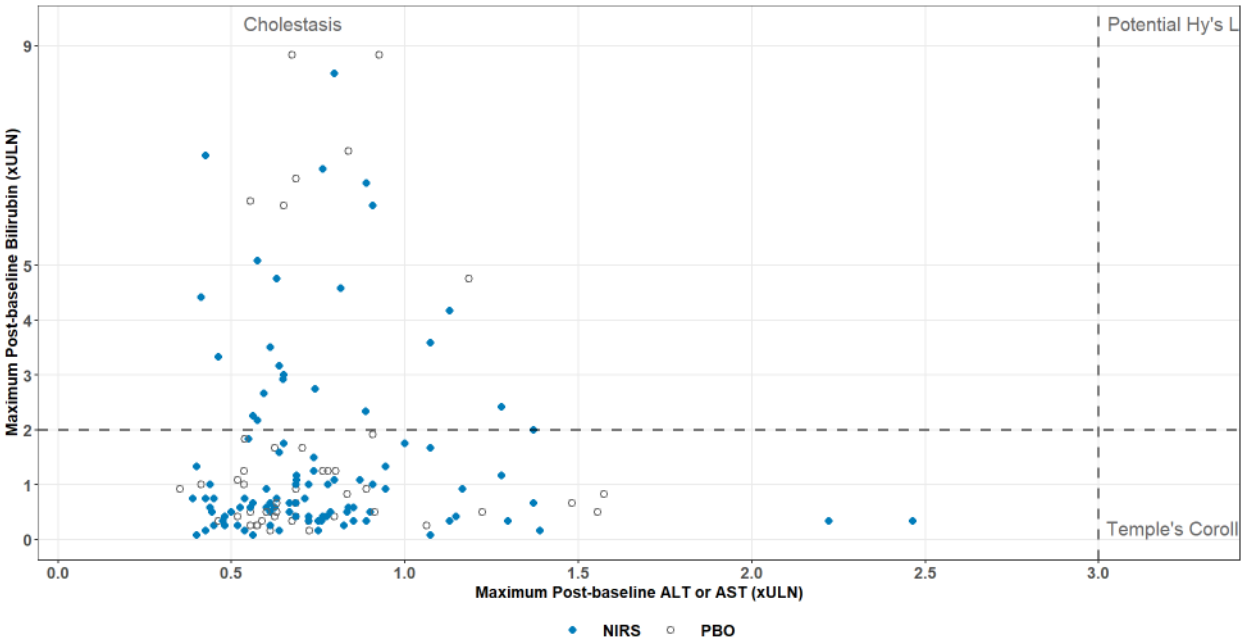
Source: BLA 761328, CSR Trial 04, pages 210-211 and ae dataset

17.2.6.2. Analysis for Hepatocellular Drug-Induced Liver Injury

The following figure, Figure 52, is a plot of maximum ALT or AST compared to maximum total bilirubin values. This analysis was conducted to assess for hepatocellular injury. Hepatocellular injury is defined using Hy's law and is any postbaseline total bilirubin equal to or exceeding 2 times the upper limit of normal (ULN) within 30 days after a postbaseline, ALT or AST equal to or exceeding 3 times ULN, and ALP less than 2X ULN.

Results consistent with hepatocellular injury are identified by a red circle in the figure. No evidence of hepatocellular injury was observed in Trial 04; therefore, there are no red circles in the figure.

Figure 52. Hepatocellular Drug-Induced Liver Injury Screening Plot, Trial 04



Source: CDS: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALT or AST versus their maximum total bilirubin values in the postbaseline period.
A potential Hy's Law case (red circle) was defined as having any postbaseline total bilirubin equal to or exceeding 2X ULN within 30 days after a postbaseline ALT or AST equal to or exceeding 3X ULN, and ALP less than 2X ULN (note ALP values are not circled).
All patients with at least one postbaseline ALT or AST and bilirubin are plotted.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; NIRS, nirsevimab; PBO, placebo; TB, total bilirubin; ULN, upper limit of normal

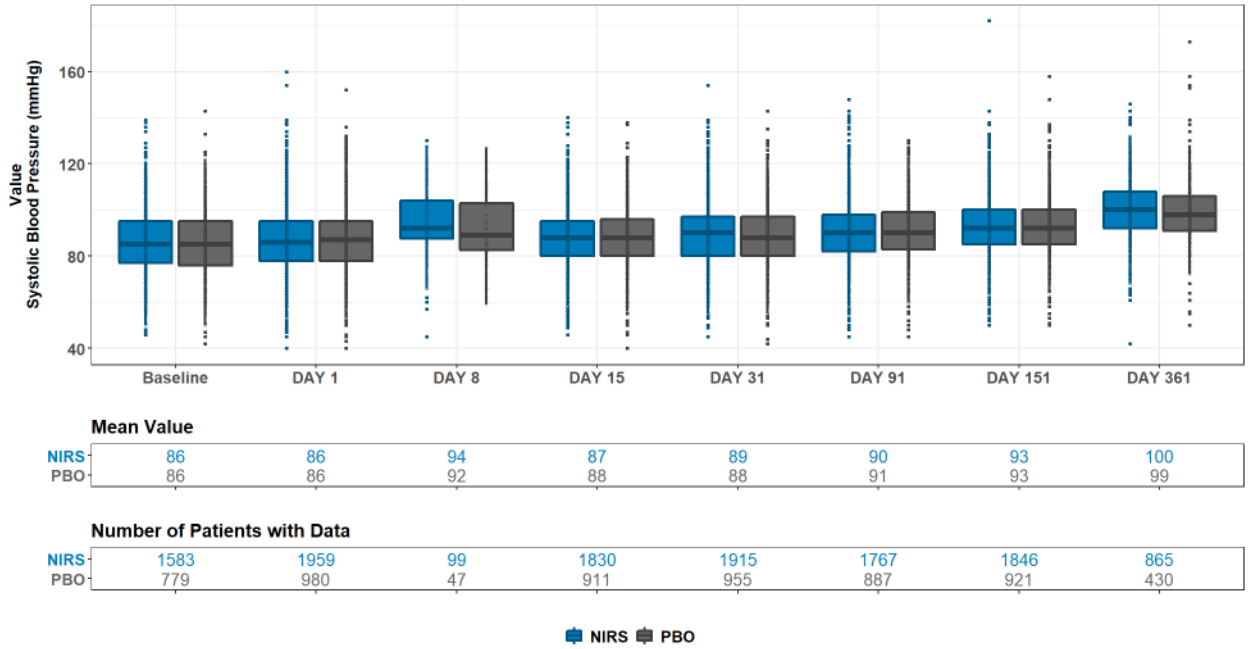
17.2.7. Vital Signs, Trial 04

17.2.7.1. Blood Pressure

The following figure, Figure 53, is a box plot comparing systolic blood pressures from subjects who received nirsevimab to those from subjects who received placebo. Mean systolic blood pressure values are provided under the box plots.

The mean values were similar in the two treatment arms at each time point. There were very few outliers. Overall, systolic blood pressures were similar between the nirsevimab and placebo arms in Trial 04.

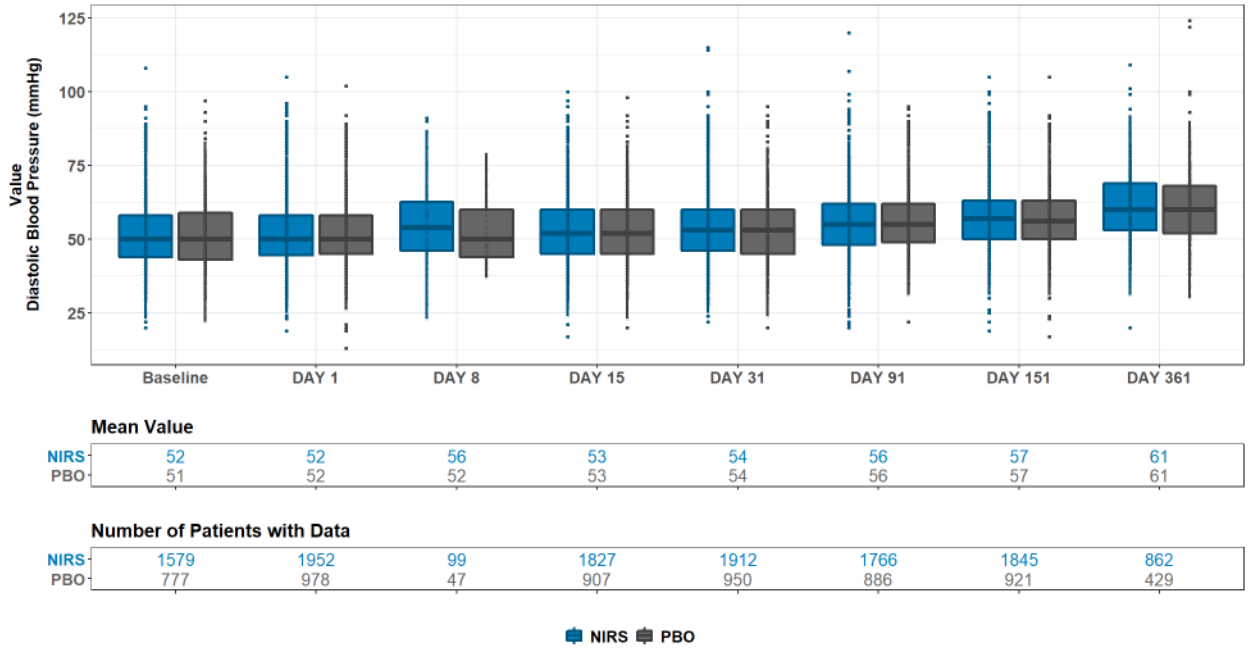
Figure 53. Median and Interquartile Range of Systolic Blood Pressure Over Time by Treatment Arm, Trial 04



Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

Figure 54 shows diastolic blood pressures for subjects in Trial 04. Diastolic blood pressure increases slowly over the first several years of life. However, since infants were enrolled at different ages, it is difficult to see an overall increase over time. The mean diastolic blood pressure was very similar between the nirsevimab and placebo arms. There were few outliers with either very high or low diastolic blood pressures.

Figure 54. Median and Interquartile Range of Diastolic Blood Pressure Over Time by Treatment Arm, Trial 04

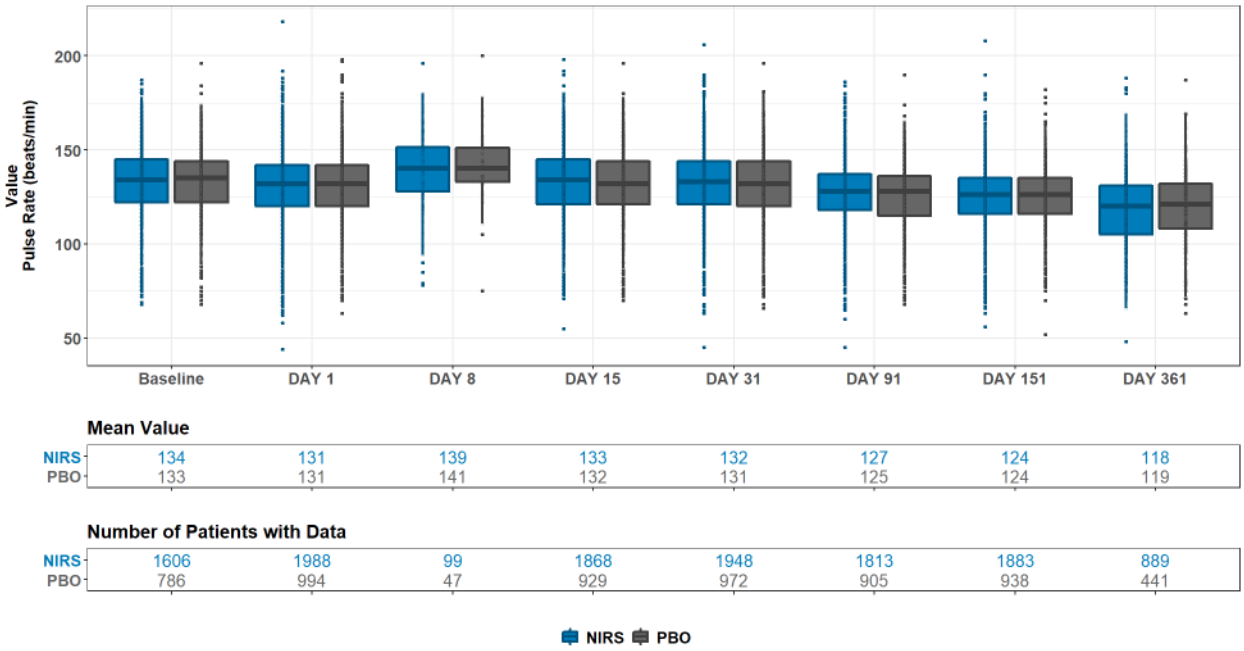


Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual points are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.2.7.2. Heart Rate

The following figure, Figure 55, compares the heart rate of subjects in the nirsevimab arm and the placebo arm in Trial 04. According to published centiles for heart rates, the mean heart rate in infants in the first year of life ranges is approximate 130 beats per min (bpm) (Fleming et al. 2011). Heart rate decreases over the first year of life. The mean heart rates in Trial 04 were within the normal range, and there is a decrease toward the end of the trial, which is consistent with infants aging over the course of the trial. Overall, the mean heart rates were similar between the nirsevimab and placebo arms, and there were very few outlying values.

Figure 55. Median and Interquartile Range of Heart Rate Over Time by Treatment Arm, Trial 04

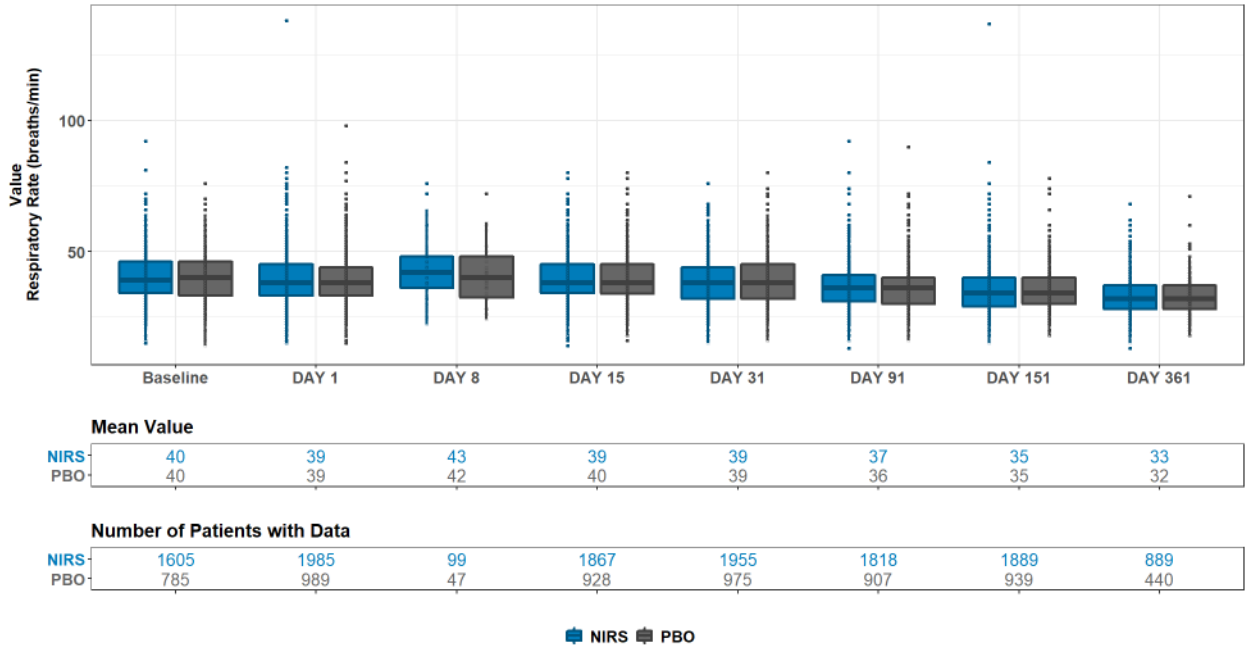


Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.2.7.3. Respiratory Rate

The mean respiratory rates at each visit are shown in the following Figure 56. The mean respiratory rate for infants is approximately 45 breaths per minute, but the respiratory rate decreases over the first year of life. The mean respiratory rates in subjects who received nirsevimab are similar to those in the placebo arms and are within normal values reported in the literature (Fleming et al. 2011).

Figure 56. Median and Interquartile Range of Respiratory Rate Over Time by Treatment Arm, Trial 04



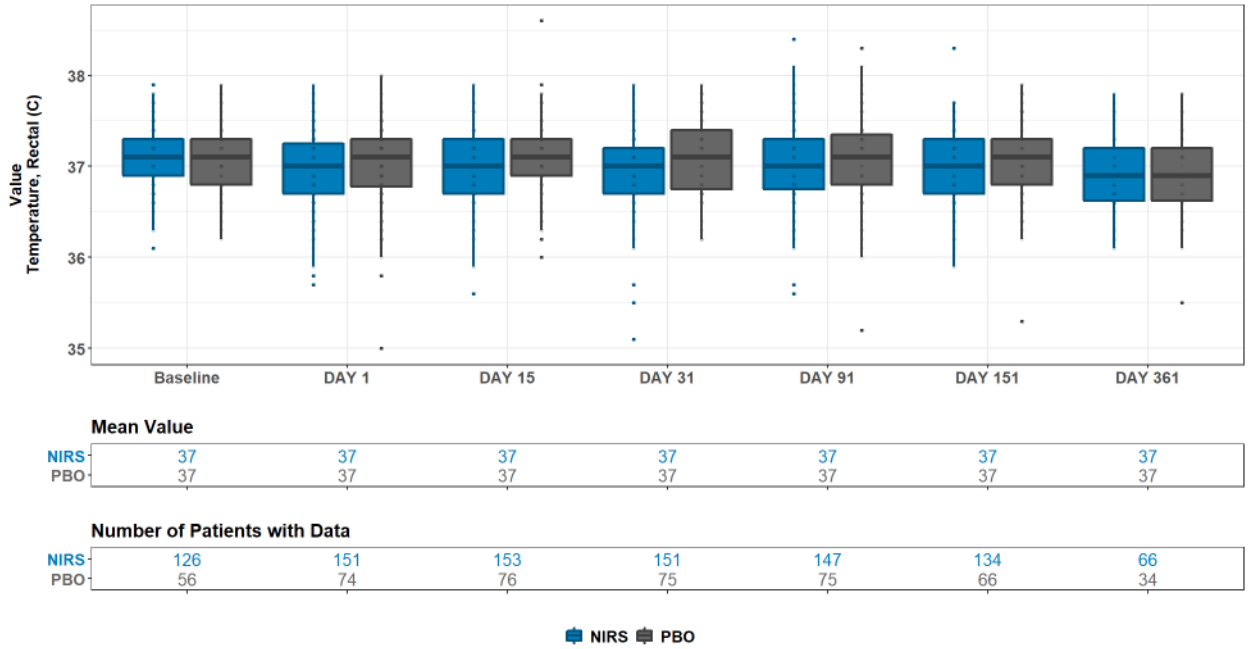
Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.2.7.4. Temperature

Mean and median temperatures are shown in Figure 57. The number of subjects who had their temperature taken was only a small percentage of subjects enrolled. For example, at the baseline visit, only 6.3% of subjects who received nirsevimab had their temperature taken. Temperature measurement at baseline and all visits was prespecified in protocol. The reason for not capturing temperature in case report forms was not provided, and most subjects had their other vital signs monitored.

The mean temperature for both treatment arms at every visit was 37° C. The median values in the box plots are similar between the nirsevimab and placebo arms at each trial visit. There are very few high temperatures outliers. Overall, the temperatures appear similar between the nirsevimab and placebo arm.

Figure 57. Median and Interquartile Range of Body Temperature Over Time by Treatment Arm, Trial 04



Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.2.8. Conclusion

In summary, similar percentage of subjects had reported adverse events, adverse reactions, and serious adverse events in the nirsevimab and placebo treatment arms. There was a slight increase in the number of infants with the adverse event of pyrexia in the nirsevimab arm compared to the placebo arm. There was also an increase in rashes considered drug-related in the nirsevimab arm compared to the placebo arm. Finally, injection site reactions were observed in the nirsevimab arm but not the placebo arm. Although there were four deaths in the nirsevimab arm and none in the placebo arm, the four deaths represent a small percentage of the trial population who received nirsevimab (0.2%). Importantly, the deaths were highly unlikely to be related to nirsevimab. Overall, safety findings were similar in the nirsevimab arm and the placebo arm, however, it is possible that fever may be observed in some term infants who receive nirsevimab postmarketing. In addition, rash and injection site reactions may occur in a small percentage of subjects who receive nirsevimab postmarketing.

17.3. Pooled Subgroup Analyses for Trials 03 and 04

The pooled safety data for Trials 03 and 04, except for subgroup analyses, are presented in Section 7.6.1.

The safety database for the pooled analysis include the following: Trial 03 subjects who weighed <5 kg and received the recommended dose (i.e., 572 in the nirsevimab arm, and 288 in the placebo arm); plus, all Trial 04 subjects (i.e., n=1998 in the nirsevimab arm, and 996 in the placebo arm).

There was no difference in the percentage of subjects with adverse events by sex. The percentage of subjects with AEs by age stratum was similar for the nirsevimab arm and placebo arm for each individual age stratum. The percentage of subjects with AEs in the nirsevimab arm was highest in the subgroup of infants >3 months to ≤6 months; there was no correlation with age and the percentage of subjects with AEs. There were fewer subjects with AEs in the White subgroup compared to other racial subgroups; the reason is unclear but could be related to multiple factors including better access to healthcare. The percentage of subjects in the nirsevimab arm with AEs was higher than the percentage of subjects in the placebo arm with AEs for the American Indian or Alaskan Native, Asian, and Native Hawaiian or Other Pacific Islander subgroups. The reason for the difference is likely due to the small sample size in each of these subgroups. There was no difference in adverse events by Hispanic/Latino ethnicity. There were more AEs in U.S. subjects compared to non-U.S. subjects. The reason for this difference is unclear but could be related to health care access.

Table 111. Overview of Treatment-Emergent Adverse Events by Demographic Subgroup, Pooled Trials 03 and 04

	Treatment-Emergent Adverse Events	
Characteristic	**Treatment Arm N=2570 n/N_s (%)**	**Placebo N=1284 n/N_s (%)**
Sex, n (%)		
Male	1151/1362 (84.5)	541/647 (83.6)
Female	1007/1208 (83.4)	519/637 (81.5)
Age group, years, n (%)		
≤3 months	1404/1675 (83.8)	688/828 (83.1)
>3 months to ≤6 months	616/716 (86.0)	300/362 (82.9)
>6 months	138/179 (77.1)	72/94 (76.6)
Race, n (%)		
American Indian or Alaska Native	87/92 (94.6)	43/52 (82.7)
Black or African American	387/416 (93.0)	166/178 (93.3)
Multiple	21/25 (84.0)	9/11 (81.8)
White	1097/1441 (76.1)	564/740 (76.2)
Asian	98/111 (88.3)	44/56 (78.6)
Native Hawaiian or Other Pacific Islander	20/21 (95.2)	8/11 (72.7)
Other	444/460 (96.5)	226/236 (95.8)
Missing	4/4 (100)	0/0 (NA)
Ethnicity, n (%)		
Hispanic or Latino	677/793 (85.4)	309/375 (82.4)
Not Hispanic or Latino	1476/1772 (83.3)	749/907 (82.6)
Not reported or unknown	5/5 (100)	2/2 (100)
United States vs. non-United States		
United States	446/493 (90.5)	206/234 (88.0)
Non-United States	1712/2077 (82.4)	854/1050 (81.3)

Source: CDS: adae.xpt; Software: R.
Abbreviations: CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects with adverse event; N_s, total number of subjects for each specific subgroup and were assigned to that specific arm

17.4. Safety Results, Trial 05

Safety monitoring and safety results for Trial 05 are discussed in Section 7.6 of this review. Additional analyses for Trial 05 that were not included in Section 7.6 are discussed here, including adverse reactions, deaths narratives, subgroup analyses, laboratory results, and vital signs.

17.4.1. Season 1, Trial 05

17.4.1.1. Deaths, Trial 05, First RSV Season

There were five deaths (0.8%) in the nirsevimab arm and one death (0.3%) in the palivizumab arm. See Table 112. Deaths in the nirsevimab arm include the following.

- A White male was enrolled in Trial 05 at one month of age. He was a born at 32 weeks GA and was enrolled in the preterm cohort. His neonatal course was complicated by respiratory distress syndrome, jaundice, and congenital CMV. On Day 29 he was admitted to an outside hospital with acute bronchiolitis and severe protein calorie malnutrition. He was discharged against medical advice, but his mother brought him back to the hospital with vomiting and weight loss. On Day 52 he experienced respiratory arrest while in the hospital and died. His autopsy revealed respiratory failure with acute bronchiolitis.
- A male infant from the Ukraine was enrolled in the preterm cohort at 7.5 months of age. He was born at 29 weeks GA and was a twin. His history was complicated by neonatal encephalopathy, retinopathy of prematurity, ASD, and VSD. He and his twin were both diagnosed with COVID-19 and admitted to the ICU on Day 149. The infant died due to COVID-19 on Day 162.
- A White female from Hungary was enrolled in the CLD/CHD cohort at 12 weeks of age. She had complicated cardiac disease with coarcation of the aorta, VSD, patent ductus arteriosus, and left superior vena cava. Her history was also complicated by dysgenesis of the corpus callosum, congenital renal disease, hypothyroidism, failure to thrive, and hypertonia. She died suddenly at home on Day 18. She had been seen at clinic 4 days before her death and she was stable at that time. Her death certificate said that her death was due to respiratory failure and bronchopneumonia. No other information was available.
- A Hispanic female was enrolled in the CLD/CHD cohort in Mexico at 2 months of age. She had Down Syndrome, ASD, VSD, and hypothyroidism. She was admitted to the hospital for cardiac failure and was diagnosed with septic shock. She died of cardiogenic shock on Day 66.
- A White male from Russia was enrolled into the CLD/CHD cohort at 6.5 months of age. He had pulmonary atresia, VSD, and congenital arterial anomalies. She had been diagnosed with cardiac insufficiency and hypoxia and died at home on Day 19. Her autopsy revealed cardiac failure.

The death in the placebo arm was in a White female enrolled in the CLD/CHD cohort at 23 days of age. She had PDA, ASD, a chromosomal anomaly, and agenesis of the corpus callosum. On day 29 after her previous dose of palivizumab, she developed respiratory symptoms and was hospitalized. Her condition deteriorated and she developed multi-organ failure. She died on Day 155. An autopsy was not done.

Although there were five deaths in the nirsevimab arm and one in the palivizumab arm; the percentage of deaths was <1% in each treatment arm. All of the infants who died had complicated histories and/or serious underlying disease. Of the deaths in the nirsevimab arm, three infants had severe underlying cardiac disease, death in one was complicated by severe protein calorie malnutrition, and one died due to COVID-19. All of the deaths had underlying causes and none of the deaths appeared to be related to nirsevimab.

Table 112. Deaths, Trial 05 – Season 1

	Overall		Preterm		CLD/CHD	
Preferred Term	**NIRS N=614 n (%)**	**PALI N=304 n (%)**	**NIRS N=406 n (%)**	**PALI N=206 n (%)**	**NIRS N=208 n (%)**	**PALI N=98 n (%)**
Any AE leading to death	5 (0.8)	1 (0.3)	2 (0.5)	0	3 (1.4)	1 (1.0)
Cardiac failure congestive	1 (0.2)	0	0	0	1 (0.5)	0
Cardiogenic shock	1 (0.2)	0	0	0	1 (0.5)	0
COVID-19	1 (0.2)	0	1 (0.2)	0	0	0
Pneumonia	1 (0.2)	0	0	0	1 (0.5)	0
Bronchiolitis	1 (0.2)	1 (0.3)	1 (0.2)	0	0	1 (1.0)

Source: adae.xpt; Software: R
Abbreviations: AE, adverse event; CHD, congenital heart disease; CLD, chronic lung disease; N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; PALI, palivizumab

17.4.1.2. Subgroup Analyses, Trial 05, First RSV Season

An analysis of adverse events by demographic subgroup is shown in Table 113. The percentage of subjects with adverse events by sex is similar in the overall population. There are no increases in AEs in subjects who received nirsevimab in either the female or male subgroups for the preterm or the CLD/CHD cohort. There was a higher percentage of subjects in the nirsevimab arm with AEs in the subgroup of subjects >3.0 months to ≤6-month age group for both the overall population and the preterm cohort. The reason is unclear, and there was no trend in the percentage of subjects with AEs across age groups. As in the other trials, there was a lower percentage of subjects with AEs in the White subgroup compared to other racial subgroups. The reason is unclear but could be related to multiple factors including access to health care. There were more AEs in the Asian and Black/African American subgroups who received nirsevimab compared to those who received palivizumab. However, the numbers of subjects in both subgroups were small. There was no increase in adverse events in Hispanics and Latinos in the nirsevimab arm compared to placebo arm. The percentage of subjects with adverse events in the nirsevimab arm was not increased compared to placebo arm in subjects in the U.S. or in those who were non-U.S. subjects. There were more subjects with AEs in the U.S. subgroup than in the non-U.S. subgroup. The reason for this is unclear.

Table 113. Adverse Events by Demographic Subgroup, Trial 05 Season 1

	Overall		Preterm		CLD/CHD	
	NIRS N=614	PALI N=304	NIRS N=406	PALI N=206	NIRS N=208	PALI N=98
Characteristic	**n/N_s (%)**	**n/N_s (%)**	**n/N_s (%)**	**n/N_s (%)**	**n/N_s (%)**	**n/N_s (%)**
Sex, n (%)						
Female	212/296 (71.6)	92/131 (70.2)	143/201 (71.1)	60/92 (65.2)	69/95 (72.6)	32/39 (82.1)
Male	232/318 (73.0)	123/173 (71.1)	144/205 (70.2)	81/114 (71.1)	88/113 (77.9)	42/59 (71.2)
Age group, months, n (%)						
≤3.0 months	185/273 (67.8)	103/140 (73.6)	139/214 (65.0)	80/111 (72.1)	46/59 (78.0)	23/29 (79.3)
>3.0 to ≤6.0 months	162/209 (77.5)	71/101 (70.3)	98/125 (78.4)	39/59 (66.1)	64/84 (76.2)	32/42 (76.2)
>6.0 months	97/132 (73.5)	41/63 (65.1)	50/67 (74.6)	22/36 (61.1)	47/65 (72.3)	19/27 (70.4)
Race, n (%)						
American Indian or Alaska Native	7/11 (63.6)	4/5 (80.0)	7/11 (63.6)	4/5 (80.0)	0/0 (NA)	0/0 (NA)
Asian	33/36 (91.7)	11/14 (78.6)	23/26 (88.5)	7/9 (77.8)	10/10 (100)	4/5 (80.0)
Black or African American	55/59 (93.2)	26/29 (89.7)	45/49 (91.8)	21/24 (87.5)	10/10 (100)	5/5 (100)
Multiple	5/6 (83.3)	4/4 (100)	2/3 (66.7)	2/2 (100)	3/3 (100)	2/2 (100)
Native Hawaiian or Other Pacific Islander	4/4 (100)	1/1 (100)	3/3 (100)	1/1 (100)	1/1 (100)	0/0 (NA)
Other	15/17 (88.2)	6/6 (100)	8/10 (80.0)	6/6 (100)	7/7 (100)	0/0 (NA)
White	325/481 (67.6)	162/244 (66.4)	199/304 (65.5)	99/158 (62.7)	126/177 (71.2)	63/86 (73.3)
Missing	0/0 (NA)	1/1 (100)	0/0 (NA)	1/1 (100)	0/0 (NA)	0/0 (NA)
Ethnicity, n (%)						
Hispanic or Latino	69/99 (69.7)	31/41 (75.6)	51/77 (66.2)	25/35 (71.4)	18/22 (81.8)	6/6 (100)
Not Hispanic or Latino	375/515 (72.8)	183/262 (69.8)	236/329 (71.7)	115/170 (67.6)	139/186 (74.7)	68/92 (73.9)
Missing	0/0 (NA)	1/1 (100)	0/0 (NA)	1/1 (100)	0/0 (NA)	0/0 (NA)
Is in United States, n (%)						
United States	69/81 (85.2)	32/35 (91.4)	26/33 (78.8)	16/18 (88.9)	43/48 (89.6)	16/17 (94.1)
Non-United States	375/533 (70.4)	183/269 (68.0)	261/373 (70.0)	125/188 (66.5)	114/160 (71.2)	58/81 (71.6)

Source: adae.xpt; Software: R
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; N, number of subjects in treatment arm; n, number of subjects with adverse event; NA, not applicable; NIRS, nirsevimab; N_s, total number of subjects for each specific subgroup and were assigned to that specific arm; PALI, palivizumab

17.4.1.3. Laboratory Results, Trial 05, First RSV Season

17.4.1.3.1. Abnormal Laboratory Results

Laboratory safety monitoring (complete blood count with differential and platelet count, AST, ALT, total bilirubin, and creatinine) was conducted at Japanese trial sites only. A total of 33 subjects (3.6% of the total trial population) including 24 in the nirsevimab arm and 9 in the palivizumab arm, had laboratory testing. It is difficult to draw any conclusions regarding laboratory abnormalities and nirsevimab given the small percentage of subjects with laboratory values reported.

Blood for laboratory testing was collected at baseline and at Days 8, 31, and 151. Laboratory toxicity was graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Grade 3 and 4 laboratory abnormalities are shown in Table 114.

Increased bilirubin levels (≥ Grade 3) were reported in 12 infants (50%) in the nirsevimab arm and in 8 infants in the palivizumab arm (98%). Of the 12 infants in the nirsevimab arm, 8 were in the preterm cohort, and in the palivizumab arm, 5 of the 8 infants with hyperbilirubinemia were preterm. Neonatal hyperbilirubinemia is more common in preterm infants, so an increase in this population is not unexpected. Ten of the 12 infants who received nirsevimab and had hyperbilirubinemia had increased bilirubin levels (Grade 3 or 4) at baseline. Increased total bilirubin values were reported at the Day 8 and 31 visits only; all subjects had a decrease in bilirubin levels after Day 31. Eight of the 12 infants were enrolled in the first month of life, and the other 4 infants were <2 months of age. Grade 4 bilirubin levels ranged from 102.6 to 263.34µMol/L. Upper limit of normal for the testing laboratory was 20.52µMol/L. There were no associated increases in ALT or AST.

Only one subject in the nirsevimab arm had jaundice (Grade 1) reported as an AE. There were no AEs of hyperbilirubinemia.

There one infant with Grade 3 decrease in hemoglobin. The infant was in the nirsevimab arm. The infant had a normal hemoglobin at baseline, and the hemoglobin decreased over time until at Day 151 it was Grade 3 (8.1 g/L). The infant was diagnosed with iron deficiency anemia and treated with iron.

Table 114. Number of Subjects With Grade 3 and 4 Laboratory Abnormalities, Trial 05, Season 1

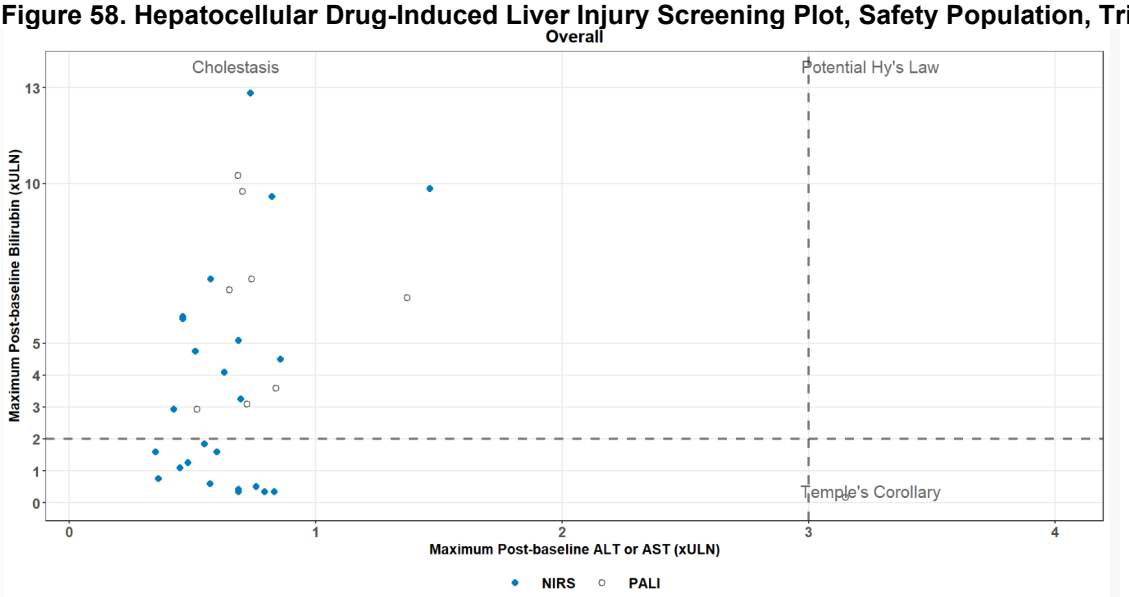
	Overall		Preterm		CLD/CHD	
Laboratory Abnormality	Nirsevimab N=24	Pali. N=9	Nirsevimab N=16	Pali. N=5	Nirsevimab N=8	Pali. N=4
Hemoglobin						
Grade 3	0	0	1	0	0	0
Bilirubin						
Grade 3	5	3	3	1	2	2
Grade 4	7	5	5	4	2	1

Source: BLA 761328, CSR Trial 05, pages 206-207 and ae dataset
Abbreviations: N, number of subjects with available laboratory data; Pali., palivizumab

17.4.1.3.2. Analysis for Hepatocellular Drug-Induced Liver Injury

The following Figure 58 is a plot of maximum ALT or AST compared to maximum total bilirubin values. This analysis was conducted to assess for hepatocellular injury. Results consistent with hepatocellular injury are identified by a red circle. No evidence of hepatocellular injury was observed in Trial 05; therefore, there are no red circles in Figure 58.

Figure 58. Hepatocellular Drug-Induced Liver Injury Screening Plot, Safety Population, Trial 05



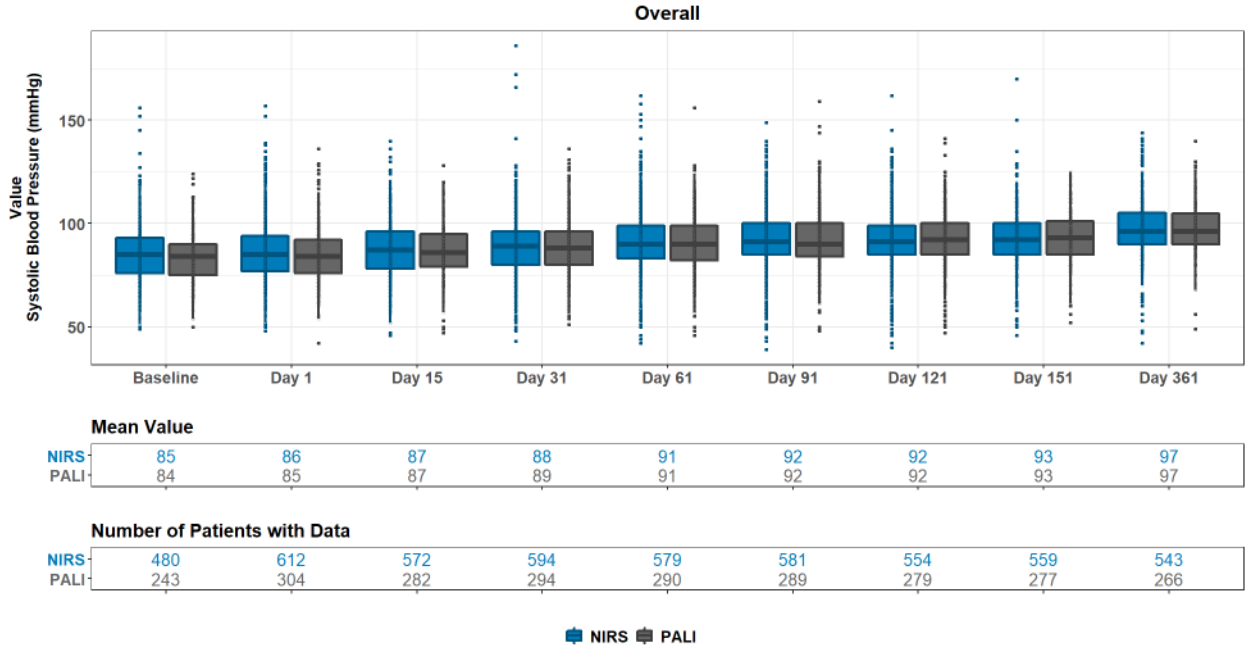
Source: CDS: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALT or AST versus their maximum total bilirubin values in the postbaseline period.
A potential Hy's Law case (red circle) was defined as having any postbaseline total bilirubin equal to or exceeding 2X ULN within 30 days after a postbaseline ALT or AST equal to or exceeding 3X ULN, and ALP less than 2X ULN (note ALP values are not circled).
All patients with at least one postbaseline ALT or AST and bilirubin are plotted.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; NIRS, nirsevimab; PBO, placebo; TB, total bilirubin; ULN, upper limit of normal

17.4.1.4. Vital Signs, Trial 05, First RSV Season

17.4.1.4.1. Blood Pressure

Systolic blood pressures at each visit are shown for the overall population of Trial 05 in the first RSV season in Figure 59. It is possible that there some of the blood pressure outliers are due to the subgroup of subjects with congenital heart disease. However, the mean and median systolic blood pressures are similar in the two treatment arms at each trial visit.

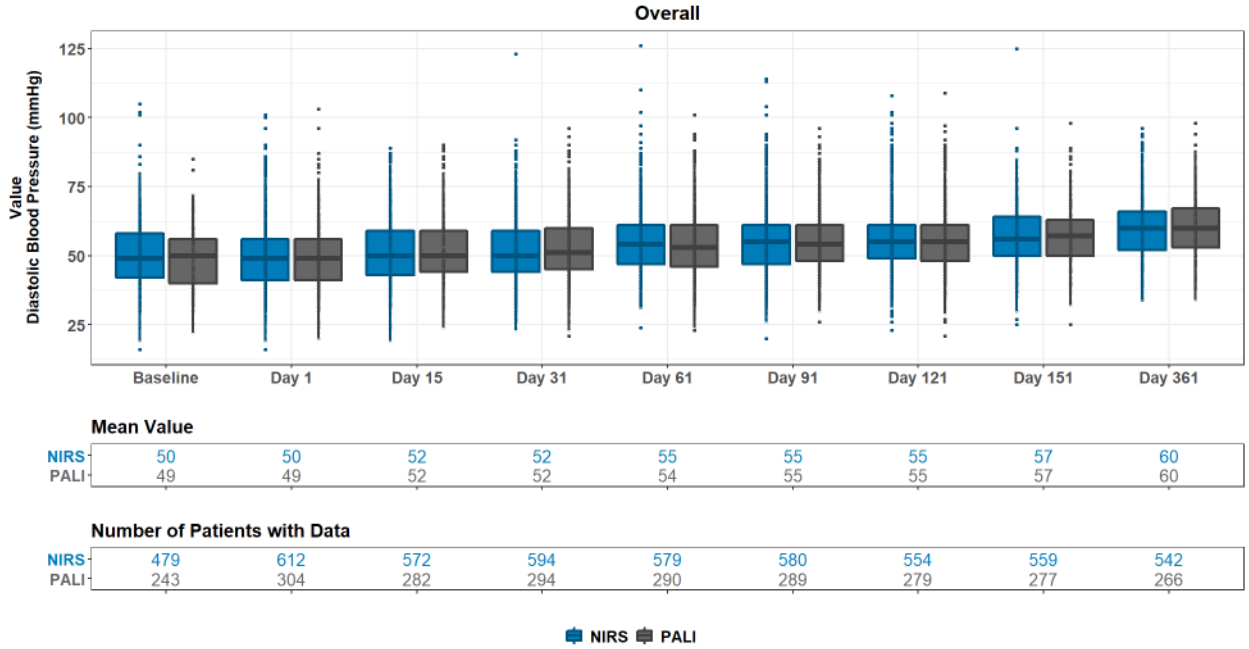
Figure 59. Median and Interquartile Range of Systolic Blood Pressure Over Time by Treatment Arm, Trial 05 – Season 1



Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

The diastolic blood pressures for Trial 05, first RSV season, are shown in Figure 60. The mean and medians are similar in the nirsevimab and palivizumab arms. The outlier values may be related to infants with congenital heart disease, but any effect of congenital heart disease seems to be offset by randomization.

Figure 60. Median and Interquartile Range of Diastolic Blood Pressure Over Time by Treatment Arm, Trial 05 – Season 1

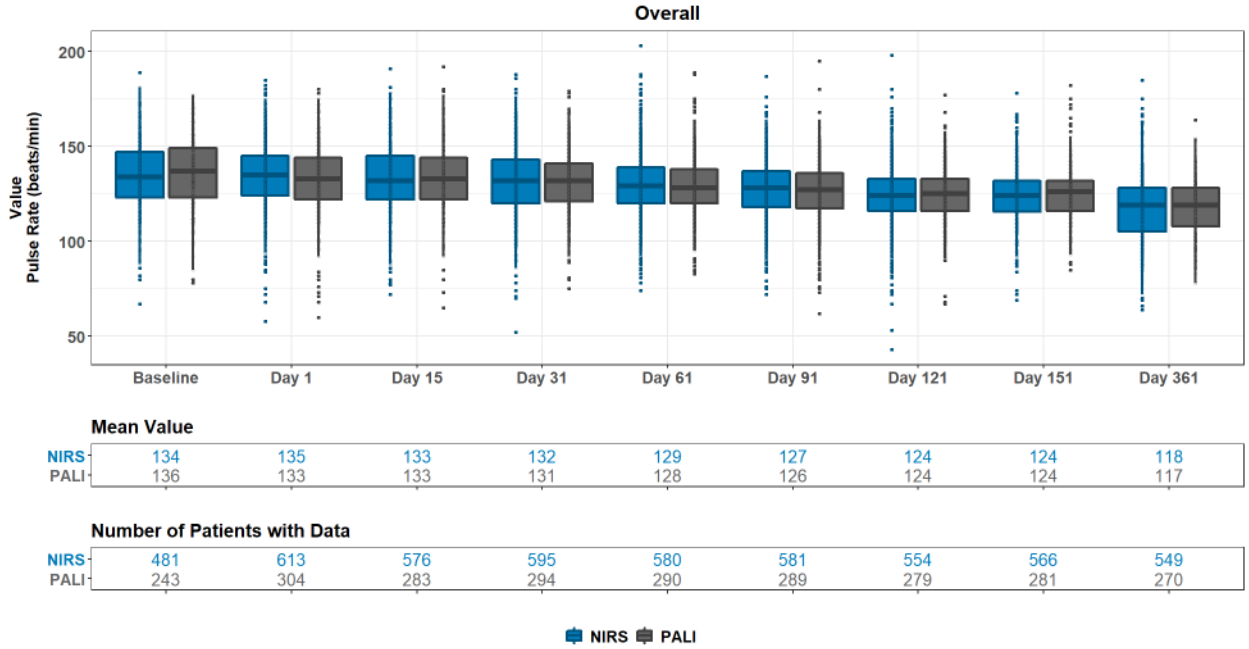


Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.4.1.4.2. Heart Rate

Mean and median heart rates for the overall population of Trial 05, first RSV season, are shown in Figure 61. The means and medians are similar between the treatment arms at each trial visit. The heart rate in both arms decreases slightly over time, which reflects the normal changes observed in heart rate in infants over the first year of life.

Figure 61. Median and Interquartile Range of Heart Rate Over Time by Treatment Arm, Trial 05 – Season 1

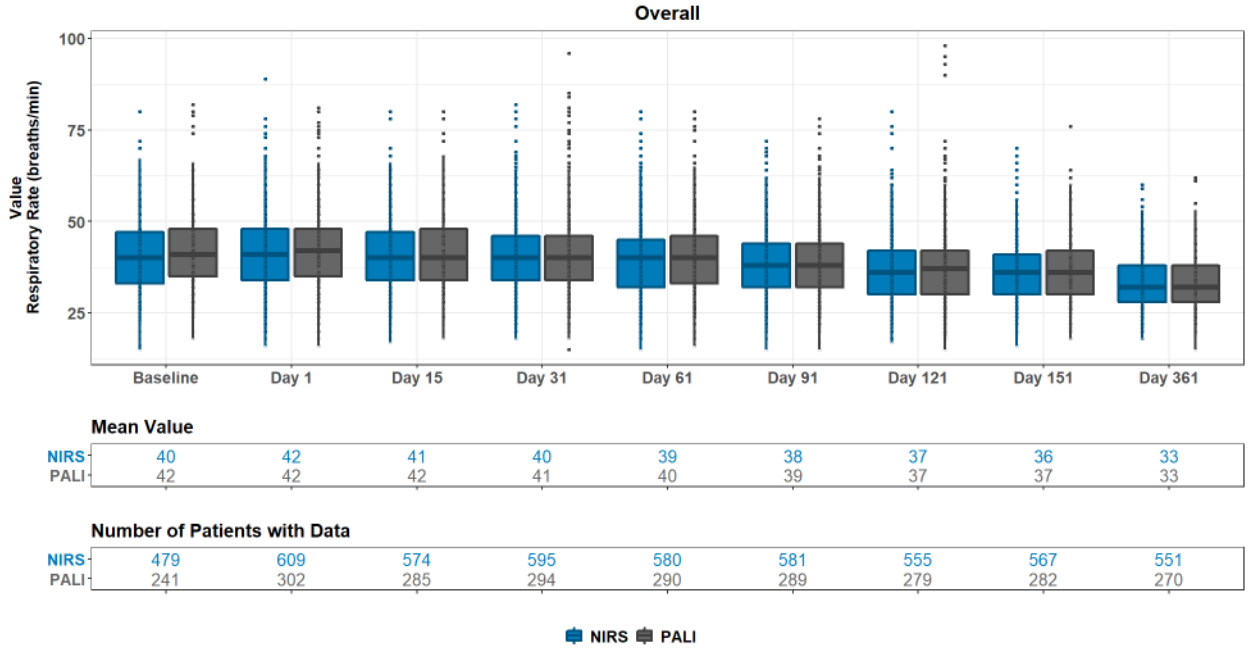


Source: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.4.1.4.3. Respiratory Rate

The respiratory rates at each trial visit in Trial 05, first RSV season are shown in Figure 62. The mean and median respiratory rates are similar between the nirsevimab and palivizumab arm at each visit. The mean respiratory rate decreases slightly over time, which is consistent with respiratory rate changes in infants over the first year of life. There are multiple outliers and the reason for these are unclear; it is possible that some outliers are related to chronic lung disease or congenital heart disease.

Figure 62. Median and Interquartile Range of Respiratory Rate Over Time by Treatment Arm, Trial 05 – Season 1

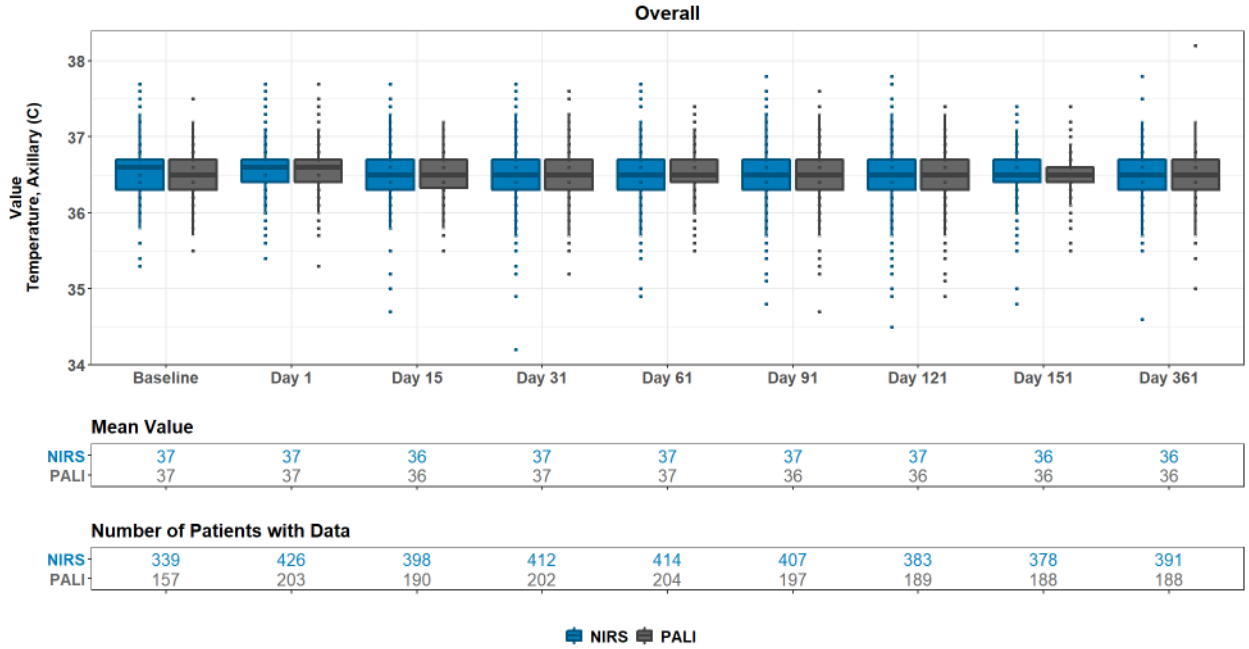


Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.4.1.4.4. Temperature

Body temperature for all trial visits in Trial 05, first RSV season is shown in Figure 63. The mean temperature in the nirsevimab and palivizumab arms is 37°-38° C at each trial visit. The median temperature is similar between the treatment arms at each trial visit. There are a considerable number of outliers. Some of the lower temperatures may be related to prematurity, as premature infants can have difficulties maintaining a stable body temperature. The reason for the higher numbers could be related to individual subjects with pyrexia. Overall, the temperature results are similar for the nirsevimab and palivizumab arms.

Figure 63. Median and Interquartile Range of Body Temperature Over Time by Treatment Arm, Safety Population, Trial 005 – Season 1



Source: CDS: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5X the interquartile range; individual outliers are those beyond this range.
Abbreviations: NIRS, nirsevimab; PBO, placebo

17.4.2. Trial 05, Second RSV Season

Safety monitoring and safety results for Trial 05, second RSV season, were discussed in Section 7.6 of this review. Additional analyses, including subgroup analyses, laboratory safety monitoring, and vital signs, for Trial 05 are discussed in the following section.

17.4.2.1. Deaths, Trial 05, Second RSV Season

There were no deaths in Season 2 of Trial 05.

17.4.2.2. Subgroup Analyses, Trial 05, Second RSV Season

There was no difference in the percentage of subjects with AEs by sex. On comparison of the percentages of subjects with AEs in the nirsevimab compared to the percentage of subjects with AEs in the palivizumab arm for each age subgroup, there were only slight differences between the two treatment arms. Unlike in other trials, the percentage of subjects with AEs was lower in the >3-month to ≤6-month age subgroup compared to the other two age groups. The reason for this is unclear and may be due to chance. The number of subjects who received palivizumab in the second RSV season was so small, and the number of subjects in the palivizumab arm in each age subgroup is ≤2. As a result, it is difficult to distinguish any differences in AEs by treatment arm. The percentage of subjects in the nirsevimab with AEs was lower in the White subgroup than for other racial subgroups. However, the number of subjects in other racial subgroups was

very small (N=1 to 13). There was a slight increase in AEs in Hispanics or Latinos in the nirsevimab arm; however, this also was likely due to small subject numbers with only 21 Hispanics or Latinos in the nirsevimab arm. As in other trials, the number of U.S. subjects with AEs was higher than the number of non-U.S. subjects with AEs in the nirsevimab arm. This is also likely due to smaller numbers of subjects with 44 subjects enrolled in the U.S. and 176 subjects enrolled outside the U.S.

Table 115. Adverse Events by Demographic Subgroup, Trial 05 - Season 2

Characteristic	PALI/PALI N=42 n/N_s (%)	All NIRS N=220 n/N_s (%)
Sex, n (%)		
Female	9/15 (60.0)	61/96 (63.5)
Male	20/27 (74.1)	94/124 (75.8)
Age group, years, n (%)		
≤3.0 months	11/14 (78.6)	43/59 (72.9)
>3.0 to ≤6.0 months	11/19 (57.9)	60/95 (63.2)
>6.0 months	7/9 (77.8)	52/66 (78.8)
Race, n (%)		
Asian	1/2 (50.0)	13/13 (100)
Black or African American	1/1 (100)	11/11 (100)
Multiple	0/1 (0)	3/3 (100)
Native Hawaiian or Other Pacific Islander	0/0 (NA)	1/1 (100)
Other	0/0 (NA)	3/5 (60.0)
White	27/38 (71.1)	124/187 (66.3)
Ethnicity, n (%)		
Hispanic or Latino	0/2 (0)	16/21 (76.2)
Not Hispanic or Latino	29/40 (72.5)	139/199 (69.8)
Is in United States, n (%)		
United States	1/4 (25.0)	40/44 (90.9)
Non-United States	28/38 (73.7)	115/176 (65.3)

Source: adae.xpt; Software: R
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; NIRS, nirsevimab; N_s, total number of subjects for each specific subgroup and were assigned to that specific arm; PALI, palivizumab

17.4.2.3. Laboratory Results, Trial 05, Second RSV Season

Laboratory testing was only conducted at the Japanese sites. In the second RSV season of Trial 05, only 12 subjects had laboratory monitoring. There were no Grade 3 or 4 laboratory abnormalities reported in Season 2.

17.4.2.4. Vital Signs, Trial 05, Second RSV Season

These analyses were limited by the small number of subjects and available data in the second RSV season of Trial 05. Analyses were conducted for blood pressure and pulse only.

17.4.2.4.1. Blood Pressure

Blood pressure was monitored at each trial visit; however, there was more variation in blood pressure results in Season 2, likely due to small numbers. Overall, the mean systolic and diastolic blood pressures were similar in subjects who received nirsevimab and in those who received palivizumab during RSV Season 2 of Trial 05.

17.4.2.4.2. Heart Rate

The difference in heart rate from baseline to values at other visits was compared. Heart rates for subjects who received nirsevimab and for those who received palivizumab were similar at each visit.

17.4.2.5. Conclusion

A comparison of safety in nirsevimab and palivizumab arms for the second RSV season of Trial 05 is limited by the small numbers in the palivizumab arm. For example, there were no serious adverse events in the palivizumab arm. In addition, in subjects who received nirsevimab, there were no adverse reactions, no injection site reactions, and no AESIs in the second RSV seasons. Because of the COVID-19 pandemic, some trial visits were conducted by telemedicine and some visits were missed or delayed. This also may have affected the safety results. However, no safety concerns were identified.

17.5. Safety Results, Trial 08

Trial 08 is a phase 2, open-label, uncontrolled, single arm, safety, PK, and effectiveness trial of nirsevimab in immunocompromised subjects. The primary objective of the trial is the evaluation of safety. The assessment of efficacy, as measured by the incidence of MA RSV LRTI through Day 151, is a secondary objective. Note, this trial is ongoing.

The trial will enroll immunocompromised subjects in their first or second year of life. Subjects in their first year of life will receive a weight-based dose of nirsevimab on Day 1 (a single 50 mg intramuscular dose for infants weighing less than 5 kg and a single 100 mg intramuscular dose for infants weighing 5 kg or more). Subjects in their second year of life will receive a single 200 mg intramuscular dose on Day 1. Subjects will be monitored for 60 minutes postdose. Subjects will be seen at the trial site on Days 31, 91, 151, and 361. Subjects will be contacted by telephone on Day 8, weekly from Days 1 to 151 and monthly from Days 151 to 361 to collect information of adverse events and MA respiratory illnesses. Subjects will participate for one year; either in their first or second year of life.

The trial will enroll neonates, infants, or children ≤24 months of age. Infants in their first year of life must be entering their first RSV season, and infants in their second year of life must be

entering their second RSV season. Subjects must meet at least one of the following conditions at the time of enrollment:

- Combined immunodeficiency (severe combined immunodeficiency, X-linked hyper-immunoglobulin syndrome, etc.), antibody deficiency (X-linked agammaglobulinemia, common variable immunodeficiency, non-x-linked-hyper-IgM syndromes, etc.), or other immunodeficiency (Wiskott-Aldrich syndrome, DiGeorge syndrome, etc.)
- Human immunodeficiency virus infection
- History of organ or bone marrow transplantation
- Receiving immunosuppressive chemotherapy
- Receiving systemic high-dose corticosteroid therapy, or
- Received other immunosuppressive therapy such as azathioprine, methotrexate, mizoribine, cyclosporine, etc.

Neonates, infants, and children will be excluded from trial participation if they are eligible for palivizumab by local guidelines or if they require oxygen supplementation, mechanical ventilation, extracorporeal membrane oxygenation, continuous positive airway pressure, or other mechanical respiratory or cardiac support.

Safety follow-up is until 361 days. All analyses are descriptive.

This trial is ongoing, and at the time of database lock, 60 subjects had been enrolled. The trial will enroll a total of 100 subjects. No cases of MA RSV LRTI were reported at the time of database lock.

17.5.1. Baseline Characteristics

At the time of the database cutoff, 60 subjects had been enrolled. The first subject was enrolled on August 19, 2020 and the database lock date was June 27, 2022.

Of the 60 subjects, 35 (58.3%) were <12 months of age at enrollment and 25 (41.7%) were ≥12 months of age. Fifty-two subjects (87%) had completed Day 151 follow-up, and 10 subjects (17%) had completed Day 361 follow-up. Two subjects prematurely discontinued the trial. One subject was discontinued due to death, which is described in Section 17.5.3. The other subject discontinued this trial in order to participate in another trial.

The mean age was 11.4 months; the median age was 10.9 months (range of 0.7 to 23.9 months). Seventy percent of subjects were male. Most subjects are White (40%) or Asian (47%). The reason for immunocompromise is provided in the following table. Subjects could have more than one reason for immunocompromise, so the percentages in Table 116 do not add up to 100%. The most common reason for immunocompromise was primary immunodeficiency. Receipt of immunosuppressive medication was also common with 17 subjects (28%) receiving high-dose steroids, 9 (15%) receiving chemotherapy, and 9 (15%) receiving other immunosuppressive therapy.

Table 116. Reasons for Immunocompromise, Trial 08

Reason for Immunocompromise	Number (%)
Primary immunodeficiency	28 (46.7%)
Receiving systemic high-dose corticosteroids	17 (28.3%)
History of organ or bone marrow transplant	12 (20.0%)
Receiving immunosuppressive chemotherapy	9 (15.0%)
Receiving other immunosuppressive therapy	9 (15.0%)
Diagnosis of HIV	1 (1.7%)

Source: BLA 761328, CSR Trial 08, Table 11, page 56.
Abbreviation: HIV, human immunodeficiency virus

17.5.2. Overview of Treatment-Emergent Adverse Events Summary, Trial 08

Most subjects (80%) reported at least one event, and 30% reported a serious adverse event (Table 117). Most adverse events were moderate or severe in intensity. There was one death. The number of SAEs and severity of adverse events is consistent with the trial population, who have serious underlying diseases.

Table 117. Overview of Adverse Events, Trial 08

Event Category	Nirsevimab N=60 n (%)
SAE	18 (30%)
SAEs with fatal outcome	1 (1.7%)
Any AE	48 (80.0%)
Severe and worse	19 (31.7%)
Moderate	22 (36.7%)
Mild	7 (11.7%)

Source: BLA 761328, CSR Trial 08, Table 18, page 75.

17.5.3. Deaths, Trial 08

There was a single death in Trial 08. A White female with malignant astrocytoma was enrolled in Trial 08 at 10.7 months of age. Her past medical history was also complicated by protein calorie malnutrition. She received a single dose of nirsevimab on Day 1. She bled into her tumor and died on Day 125. This death was judged as not related to nirsevimab, and the review team agrees with that assessment.

17.5.4. Serious Treatment-Emergent Adverse Events, Trial 08

Serious adverse events were reported in 18 (30%) of subjects in Trial 08. All SAEs were reported in a single subject each, except for the following SAEs, which were reported in 2 subjects (3.3%) each: COVID-19, gastrointestinal viral infection, lower respiratory tract infection, viral upper respiratory tract infection, and nephrotic syndrome. The SAEs of nephrotic syndrome were related to the subjects' underlying diseases. Other than nephrotic syndrome, the SAEs are consistent with those reported in other trials in both nirsevimab and placebo arms.

17.5.5. Treatment-Emergent Adverse Events, Trial 08

Treatment-emergent adverse events reported in more than 5% of subjects in Trial 08 are shown in the following table (Table 118). As in the trials of otherwise healthy infants and children, the most common TEAE was upper respiratory tract infection. However, some AEs, such as febrile neutropenia, were clearly related to the underlying disease. The trial was clearly affected by COVID-19 with 10% of subjects diagnosed with COVID-19 during trial participation. Overall, TEAEs in Trial 08 were a combination of adverse events commonly observed in childhood, such as URTI and diaper dermatitis, and of other adverse events that are associated with immunosuppression and immunosuppressive therapy, such as febrile neutropenia and vomiting.

Table 118. Treatment-Emergent Adverse Events Reported in >5% of Subjects, Trial 08

Event Category	Nirsevimab N=60 n (%)
Any adverse event	48 (80%)
Upper respiratory tract infection	21 (35%)
Pyrexia	17 (28%)
Vomiting	14 (23%)
Diarrhea	12 (20%)
Diaper dermatitis	9 (15%)
Rhinorrhea	7 (12%)
COVID-19	6 (10%)
Viral upper respiratory tract infection	6 (10%)
Febrile neutropenia	5 (8%)
Lower respiratory tract infection	5 (8%)
Otitis media	5 (8%)
Viral gastroenteritis	4 (7%)
Infantile eczema	4 (7%)
Rash	4 (75)

Source: BLA 761328, CSR Trial 08, Table 14.3.1.1.1_a., page 251.
Abbreviations: COVID-19, coronavirus disease 2019; N, number of subjects; n, number of subjects with specific adverse event

There were seven adverse events that were judged as nirsevimab-related. These included four subjects with pyrexia and one subject each with rash, erythema, and abdominal pain. Rash and pyrexia have been reported with nirsevimab in other trials.

17.5.6. Conclusion

Adverse events and serious adverse events were observed commonly in subjects in Trial 08; however, some AEs and SAEs were clearly related to underlying diseases. The majority of the adverse events and serious adverse events were consistent with common childhood illness and with AEs observed in other trials of nirsevimab. Overall, safety was difficult to interpret in Trial 08 because of the serious underlying conditions and the single arm trial design.

17.6. Injection Site Reactions in Trials 03, 04, and 05

Injection site reactions were reported as adverse events by the investigator. Diary cards were not used to collect information on injection site reactions. The first visit after dose administration was on Day 8, so it was possible that injection site reactions were missed. Injection site reactions in the three main trials are shown in Table 119. Season 2 of Trial 05 was not included in Table 119, because no injection site reactions were reported in Season 2.

Injection site reactions were reported with the preferred term of injection site reaction or by the symptoms or signs at the injection site: pain, induration, or erythema. Overall, any type of injection site reaction was reported in 0.3% to 0.6% of subjects who received nirsevimab. The most common injection site reactions were pain and induration. All injection site reactions were mild in intensity. Injection site reactions do not appear to be common or serious adverse reactions associated with nirsevimab, but they may have been underreported in the trials.

Table 119. Injection Site Reactions, Trials 03, 04, and 05

	Trial 03		Trial 04		Trial 05, Season 1	
Signs or Symptoms of Injection Site Reaction	Nirsevimab N=968	Placebo N=479	Nirsevimab N=1998	Placebo N=996	Nirsevimab N=614	Palivizimab N=304
Injection site reaction	1 (0.1%)	0	0	0	1 (0.2%)	0
Injection site pain	2 (0.2%)	1 (0.2%)	4 (0.2%)	0	0	0
Injection site induration	3 (0.3%)	0	2 (0.1%)	0	1 (0.2%)	1 (0.3%)
Injection site erythema	0	0	0	0	1 (0.2%)	1 (0.3%)

Source: BLA 761328, CSRs Trials 03, 04, and 05 and ae datasets.
Abbreviation: N, number of subjects

17.7. New Onset Chronic Diseases in Trials 03, 04, and 05

Information on new onset chronic diseases (NOCDs) was collected in each of the three main trials of nirsevimab (Trials 03, 04, and 05). The protocol defined a new onset chronic disease as a newly diagnosed medical condition that is of a chronic, ongoing nature. NOCD must not have existed at baseline and had to be judged by the investigator as significant. For example, eczema could not be considered a NOCD. Information on NOCD is typically collected in trials of vaccines because vaccines may affect the immune system. However, nirsevimab is a monoclonal antibody that is specific for RSV and is less likely to have a nonspecific effect on the immune system.

New onset chronic diseases reported in these trials are shown in Table 120. The majority of NOCDs in both the nirsevimab group and the control groups were asthma (including wheezing and chronic bronchitis). Asthma was reported in 0.2% of subjects in both the nirsevimab and control groups. Overall, the percentage of NOCDs was low and was similar in the nirsevimab arms and the control arms. There was no evidence of an increase in NOCDs associated with nirsevimab.

Table 120. New Onset Chronic Diseases, Trials 03, 04, and 05

New Onset Chronic Disease	Trial 03 Nirs. N=968	Trial 03 Placebo N=479	Trial 04 Nirs. 1998	Trial 04 Placebo N=996	Trial 05, Season 1 Nirs N=614	Trial 05, Season 1 Pali. N=304
Asthma	3 (0.3%)	2 (0.4%)	2 (0.1%)	0	1 (0.2%)	0
Infantile asthma	0	1 (0.2%)	0	0	0	0
Chronic bronchitis	0	1 (0.2%)	0	0	0	0
Wheezing	1 (0.1%)	0	0	0	0	0
Hypothyroidism	0	0	0	2 (0.2%)	0	0
PFAPA syndrome	0	0	1 (0.1%)	0	0	0
Urinary calculi	0	0	0	0	1 (0.2%)	0

Source: BLA 761328, CSRs Trials 03, 04, and 05 and ae datasets.
Abbreviations: Nirs., nirsevimab; Pali., palivizumab; PFAPA syndrome, periodic fever, aphthous stomatitis, pharyngitis, and adenitis

18. Clinical Virology

18.1. RSV Surveillance Studies, 2015 to 2021

Study reports: ID8897-sVAP-002 amend 1; ID8897-0102.

Key to the effectiveness of monoclonal antibodies being used for immunoprophylaxis of RSV is that the prevalent viral variants in circulation maintain susceptibility to neutralization. The phase 3 clinical trial of suptavumab, targeting site V of prefusion F protein, failed to meet the primary endpoint of prevention of hospitalization or MA LRTI; this was attributed mainly to the emergence in 2015 and subsequent predominance of L172Q+S173L substitutions in RSV B strains, which caused loss of binding and neutralization activity (Simoes et al. 2021). For nirsevimab and palivizumab, binding affinity to RSV F appears to correlate with neutralization activity and is impacted by amino acid substitutions in the respective binding sites (Section 20.7). Hence, to determine whether these mAbs maintain activity from one season to the next it is important to monitor contemporary and circulating clinical variants through sequencing of the F protein and phenotyping of any novel substitutions within and potentially outside of the antibody binding sites.

The Applicant initially examined the breadth of antiviral activity of nirsevimab against geographically and temporally diverse clinical isolates collected from 2003 to 2017 (Section 20.6). In 2015, an RSV surveillance program in the U.S. was initiated, OUTSMART-RSV (Observational United States Targeted Surveillance of Monoclonal Antibody Resistance and Testing of RSV; (Ruzin et al. 2018; Bin et al. 2019)), and subsequently in 2017 an international program, INFORM-RSV (International Network For Optimal Resistance Monitoring of RSV; (Langedijk et al. 2020; Tabor et al. 2020; Wilkins et al. 2023)). Data from these ongoing studies, as well as an assessment of RSV isolates from 2015 to 2017 collected from a pilot program in South Africa (Liu et al. 2020), were submitted with the application. The isolates that were collected from these programs were subtyped through sequencing of the RSV G protein, and polymorphisms potentially impacting nirsevimab or palivizumab activity identified through sequencing of the complete RSV F protein.

A separate study report was provided for each season, for OUTSMART-RSV and INFORM-RSV studies, and a single report for the South Africa pilot study. In addition, study reports were

provided presenting an analysis of the RSV NS2 and L gene regions of RSV RNA detected by primers/probes in the RT-PCR assay used in nirsevimab clinical studies (Lyra® RSV+human metapneumovirus (hMPV) assay [Quidel assay]), for seasons assessed in the surveillance programs.

OUTSMART-RSV: ID8897O-1516; ID8897O-1617; ID8897O-1718; ID8897O-1819; ID8897O-1920; ID8897O-2021

INFORM-RSV: ID8897I-1718; ID8897I-1819; ID8897I-1920; ID8897I-2021

South Africa pilot study: ID8897-0027 amend 2

NS2 and L gene analyses: ID8897O-BAR-Q-1617; ID8897O-BAR-Q-1718; ID8897O-BAR-Q-1819; ID8897I-BAR-Q-1718; ID8897I-BAR-Q-1819; ID8897I-BAR-Q-1920; ID8897I-BAR-Q-2021

18.1.1. Objectives

The purpose of the surveillance programs is to characterize RSV seasonal variants circulating in the northern and southern hemispheres and determine the prevalence of variants harboring nirsevimab or palivizumab resistance-associated substitutions. The key objectives, as stated in the study reports, are:

- To evaluate the genetic variability of F protein sequences, including the nirsevimab and palivizumab binding site, from recent circulating RSV A and RSV B strains
- To track the emergence, geographic distribution, and temporal frequency of RSV variants harboring prevalent F sequence variations and any nirsevimab and/or palivizumab binding site substitutions, including resistance-associated substitutions
- To evaluate cell culture neutralization susceptibility of recombinant RSV (rRSV) variants containing prevalent RSV F protein sequence variations and any nirsevimab and/or palivizumab binding site substitutions

The populations and countries included in the OUTSMART-RSV and INFORM-RSV programs are shown in Table 121. Also shown is SEARCH-RSV, a similar program being conducted in China, but with no data submitted with the application.

Table 121. Summary of RSV Molecular Surveillance Studies Supporting Nirsevimab

Study	Study Design	Population	RSV (+) Sample Size[a]	Study Period
OUTSMART - RSV	PS, MC, PV, OB	Infants (<2 yoa), children (2-12 yoa), adolescents (12-21 yoa), adults (≥21 yoa)	Total planned: >6,500 (50 samples/site/year)[b]	2015 to 2022+
INFORM-RSV	PS, MC, PV, OB	Infants (<2 yoa) and young children (2-5 yoa)	Total planned: >4,000 (50-100 samples/site/year)[c]	2017 to 2022+
SEARCH-RSV	PS, MC, PV, OB	Infants (<2 yoa) and young children (2-5 yoa)	Total planned: >1,200 (50 samples/site/year)[d]	2020 to 2024+

Source: page 10, study report ID8897-sVAP-002 amend 1
[a] First 10–20 RSV-positive nasal samples/month/site x ~5 months/year (typical length of RSV season)
[b] OUTSMART-RSV (USA); 4 geographical regions (West, Midwest, Northeast, South) at ~25-27 sites/year
[c] INFORM-RSV (global); GBR, ESP, NLD, FIN, JPN, BRA, ZAF, AUS (2017-2022+); CAN, FRA, GER, ITA (2018-2022+); KOR, TWN, RUS, MEX, CHL (2019-2022+) at 1 site/country except CAN with 2 sites
[d] SEARCH-RSV (CHN); 6 geographical regions (Northwest, North, Northeast, Southwest, South Central, East) at 6 sites/year
Abbreviations: INFORM-RSV, International Network For Optimal Resistance Monitoring of RSV; MC, multicenter; OB, observational; OUTSMART-RSV, Outsmart United States Targeted Surveillance of Monoclonal Antibody Resistance and Testing of RSV; PS, prospective; PV, passive; RSV, respiratory syncytial virus; RSV (+), RSV-positive; SEARCH-RSV, Surveillance, Epidemiology and Research of China Hospital-Associated RSV; yoa, years of age

18.1.2. Methodology

Sample Collection and Processing

For the prospective surveillance studies, respiratory secretions from inpatient or outpatient subjects seeking medical attention for an upper or lower respiratory tract infection were tested at local and/or central laboratories for RSV using a variety of diagnostic assays based on individual institutional protocols. All samples were shipped on dry ice to a central repository for storage and processing (b) (4) for OUTSMART-RSV and South Africa pilot programs, and (b) (4) for INFORM-RSV). Samples for OUTSMART-RSV and the South Africa pilot program were transferred to AstraZeneca/MedImmune (CA, USA) for sequencing and data analysis, and samples for INFORM-RSV were sequenced at (b) (4).

None of the subjects included in these studies were enrolled in clinical trials of nirsevimab. Nasal samples which were positive for RSV and anonymized demographic data were collected. In accordance with the study protocol, the first 10 to 20 RSV-positive nasal samples/month/site over the course of the typical 5-month RSV season were submitted each year for resistance analyses.

Nasal samples obtained using a flocked swab with a plastic shaft were collected in universal transport medium (UTM) or viral transport medium (VTM) and stored at -20°C or -70°C, then shipped on dry ice to the sequencing laboratory. Nucleic acids were extracted from samples using the Nuclisens easyMAG® (bioMerieux, Inc., Durham, NC) assay for OUTSMART-RSV and the South Africa pilot study, or the MagNA Pure LC kit (Roche Molecular Systems, Pleasanton, CA) for the INFORM-RSV study.

Reference ID: 5208810

RT-PCR Amplification of RSV F and G Genes and Next-Generation Sequencing (NGS)

RSV RNA was reverse transcribed, and the F and G genes amplified for sequencing. For OUTSMART-RSV and South Africa studies, the SuperScript™ III One-Step RT-PCR System (Life Technologies, Carlsbad, CA) was used for gene amplification.

For OUTSMART-RSV 2015/2016 (study report: ID8897O-1516), the F and G genes were amplified by RT-PCR separately. To amplify full-length F gene, RSV A and RSV B strain specific primers were used (RSV-AF-Fwd 8059: 5' CTCTGGGGCAAATAACAATGG 3'; RSV-AF-Rev1055: 5' AGTGTAAGTGAGATGGTTTATAGATG 3'; RSV-BF-Fwd-v2: 5' CTGGGGCAAATAACCATGG 3'; RSV-BF-Rev-v2: 5' GGTAGCATGATGTGAGGAAATG 3'). For this study only, Sanger sequencing of RT-PCR products was used, using the strain specific primers for RSV A and RSV B, and the internal sequencing primers for both RSVA and RSV B genes: RSVF-6418 Forward 5' TCAATGATATGCCTATAACAAATGATC 3'; RSVF-6637 Reverse 5', CCARCARGGWGTATCWATWACACCAT 3'.

For OUTSMART-RSV (2016 to 2021) and South Africa studies, the RSV G second hypervariable region (HVR2) through the full-length F from RSV A and B genomes was amplified by RT-PCR as a single 2.5 kb fragment, using the primers shown in Table 122. Products were sequenced using NGS.

For INFORM-RSV studies, multiplexed TaqMan RT-PCR analysis of the RSV N gene using RSV A and RSV B specific primer/probe mixes (Langedijk et al. 2020) was used for initial subtyping and quantification, then one-Step RT-PCR System (Invitrogen, Carlsbad, CA USA) used to amplify 4 overlapping fragments covering the full-length genome of RSV (15 kb). Table 122 also lists the whole genome amplification primers used in the INFORM-RSV studies.

Table 122. RSV HVR2 G-F and Whole Genome RT-PCR Amplification Primers

Study	Process	Primer Name/Position	Oligonucleotide Sequence (5'-3')
OUTSMART- RSV SEARCH-RSV	RSV G HVR2-F	RSV_F5109–5129Y	AGTGTTCAAYTTYGTWCCYTG
		RSV_R7654–7634	YTACCATTCAAGCAATGACCTC
INFORM-RSV	RSV WG	RSVA-fragment1-Fw	AAAAATGCGTACWACAAACTTGC
		RSVA-fragment1-Rev	GTTGGTCCTTGGTTTTGGAC
		RSVA-fragment2-Fw	CACAGTGACTGACAACAAAGGAG
		RSVA-fragment2-Rev	GCTCATGGCAACACATGC
		RSVA-fragment3-Fw	CGAGGTCATTGCTTGAATGG
		RSVA-fragment3-Rev	CACCACCACCAAATAACATGG
		RSVA-fragment4-Fw	AGGGTGGTGTCAAAAACTATGG
		RSVA-fragment4-Rev	ACGAGAAAAAAAGTGTCAAAAACT
		RSVB-fragment1-Fw	AAAAATGCGTACTACAAACTTGC
		RSVB-fragment1-Rev	TTGTGCTTGGCTTGTTGTTC
		RSVB-fragment2-Fw	AAGGGTTAGCCCATCCAAMC
		RSVB-fragment2-Rev	TGCTAAGGCTGATGTCTTTCC
		RSVB-fragment3-Fw	GTCCTCGTCTGARCAAATTGC
		RSVB-fragment3-Rev	TAGGTCCTCTTTCACCACGAG

Study	Process	Primer Name/Position	Oligonucleotide Sequence (5'-3')
		RSVB-fragment4-Fw	GAGGGATCCACAGGCTTTAGG

Source: page 21, study report ID8897-sVAP-002 amend 1
"R" and "Y" in the nucleotide sequence refers to any purine and any pyrimidine, respectively
"W" refers to A-T weak bonds (2 H bonds)
"M" refers to adenine or cytosine
Abbreviations: RSV HVR2, respiratory syncytial virus second hypervariable regions; RT-PCR, reverse transcriptase-polymerase chain reaction

For all NGS studies, libraries were constructed from the normalized RT-PCR amplicon using the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA). Illumina sequencing adapters and barcodes were added to the tagmented DNA via PCR amplification using unique custom oligo sequences ([redacted] (b) (4)), and the DNA sequenced.

Raw sequencing reads were assembled into RSV G HVR2 - F contigs, and RSV subtypes assigned based on alignment with reference sequences from Netherlands RSV A/13-5275 (GenBank accession number KX858754.1) and RSV B/13-1273 (GenBank accession number KX858755.1) clinical isolates collected in 2013, and a 2014 reference sequence database of 11 RSV A genotypes and 23 RSV B genotypes (Tabatabai et al. 2014).

Analysis of F Protein Amino Acid Substitutions

RSV F gene sequences in FASTA format were translated into amino acids sequences, then aligned with reference sequences from Netherlands clinical isolates RSV A/13-5275 (GenBank accession number KX858754.1) and RSV B/13-1273 (GenBank accession number KX858755.1). Amino acid variation frequency in the F protein was calculated from the number of sequences harboring a substitution divided by the number of sequences in the RSV A or RSV B datasets.

Frequency tables for variants were generated to show amino acid substitution(s) identified in the full-length RSV F protein (AA 1-574), the extracellular region of the mature RSV F protein (AA 24-109 and AA 137-524), antigenic sites (Ø and I–V) (Gilman et al. 2016), and the nirsevimab (AA 62-69 and AA 196-212) and palivizumab (AA 262-275) binding sites.

Individual polymorphisms in the full-length F protein (AA 1–574) and co-occurring polymorphisms in the extracellular region of the mature RSV F protein (AA 24-109 and AA 137-524) which were detected at ≥10% prevalence within an RSV season and/or ≥3-fold increase above ≥1% from the previous RSV season were also phenotypically assessed.

Generation of Recombinant RSV Using Reverse Genetics

Amino acid changes of interest were engineered into the RSV A or RSV B F gene with standard techniques, using full-length rRSV A2-000 or RSV B9320-000 cDNA. These rRSV strains are versions of RSV A2 and RSV B9320 modified to include F protein E66 and N197 residues, respectively, which are more representative of contemporary isolates, and consistent with the Netherlands RSV A/13-5275 and Netherlands RSV B/13-1273 reference strains. Recombinant RSV was rescued following transfection into BSR-T7 cells as described in Section 20.7.1, using the approach of (Hotard et al. 2012), then plaque purified in Vero cells and expanded in HEp-2 cells. Recombinant viral RNAs were confirmed by sequencing.

Microneutralization Assay

The activity of nirsevimab and palivizumab against RSV variants was determined by microneutralization assay as described in Section 20.6. Fold-changes in susceptibility were calculated by comparison with the EC_{50} value against the corresponding rRSV A2-000 or rRSV B9320-000 reference strain. All rRSV variants were tested against nirsevimab and palivizumab to assess cross-resistance. According to the Applicant's validation report, ≥5.0-fold shifts in neutralization susceptibility could be detected with 99.2% confidence. It is not known what fold-shift in susceptibility is clinically relevant; breakthrough infections were seen with variants which did not have reduced susceptibility to nirsevimab, and there were no variants or substitutions which were clearly associated with breakthrough infections in nirsevimab-treated subjects (see Section 18.6).

18.1.3. Results

Sample Collection

Samples testing positive for RSV were collected between February 2015 and December 2021 from sites/countries participating in the OUTSMART-RSV, INFORM-RSV and South Africa pilot study. From the samples received (n=7,547), sequence and assembly of the RSV G HVR2 – F regions was successful for 5,735, and of these 2,875 (50.7%) were RSV A and 2,800 (49.3%) were RSV B, and 60 had partial length F or missing metadata.

Table 123 shows the number of samples collected in each program for each RSV season evaluated. In total, 3,921 RSV isolates were collected from the OUTSMART-RSV study (RSV A: 1954; RSV B: 1967), 1,607 from the INFORM-RSV study (RSV A: 847; RSV B: 760), and

147 from the pilot South Africa study (RSV A: 74; RSV B: 73). Since the beginning of 2020, the SARS-CoV-2 pandemic impacted sample collection, accounting for the low number of samples collected from the 2020-2021 season compared to prior seasons.

Figure 64 shows the varying incidence of RSV A and RSV B subtypes across the 6 seasons evaluated, showing alternating predominance of the two subtypes. These findings are consistent with other studies in the U.S. (Adhikari et al. 2022), and in other countries (Zlateva et al. 2007; Staadegaard et al. 2021), showing subtype predominance varying across seasons. The determination of subtype predominance is less clear for 2020-2021 because of the impact of the SARS-CoV-2 pandemic on sample numbers.

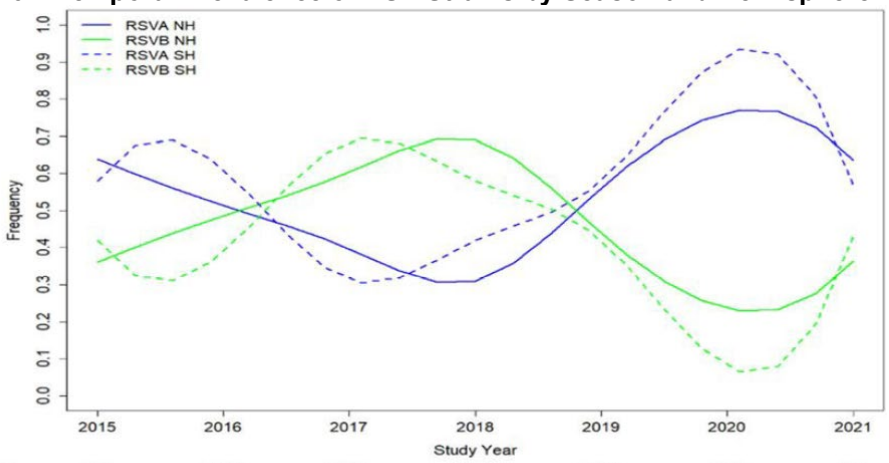
Table 123. Global RSV Strains Collected by Study, Season, and Hemisphere

RSV Season	Hemisphere	Countries	Study Report	RSV G HVR2 – F Sequences	
				RSV A (N=2,875)	RSV B (N=2,800)
2015-2016	NH	USA	ID8897O-1516	179	122
	SH	ZAF	ID8897-0027	29	21
	Total NH+SH			208	143
2016-2017	NH	USA	ID8897O-1617	387	457
	SH	ZAF	ID8897-0027	30	19
	Total NH+SH			417	476

RSV Season	Hemisphere	Countries	Study Report	RSV G HVR2 – F Sequences RSV A (N=2,875)	RSV B (N=2,800)
2017-2018	NH	ESP, GBR, FIN, JPN, NLD, USA	ID8897O-1718 ID8897I-1718	260 46	615 171
	SH	AUS, BRA, ZAF	ID8897I-1718 ID8897-0027	81 15	112 33
	Total NH+SH			402	931
2018-2019	NH	CAN, DEU, ESP, FIN, FRA, GBR, NLD, USA	ID8897O-1819 ID8897I-1819	359 98	540 181
	SH	AUS, BRA, ZAF	ID8897I-1819	113	82
	Total NH+SH			570	803
2019-2020	NH	CAN, DEU, ESP, FIN, FRA, GBR, ITA, JPN, KOR, MEX, NLD, RUS, TWN, USA	ID8897O-1920 ID8897I-1920	762 287	207 146
	SH	AUS, ZAF	ID8897I-1920	50	3
	Total NH+SH			1,099	356
2020-2021	NH	CAN, FRA, GBR, NLD, TWN, USA	ID8897O-2021 ID8897I-2021	7 29	26 21
	SH	ZAF	ID8897I-2021	43	44
	Total NH+SH			179	91

Source: page 17, study report ID8897-0102
Abbreviations: AUS, Australia; BRA, Brazil; CAN, Canada; DEU, Germany; ESP, Spain; FIN, Finland; FRA, France; GBR, United Kingdom, HVR2, second hypervariable regions; ITA, Italy; JPN, Japan; KOR, South Korea; MEX, Mexico; N, number of subjects; NH, northern hemisphere; NLD, Netherlands; RSV, respiratory syncytial virus; RUS, Russia; SH, southern hemisphere; TWN, Taiwan; USA, United States; ZAF, South Africa

Figure 64. Temporal Prevalence of RSV Strains by Season and Hemisphere



RSVA NH	23	313	365	358	747	626	82
RSVB NH	13	296	555	797	585	193	47
RSVA SH	29	30	15	81	113	35	58
RSVB SH	21	19	33	112	82	3	44

Source: page 18, study report ID8897-0102
Abbreviations: NH, northern hemisphere; RSV, respiratory syncytial virus; SH, southern hemisphere

Conservation of Full-Length RSV F Protein Sequences From 2015 to 2021

To assess the natural variation of RSV F protein across seasons, the sequences from the surveillance programs were compared with reference isolates from 2013: Netherlands RSV A/13-5275 and RSV B/13-1273. Amino acid substitutions in F protein were seen at 221 of 574 positions (38.5%) in RSV A and 230 of 574 positions in RSV B (Table 124). From 2015-2021, there was some variability in the frequency of substitutions observed, likely to be mostly accounted for by the different number of samples collected each season.

Across 2015 to 2021 RSV seasons, most RSV F protein amino acid residues were highly conserved, with a total of 554/574 (96.5%) of RSV A sequences, and 550-574 (95.8%) RSV B sequences showing >99% conservation based on identity (Table 125).

Table 124. Frequency of Amino Acid Variation Within Full-Length RSV F Protein Sequences (RSV A, N=2,875; RSV B, N=2,800), 2015 – 2021

	Frequency, n/N (%)[a]							
Hemisphere	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2015-2021**
RSV A								
NH	11/574 (1.92%)	64/574 (11.15%)	65/574 (11.32%)	81/574 (14.11%)	119/574 (20.73%)	102/574 (17.77%)	26/574 (4.53%)	204/574 (35.54%)
SH	8/574 (1.39%)	13/574 (2.26%)	8/574 (1.39%)	27/574 (4.70%)	33/574 (5.75%)	11/574 (1.92%)	22/574 (3.83%)	80/574 (13.94%)
NH+SH	18/574 (3.14%)	69/574 (12.02%)	68/574 (11.85%)	93/574 (16.20%)	131/574 (22.82%)	105/574 (18.29%)	43/574 (7.49%)	221/574 (38.50%)
RSV B								
NH	12/574 (2.09%)	85/574 (14.81%)	104/574 (18.12%)	128/574 (22.3%)	104/574 (18.12%)	64/574 (11.15%)	18/574 (3.14%)	218/574 (37.98%)
SH	18/574 (3.14%)	14/574 (2.44%)	23/574 (4.01%)	36/574 (6.27%)	30/574 (5.23%)	7/574 (1.22%)	15/574 (2.61%)	75/574 (13.07%)
NH+SH	25/574 (4.36%)	90/574 (15.68%)	109/574 (18.99%)	135/574 (23.52%)	113/574 (19.69%)	64/574 (11.15%)	25/574 (4.36%)	230/574 (40.07%)

Source: page 20, study report ID8897-0102
[a] Frequency of RSV F protein residues (n) with any genetic diversity among all residues (N) in full-length RSV F protein (AA 1–574)
Abbreviations: N, number of subjects; n, number of subjects with specific protein sequence; NH, northern hemisphere; RSV, respiratory syncytial virus; SH, southern hemisphere

Table 125. Temporal Frequency of Amino Acid Conservation (>99%) Within Full-Length RSV F Protein Sequences (RSV A, N=2,875; RSV B, N=2,800), 2015 – 2021

	Frequency, n/N (%)[a]							
Hemisphere	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2015-2021**
RSV A								
NH	563/574 (98.08%)	560/574 (97.56%)	554/574 (96.52%)	552/574 (96.17%)	555/574 (96.69%)	557/574 (97.04%)	548/574 (95.47%)	555/574 (96.69%)
SH	566/574 (98.61%)	561/574 (97.74%)	566/574 (98.61%)	547/574 (95.30%)	563/574 (98.08%)	563/574 (98.08%)	552/574 (96.17%)	561/574 (97.74%)
NH+SH	556/574 (96.86%)	559/574 (97.39%)	554/574 (96.52%)	553/574 (96.34%)	555/574 (96.69%)	557/574 (97.04%)	553/574 (96.34%)	554/574 (96.52%)
RSV B								
NH	562/574 (97.91%)	548/574 (95.47%)	554/574 (96.52%)	558/574 (97.21%)	554/574 (96.52%)	546/574 (95.12%)	556/574 (96.86%)	553/574 (96.34%)
SH	556/574 (96.86%)	560/574 (97.56%)	551/574 (95.99%)	557/574 (97.04%)	544/574 (94.77%)	567/574 (98.78%)	559/574 (97.39%)	553/574 (96.34%)
NH+SH	549/574 (95.64%)	548/574 (95.47%)	553/574 (96.34%)	558/574 (97.21%)	555/574 (96.69%)	546/574 (95.12%)	549/574 (95.64%)	550/574 (95.82%)

Source: pages 20-21, study report ID8897-0102
[a] Frequency of RSV F protein residues (n) with <1% genetic diversity among all residues (N) in full-length RSV F protein (AA 1–574)
Abbreviations: N, number of subjects; n, number of subjects with specific protein sequence; NH, northern hemisphere; RSV, respiratory syncytial virus; SH, southern hemisphere

Frequency of Prevalent Substitutions From 2015-2021

Table 126 shows the temporal frequency of amino acid substitutions seen in at least 10% of isolates across RSV seasons and/or increased ≥3-fold above ≥1% from the preceding season. For RSV A, only a T122A substitution was seen as prevalent across seasons, which differs from the reference RSV A/13-5275 isolate but occurs in the laboratory reference sequence A000. Hence, there was no fold change in susceptibility to nirsevimab or palivizumab.

For RSV B, three substitutions emerged and became predominant over the course of the surveillance programs, K191R, I206M and Q209R. Of these, only I206M showed a reduction in susceptibility (5-fold) to nirsevimab when tested in a recombinant RSV assay; however, this substitution has been rarely (0.64%) detected in the absence of Q209R, and together, these substitutions do not confer a loss of susceptibility to nirsevimab (Table 126).

Other prevalent substitutions seen in RSV B isolates include F15L, A103V, L172Q and S173L, which were all seen at ≥10% frequency from 2015. These polymorphisms occur outside of the nirsevimab and palivizumab binding sites, and do not impact susceptibility to either mAb.

Table 126. Temporal Frequency (n/N, %[a]) of Prevalent Amino Acid Changes in Full-Length RSV F Protein Sequences, 2015 – 2021

Amino Acid Change[b]	2015	2016	2017	2018	2019	2020	2021	2015-2021	Fold Reduction in Susceptibility[c]	
RSV A	**(N=52)**	**(N=343)**	**(N=380)**	**(N=439)**	**(N=860)**	**(N=661)**	**(N=140)**	**(N=2,875)**	**Nirsevimab**	**Palivizumab**
T122A[d]	3.85%	3.21%	3.16%	8.88%	11.74%	8.93%	77.86%	11.58%	1.0	1.0
RSV B	**(N=34)**	**(N=315)**	**(N=588)**	**(N=909)**	**(N=667)**	**(N=196)**	**(N=91)**	**(N=2,800)**	**Nirsevimab**	**Palivizumab**
F15L[e]	94.12%	100%	99.66%	99.56%	99.40%	100%	100%	99.57%	1.0	1.0
A103V	41.18%	96.51%	99.32%	99.89%	99.25%	100%	98.90%	98.50%	0.9	1.8
L172Q	41.18%	95.56%	99.15%	100%	100%	100%	100%	98.61%	1.1	2.3
S173L	38.24%	93.97%	98.98%	100%	100%	100%	100%	98.36%	0.9	1.9
K191R	0%	10.48%	37.59%	83.28%	96.40%	98.47%	81.32%	68.61%	1.3	2.7
I206M	0%	10.48%	37.59%	84.16%	96.40%	98.47%	81.32%	68.89%	**5.0**	2.0
Q209R	0%	10.48%	37.59%	83.61%	94.90%	96.43%	80.22%	68.18%	0.5	3.1

Source: pages 22-23, study report ID8897-0102
Bold value: ≥5-fold reduction in suscept bility
[a] Global prevalence, based on the ratio of RSV G HVR2 – F sequences containing F protein substitutions (full and mixtures) to all sequences collected
[b] Prevalent RSV F protein substitutions (≥10%) identified among seasonal strains compared to year 2013 RSV A/13-005275 and RSV B/13-001273 reference strains. rRSV variants containing individual amino acid substitutions were engineered into full-length antigenomic A2-000 (containing consensus E66 residue) or B9320-000 (containing consensus N197 residue) cDNA. nirsevimab binding site = AA 62–69 and 196–212 (Zhu et al. 2017)
[c] Reduced cell culture potency using a validated rRSV neutralization suscept bility assay (b) (4)). Fold change in half maximal effective concentration (EC50 value) of mAb required for a 50% reduction in infection compared to wild type reference strain tested in parallel on the same plate.
[d] A000 reference contains alanine (A) at position 122
[e] B000 reference strain contains leucine (L) at position 15
Abbreviations: N, number of subjects; n, number of subjects with specific amino acid change; NH, northern hemisphere; RSV, respiratory syncytial virus; SH, southern hemisphere

Conservation of Nirsevimab and Palivizumab Binding Sites From 2015 to 2021

The conservation of the nirsevimab binding site (F protein amino acids 62-69 and 196-212) based on sequences from isolates collected from 1956 to 2014 is discussed in Section 20.2. Most F protein amino acids were >99% conserved, including 24/25 positions in the RSV A binding site and 22/25 positions in the RSV B binding site.

Table 127 shows the conservation of these amino acids based on data from surveillance programs from 2015 to 2021, and Table 128 shows the conservation of amino acids in the palivizumab binding site for the same years. Most amino acids in the nirsevimab binding site have remained >99% conserved since 2015, including all positions for RSV A and 22 of 25 positions for RSV B. Of the three RSV B positions with ≤99% conservation, I211 was 98.8% conserved, and I206 and Q209 were only approximately 31% conserved because of the emergence and predominance of I206M+Q209R co-occurring substitutions.

Substitutions which were selected with nirsevimab in cell culture (Section 20.7; (Zhu et al. 2017; Zhu et al. 2018)) have been seen rarely since 2015. K68N and N201S substitutions were seen in RSV B at <0.05% frequency and have not persisted (Table 127). Likewise, palivizumab resistance-associated substitutions L272M, K272T and S275F (AstraZeneca 1998; Zhu et al. 2011; Zhu et al. 2012) were seen at <0.05% frequency in RSV A sequences from 2015 to 2021 (Table 128). Other substitutions at K272 (i.e., K272N/Q/R in RSV B) were also seen at low frequency.

Table 127. Conservation of the Nirsevimab Binding Site in RSV F Protein Sequences, 2015 – 2021

Amino Acid Position	RSV A (n=2,875)			RSV B (n=2,800)		
	Amino Acid[a]	Conservation (%)	Substitution[c] (Frequency, %)	Amino Acid[b]	Conservation (%)	Substitution[c] (Frequency, %)
62	S	99.97	G/S (0.03)	S	100.00	-
63	N	100.00	-	N	99.96	S (0.04)
64	I	99.97	V (0.03)	I	100.00	-
65	K	99.86	Q (0.03), R (0.10)	K	99.89	R (0.11)
66	E	100.00	-	E	99.96	D (0.04)
67	N	100.00	-	T	99.96	A (0.04)
68	K	99.34	E (0.07), N (0.56), R (0.03)	K	99.57	N (0.32), Q (0.04), R (0.07)
69	C	100.00	-	C	100.00	-
196	K	100.00	-	K	100.00	-
197	N	99.79	D (0.03), H (0.03), K (0.14)	N	99.57	D (0.39), S (0.04)
198	Y	100.00	-	Y	100.00	-
199	I	99.97	M (0.03)	I	100.00	-
200	D	99.97	N (0.03)	N	100.00	-
201	K	100.00	-	N	99.75	S (0.21), T (0.04)
202	Q	100.00	-	Q	100.00	-
203	L	100.00	-	L	100.00	-
204	L	99.97	I (0.03)	L	100.00	-
205	P	100.00	-	P	100.00	-
206	I	99.79	T (0.17), V (0.03)	I	31.04	I/M (0.07), M (68.89)[d]
207	V	99.97	I (0.03)	V	100.00	-
208	N	100.00	-	N	100.00	-
209	K	100.00	-	Q	31.43	K (0.21), L (0.11), Q/R (0.07), R (68.18)[d]
210	Q	99.97	L (0.03)	Q	99.93	H (0.07)
211	S	100.00	-	S	98.82	I (0.04), N (1.14)
212	C	100.00	-	C	100.00	-

Source: pages 26-28, study report ID8897-0102
[a] Based on reference strain RSV A/13-005275 from 2013
[b] Based on reference strain RSV B/13-001273 from 2013
[c] RSV F protein substitutions among 2015 – 2021 RSV isolates compared to reference strains
[d] high prevalence (≥10%) polymorphisms
Abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; n, number of subjects; P, proline; Q, glutamine; R, arginine; RSV, respiratory syncytial virus; S, serine; T, threonine; V, valine; Y, tyrosine

Table 128. Conservation of the Palivizumab Binding Site in RSV F Protein Sequences, 2015 – 2021

Amino Acid Position	RSV A (n=2,875)			RSV B (n=2,800)		
	Amino Acid[a]	Conservation (%)	Substitution[c] (Frequency, %)	Amino Acid[b]	Conservation (%)	Substitution[c] (Frequency, %)
262	N	100.00	-	N	100.00	-
263	D	99.97	Y (0.03)	D	100.00	-
264	M	100.00	-	M	99.96	I (0.04)
265	P	100.00	-	P	100.00	-
266	I	100.00	-	I	100.00	-
267	T	100.00	-	T	100.00	-
268	N	100.00	-	N	100.00	-
269	D	100.00	-	D	100.00	-
270	Q	100.00	-	Q	100.00	-
271	K	100.00	-	K	100.00	-
272	K	99.93	K/M (0.03), T (0.03)	K	99.89	N (0.04), Q (0.04), R (0.04)
273	L	100.00	-	L	99.93	I (0.07)
274	M	100.00	-	M	100.00	-
275	S	99.97	F (0.03)	S	100.00	-

Source: pages 27-29, study report ID8897-0102
[a] Based on reference strain RSV A/13-005275 from 2013
[b] Based on reference strain RSV B/13-001273 from 2013
[c] RSV F protein substitutions among 2015 – 2021 RSV isolates compared to reference strains
Abbreviations: D, aspartic acid; E, glutamic acid; F, phenylalanine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; n, number of subjects; P, proline; Q, glutamine; RSV, respiratory syncytial virus; S, serine; T, threonine

Temporal Frequency and Neutralization Data for Nirsevimab and Palivizumab Binding Site Substitutions

Table 129 and Table 130 show the frequency of substitutions in the nirsevimab binding site from 2015 to 2021 in RSV A and RSV B, respectively, and phenotypic data for nirsevimab and palivizumab against these substitutions. Individual substitutions were evaluated with recombinant RSV, using antigenomic RSV A2-000 or RSV B9320-000 cDNA, which include the consensus E66 or N197 residues, respectively (Section 18.1.2).

Of the individual substitutions evaluated phenotypically, the following had ≥5-fold reduction in susceptibility to nirsevimab neutralization: K68E (12.6-fold), K68N (5.1-fold), and S275F (6.4-fold), in RSV A, and K68N (29.9-fold), N201S (126.7-fold), N201T (>405.7-fold), and I206M (5.0-fold), in RSV B. For variants with concurrent substitutions which were tested, the following had ≥5-fold reduction in susceptibility to nirsevimab neutralization: K68Q+I206M+Q209R (46.4-fold) and N201T+I206M+Q209R (>417.8-fold) in RSV B. The only substitution with reduced susceptibility to palivizumab and showed reduced susceptibility to nirsevimab was S275F (6.4-fold).

Overall, from 2015-2021, the prevalence of variants harboring substitutions with reduced susceptibility to nirsevimab and/or palivizumab was <1.0%.

Table 129. Temporal Frequency and Neutralization Data of RSV A F Variants With Nirsevimab and/or Palivizumab Binding Site Substitutions (N=2,875), 2015-2021

		Frequency n/N (%)[b]								Fold Reduction in Susceptibility[c]	
Region in F Subunit	**Amino Acid Substitution(s)[a]**	**2015 (N=52)**	**2016 (N=343)**	**2017 (N=380)**	**2018 (N=439)**	**2019 (N=860)**	**2020 (N=661)**	**2021 (N=140)**	**2015-21 (N=2,875)**	**Nirsevimab**	**Palivizumab**
	S62G/S					0.12%			0.03%	TBD	TBD
	I64V					0.12%			0.03%	1.7	1.8
	K65Q						0.15%		0.03%	TBD	TBD
F2	K65R		0.29%	0.53%					0.10%	3.9	2.2
	K68E[d]						0.30%		0.07%	12.6[e]	1.9
	K68N		0.87%	1.58%		0.35%	0.61%		0.56%	5.1[e]	2.1
	K68R					0.12%			0.03%	1.4	0.8
	N197D					0.12%			0.03%	2.5	1.2
	N197H				0.23%				0.03%	1.1	1.0
	N197K					0.47%			0.14%	1.5	1.8
	I199M				0.23%				0.03%	1.7	1.5
	D200N[d]						0.15%		0.03%	TBD	TBD
	L204I				0.23%				0.03%	2.0	4.2
F1	I206T		0.29%	0.79%	0.23%				0.17%	1.6	1.0
	I206V						0.15%		0.03%	3.2	2.3
	V207I					0.12%			0.03%	2.5	1.5
	Q210L					0.12%			0.03%	2.2	1.7
	D263Y					0.12%			0.03%	0.6	0.2
	K272K/M[d]		0.29%						0.03%	2.7	>179.9[e]
	K272T[d]		0.29%						0.03%	1.1	>213.9[e]
	S275F						0.15%		0.03%	6.4[e]	>356.1[e]

Source: page 30, study report ID8897-0102
[a] rRSV variants containing amino acid substitution(s) engineered into full-length antigenomic A2-000 (containing consensus E66 residue) or B9320-000 (containing consensus N197 residue) cDNA. Full and mixed amino acid changes identified in the nirsevimab (AA 62-69 and AA 196-212) and/or palivizumab binding site (AA 262-275) were engineered and tested in their observed context as full substitutions with and without other binding site substitutions.
[b] Global prevalence, based on the ratio of RSV G HVR2 – F sequences containing F protein substitutions (full and mixtures) to all sequences collected
[c] Fold change in half maximal effective concentration (EC50 value) of mAb required for a 50% reduction in infection compared to wild type reference strain tested in parallel on the same plate in a validated rRSV neutralization susceptibility assay [redacted] (b) (4)).
[d] Substitutions: seen in breakthrough infection variants in clinical trials of nirsevimab (Section 18.6)
[e] Values: ≥5.0-fold change (based on 99.2% statistical power to detect)
F2 = subunit, extracellular region, AA 24-109; F1 = subunit, extracellular region, AA 137-524
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus; TBD, to be determined

Table 130. Temporal Frequency and Neutralization Data of RSV B F Variants With Nirsevimab and/or Palivizumab Binding Site Substitutions (N=2,800), 2015-2021

		Frequency n/N (%)[b]								Fold Reduction in Susceptibility[c]	
Region in F Subunit	Amino Acid Substitutions(s)[a]	2015 (N=34)	2016 (N=315)	2017 (N=588)	2018 (N=909)	2019 (N=667)	2020 (N=196)	2021 (N=91)	2015-21 (N=2,800)	Nirsevimab	Palivizumab
	N63S+I206M+Q209R					0.15%			0.04%	0.6	3.7
	K65R			0.17%	0.11%				0.07%	0.8	1.5
	K65R+I206M+Q209R					0.15%			0.04%	0.5	2.4
	E66D		0.32%						0.04%	2.0	1.7
F2	T67A+I206M+Q209R					0.15%			0.04%	1.1	7.1[f]
	K68N					0.15%			0.04%	29.9[f]	2.0
	K68N+I206M+Q209R					0.90%	1.02%		0.29%	TBD	TBD
	K68Q+I206M+Q209R				0.11%				0.04%	46.4[f]	1.6
	K68R+I206M+Q209R				0.11%		0.51%		0.07%	0.3	1.1
	N197D+I206M+Q209R				0.44%	0.90%	0.51%		0.39%	0.6	1.9
	N197S+I206M+Q209R				0.11%				0.04%	1.6	2.5
	N201S			0.17%					0.04%	126.7[f]	3.1
	N201S+Q209K	2.94%	0.32%	0.51%					0.18%	0.8	3.4
	N201T				0.11%				0.04%	>405.7[f]	2.8
	N201T+I206M+Q209R				0.11%				0.04%	>417.8[f]	3.2
	I206I/M				0.11%				0.04%	5.0[f]	2.0
	I206I/M+Q209Q/R			0.17%					0.04%	0.2	1.3
	I206M[d]				0.22%	1.65%	2.04%	1.10%	0.64%	5.0[f]	2.0
	I206M+Q209L				0.22%				0.07%	4.5	3.6
F1	I206M+Q209Q/R				0.11%				0.04%	0.2	1.3
	I206M+Q209R[d]		10.48%	37.41%	82.07%	91.90%	93.88%	51.65%	65.82%	0.2	1.3
	I206M+Q209R+K272N				0.11%				0.04%	0.6	>306.5[f]
	I206M+Q209R+K272Q				0.11%				0.04%	0.2	>269.0[f]
	I206M+Q209R+K272R[d]						0.51%		0.04%	TBD	TBD
	I206M+Q209R+L273I				0.11%				0.04%	0.3	2.2
	I206M+Q209R+M264I			0.17%					0.04%	0.4	2.9
	I206M+Q209R+S211I				0.11%				0.04%	1.8	2.6
	I206M+Q209R+S211N[d]				0.22%	0.60%		28.57%	1.14%	0.5	3.7
	Q209K		0.32%						0.04%	0.5	1.8

Region in F Subunit	Amino Acid Substitutions(s)[a]	Frequency n/N (%)[b] 2015 (N=34)	2016 (N=315)	2017 (N=588)	2018 (N=909)	2019 (N=667)	2020 (N=196)	2021 (N=91)	2015-21 (N=2,800)	Fold Reduction in Susceptibility[c] Nirsevimab	Palivizumab
	Q209L			0.17%					0.04%	0.4	0.8
	Q209R[d]					0.15%			0.04%	0.5	3.1
	Q210H		0.32%	0.17%					0.07%	2.3	2.5
	L273I[e]				0.11%				0.04%	QNS	QNS

Source: page 31, study report ID8897-0102; ID8897O-1718 (for N201T substitution data)
[a] rRSV variants containing amino acid substitution(s) engineered into full-length antigenomic A2-000 (containing consensus E66 residue) or B9320-000 (containing consensus N197 residue) cDNA. Full and mixed amino acid changes identified in the nirsevimab (AA 62-69 and AA 196-212) and/or palivizumab binding site (AA 262-275) were engineered and tested in their observed context as full substitutions with and without other binding site substitutions. Individual amino acid changes, not observed on their own without other binding site substitutions, were also tested (bold).
[b] Global prevalence, based on the ratio of RSV G HVR2 – F sequences containing F protein substitutions (full and mixtures) to all sequences collected
[c] Fold change in half maximal effective concentration (EC50 value) of mAb required for a 50% reduction in infection compared to wild type reference strain tested in parallel on the same plate in a validated rRSV neutralization susceptibility assay [redacted] (b) (4)).
[d] Substitutions: seen in breakthrough infection variants in clinical trials of nirsevimab (Section 18.6)
[e] This variant was unable to be rescued and did not generate sufficient stock for susceptibility testing
[f] Values: ≥5.0-fold change (based on 99.2% statistical power to detect)
F2 = subunit, extracellular region, AA 24-109; F1 = subunit, extracellular region, AA 137-524
Abbreviations: N, number of subjects; QNS, quantity not sufficient; RSV, respiratory syncytial virus; TBD, to be determined

18.1.4. Conclusions

The Applicant provided an integrated analysis of OUTSMART-RSV, INFORM-RSV and South Africa pilot surveillance programs from 2015 to 2021, showing that overall, there was a high level of conservation of amino acids in full-length F protein and in the nirsevimab binding site, with all binding site changes having >99% conservation in RSV A sequences and 22/25 positions in RSV B. The two predominant substitutions seen in RSV B, I206M and K209R, occurred in 66% of isolates from 2015 to 2021, but did not confer reduced susceptibility to nirsevimab.

A number of substitutions seen alone or concurrent with others had reduced susceptibility (≥5-fold) to nirsevimab, but generally, these variants were seen at low frequency across 2015 to 2021 seasons. The greatest fold-reductions in susceptibility to nirsevimab included N201S (126.7-fold), N201T (>405.7-fold), and N201T+I206M+Q209R (>417.8-fold) substitutions in RSV B, although it is not known what magnitude fold-reduction would impact clinical efficacy. The only substitution observed which impacted susceptibility to both palivizumab (>356-fold) and nirsevimab (6.4-fold) was S275F in RSV A, but this was only seen at low frequency (0.15%) in 2020.

18.2. Analyses of Quidel Assay Primer/Probe Binding Sites From 2016 to 2021

Study reports: ID8897O-BAR-Q-1617, ID8897O-BAR-Q-1718, ID8897O-BAR-Q-1819, ID8897I-BAR-Q-1718, ID8897I-BAR-Q-1819, ID8897I-BAR-Q-1920, ID8897I-BAR-Q-2021.

For RSV seasons from 2016 to 2021, sequence data from isolates collected as part of the OUTSMART-RSV and INFORM-RSV programs were also assessed for changes in the NS2 and L (polymerase) gene sequences to determine the potential for impacting the Lyra® RSV + hMPV RT-PCR assay (Quidel Corporation, San Diego, CA), used for RSV diagnosis in clinical trials of nirsevimab.

18.2.1. Methodology

A subset of 20 RSV A and 20 RSV B isolates per season, collected as part of OUTSMART-RSV (2016 to 2019), were randomly selected for NS2 and L gene sequencing. In addition, all 1,468 RSV isolates (759 RSV A and 709 RSV B) from INFORM-RSV (2017 to 2021) were assessed for NS2 and L gene changes.

For samples from OUTSMART-RSV, nucleic acids were extracted from nasal specimens using the Nuclisens easyMAG® instruments (bioMerieux Inc., Durham, NC) according to the manufacturer's instructions. Real-time RT-PCR was performed using NS and L-specific primers (Table 131) that anneal to the regions flanking the RT-PCR targets within the Quidel assay. The amplified NS2 and L regions were subjected to Sanger sequencing.

Table 131. RT-PCR Primers Used to Amplify NS2 and L Regions in RSV RNA From OUTSMART-RSV Program Isolates

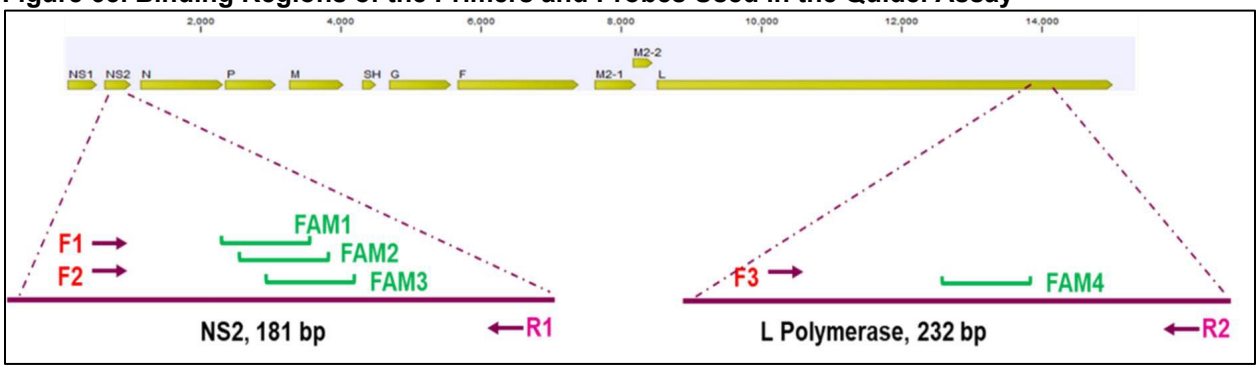
Amplicon	Primer Name	Sequence (5'-3')	Amplicon Size
NS2	NS2_F1	ACGCGAAAAAATGCGTACWAC	1200 bp
	NS2_R1201	GGTGTTTTTGCACATCATAATTGG	
L	L_F13160	CTGATCATATGTTCATTAATTTG	1460 bp
	L-R14620	CCTATTGTAAGGACTAARTAAAC	

Source: page 6, study report ID8897O-BAR-Q-1617; page 5, study report ID8897O-BAR-Q-1718
R (A or G); W (A or T)
Abbreviations: RNA, ribonucleic acid; RSV, respiratory syncytial virus; RT-PCR, reverse transcriptase-polymerase chain reaction

For samples collected in the INFORM-RSV program, nucleic acids were isolated from RSV-positive nasal specimens using the MagNA Pure LC kit (Roche Molecular Systems, Pleasanton, CA). Subtype-specific RT-PCR was performed to amplify four 3.5 to 5 kb overlapping fragments covering the full RSV genome. Amplified fragments were used for library construction with the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA), then libraries were sequenced on a NextSeq500 Illumina instrument generating paired-end, 150 bp reads.

Sequences from OUTSMART-RSV or INFORM-RSV isolates were aligned to the Quidel assay primer/probes (Figure 65, Table 132) using Geneious Prime software and/or CLC Genomic Workbench 10.0.1. software (Qiagen, Hilden, Germany). For any mismatched isolate sequences and the Quidel assay primer/probes, the melting temperature of the mismatched sequence was determined using Modified Breslauer's thermodynamics (https://horizondiscovery.com/en/products/tools/TMCalculator).

Figure 65. Binding Regions of the Primers and Probes Used in the Quidel Assay



Source: page 6, study report ID8897I-BAR-Q-2021

Table 132. Sequences of Primers and Probes Used in Quidel the Assay

RSV Gene	Primer/Probe Name	Sequence (5'-3')	Tm (°C)	Product Size (bp)
NS2	F1 primer	AACTTGATGAAAGACARGCYACA	60-67	181
	F2 primer	AACT**C**GATGA**G**AGACA**A**GC**T**ACA	62.4	
	R1 primer	GTAGGCTTAATGCCAATRCATTCTA	61-66	
	FAM 1 probe	ATGGCACTTTCCCYATGCCWAT	65-72	
	FAM 2 probe	AAAATATGGCACTTTCCCYATGCC	67-71	
	FAM 3 probe	CAAAATATRGNACWTTYCCTATGCC	60-70	

RSV Gene	Primer/Probe Name	Sequence (5'-3')	Tm (°C)	Product Size (bp)
L	F3 primer	TAGATCATTCAGGYAATACAGCMAAAT	62-68	232
	R2 primer	CCWGCTCCTTCACCTATGA	61	
	FAM 4 probe	TGCATGCTTCCTTGGCATC	68	

Source: page 6, study report ID8897I-BAR-Q-2021
F2 primer targets the same region as F1 in NS 2 gene but contains 4 base changes (in **bold**) to allow for improved detection. Y (C or T); R (A or G); M (A or C); W (A or T); N (A, C, G, or T).
Abbreviations: RSV, respiratory syncytial virus; Tm, predicted melting temperature

18.2.2. Results

NS2 and L gene sequences were aligned with the Quidel assay primer/probe sequences and melting temperatures (Tm) of mismatched sequences determined. Overall, for samples from both OUTSMART-RSV and INORM-RSV programs, most of the NS2 and L sequence variations were predicted to have a minimal impact on the binding of the NS2 and L primer and/or proves used in the Quidel assay (ΔTm = ±4°C). There were few examples of sequences which contained nucleotide changes in both NS2 and L genes, and none of these appeared likely to impact detection of the NS2 or L gene based on the design of redundant primers and/or probes used in the assay.

RSV samples from OUTSMART-RSV, 2016 to 2017 and 2017 to 2018, with detectable amounts of cDNA (n=32 and 10, respectively), were also assessed by (b) (4) using the Quidel assay. All samples tested positive for RSV, regardless of changes in the primer/probe binding regions (data not shown).

18.2.3. Conclusions

Melting temperature analyses of NS2 and L genes from representative RSV isolates from the OUTSMART-RSV program from 2016 to 2019, and all isolates from the INFORM-RSV program from 2017 to 2021, indicated that Lyra® RSV+hMPV RT-PCR assay likely retained the ability to detect RSV A and RSV B strains in circulation during the clinical trials of nirsevimab between 2016 and 2021.

18.3. Impact of Nirsevimab Resistance-Associated Substitutions on RSV Diagnostic Assays

Study reports: ID8897-0012, ID8897-0014.

An evaluation was conducted of the impact of nirsevimab resistance-associated amino acid substitutions in the RSV F protein (Section 20.7) on the sensitivity of rapid antigen assays commonly used to diagnose RSV infection, many of which depend on binding by antibodies directed against the F protein.

18.3.1. Methodology

Virus variants were titered on HEp2 cells (TCID50 assay; Section 20.6) and serially diluted to the same concentration in pooled human nasopharyngeal swab samples (in universal transport medium) from healthy adult volunteers stored in and determined to be RSV negative by the most sensitive test evaluated (Quidel Lyra® RSV+hMPV RT-PCR assay). All RSV A viruses were normalized to an initial starting concentration of 6.25 × 105 TCID50/mL and all RSV B viruses were normalized to an initial starting concentration of either 7.50×10^5 or 7.50×10^4 $TCID_{50}$/mL, then 10-fold serial dilutions performed.

Samples were then aliquoted and processed as clinical specimens according to the test manufacturer's directions. For the RT-PCR test, viruses were spiked into adult human nasal pharyngeal swab matrix at 1x the limit of detection (LoD) and 10x the LoD. For rapid antigen tests, viruses were diluted to a previously determined "optimal quantity for detection" of wild-type virus, 1.45 x 106 $TCID_{50}$/mL. The 5 antibody-based RSV antigen tests which were evaluated were: BD Directigen™ EZ RSV, BinaxNOW™ RSV, Meridian TRU RSV®, Quidel QuickVue® RSV, and Remel™ Xpect™ RSV.

To determine the impact of the different RSV variants on the limit of detection of each of the rapid antigen tests, the variants were propagated to achieve high viral titers, then assessed at different titers in each of the tests.

18.3.2. Results

A panel of six variants selected in cell culture with nirsevimab were evaluated, including RSV A_N208Y, RSV B_K68N/N201S, RSV B_K68N/N208S, RSV B_N208D, RSV B_N208S, and RSV B_N208D/E294K (Section 20.7). Note that this analysis did not include all resistance-associated substitutions seen in cell culture (i.e., RSV A with N67I+N208Y) or in clinical trials (Section 18.6; I64T, K68E, and N208K substitutions in RSV B).

Quidel RT-PCR Assay

The Quidel Lyra® RSV+hMPV RT-PCR assay failed to detect any virus diluted to the limit of detection, 0.625 (RSV A) or 0.750 (RSV B) TCID50/mL; however, all viruses at titers of 6.25 or 7.5 TCID50/mL, 10 x the LoD, were detected (Table 133). Note that the LoD is defined by the assay manufacturer as the lowest concentration at which 95% of all replicates tested positive; hence it appears that the assay was not as sensitive as expected in this experiment.

Also note that the Ct value for RSVB9320_N208S was about 3 to 6 cycles above the rest of panel, consistent with the finding that this virus stock had a lower titer than originally calculated. The Quidel Lyra® RSV hMPV RT-PCR assay performed adequately against all 6 members of the variant panel.

Table 133. Test Results of Quidel Molecular RSV+hMPV Assay to Detect RSV Variants

Virus	Substitution	1 × LoD ($TCID_{50}$/mL)	RSV	Mean Ct Value[a]	10 × LoD ($TCID_{50}$/mL)	RSV	Mean Ct Value[a]
RSV A2	wild-type	6.25×10^{-1}	-	>35	6.25×10^{0}	+	27.28
RSV A_N208Y	N208Y	6.25×10^{-1}	-	>35	6.25×10^{0}	+	26.77
RSV B9320	wild-type	7.50×10^{-1}	-	>35	7.50×10^{0}	+	26.54
RSVB_N208S	N208S	7.50×10^{-1}	-	>35	7.50×10^{0}	+	32.74
RSVB_N208D	N208D	7.50×10^{-1}	-	>35[b]	7.50×10^{0}	+	26.6
RSVB_K68N/N201S	K68N+N201S	7.50×10^{-1}	-	>35	7.50×10^{0}	+	26.04
RSVB9_K68N/N208S	K68N+N208S	7.50×10^{-1}	-	>35	7.50×10^{0}	+	28.56
RSVB_N208D/E294K	N208D+E294K	7.50×10^{-1}	-	>35	7.50×10^{0}	+	29.31

Source: page 18, study report ID8897-0012
[a] Mean values were obtained from two replicates
[b] Ct values were >35 and 32.16 for each replicate, respectively.
Abbreviations: Ct, cycle threshold; LoD, limit of detection; RSV, respiratory syncytial virus; $TCID_{50}$, tissue culture half-maximal infectious dose

Rapid Antigen Tests

The panel of RSV variants were also tested in 5 different rapid antigen assays (Table 134). All but one variant diluted to 1.45 x106 TCID50/mL were detected with each assay. Variant RSVB_N208S, which was subsequently determined to be 5-fold less concentrated than the other variants in the panel, was only detected robustly by the Quidel Quickvue® test and marginally by the Meridian Tru RSV® and Directigen™ EZ RSV test. The RSVB_N208S variant was retested based on a revised titer and each of the tests was able to detect the virus at 1.45 x 106 TCID50/mL.

Table 134. Test Results of Rapid Antigen Kits for Detection of RSV Variants With Nirsevimab Resistance-Associated Substitutions

		Rapid Antigen Kits				
Virus	Amino Acid Changes	Remel Xpect	Quidel QuickVue	Meridian TRU	Binax NOW	Directigen EZ
RSV A2	wild-type	+	+	+	+	+
RSV A_N208Y	N208Y	+	+	+	+	+
RSV B9320	wild-type	+	+	+	+	+
RSVB_N208D	N208D	+	+	+	+	+
RSV B_N208S	N208S[a]	-	+	+/-	-	+/-
RSVB_K68N/N201S	K68N+N201S	+/-	+	+	+	+
RSVB_K68N/N208S	K68N+N208S	+/-	+	+	+	+
RSVB_N208D/E294K	N208D+E294K	+	+	+	+	+

Source: pages 19-20, ID8897-0012
- = negative; +/- = too weak to call positive confidently; + = positive
[a] This variant tested positive in retest using newly determined titer
Abbreviations: RSV, respiratory syncytial virus

Impact of Variants on Rapid Antigen Test Limit of Detection

All rapid antigen kits were able to detect the six different RSV variants containing nirsevimab resistance-associated substitutions N208Y, N208D, N208S, K68N+N201S, K68N+N208S or N208D+E294K to the same limit of detection as the parental wild-type RSV A2 or RSVB9320. Viruses (Table 135).

Table 135. Virus Titer (PFU/mL) at the Limit of Detection of RSV Variants With Nirsevimab Resistance-Associated Substitutions

		Rapid Antigen Kits				
Virus	Amino Acid Changes	Remel X/pect	Quidel QuickVue	Meridian TRU	Binax NOW	Directigen EZ
RSV A2	wild-type	2.50E +06	6.25E +05	1.25E +06	6.25E +05	3.13E +05
RSV A_N208Y	N208Y	2.50E +06	6.25E +05	1.25E +06	6.25E +05	3.13E +05
RSV B9320	wild-type	2.50E +06	1.56E +05	6.25E +05	6.25E +05	3.13E +05
RSV B_N208D	N208D	2.50E +06	1.56E +05	6.25E +05	6.25E +05	3.13E +05
RSV B_N208S	N208S	2.50E +06	1.56E +05	6.25E +05	6.25E +05	3.13E +05
RSV B_N208D/E294K	N208D+E294K	2.50E +06	1.56E +05	6.25E +05	6.25E +05	3.13E +05
RSV B_K68N/N208S	K68N+N208S	2.50E +06	1.56E +05	6.25E +05	6.25E +05	3.13E +05
RSV B_K68N/N201S	K68N+N201S	1.25E +06	1.56E +05	6.25E +05	6.25E +05	3.13E +05

Source: page 7, study report ID8897-0014
Abbreviations: PFU, plaque forming units; RSV, respiratory syncytial virus

18.3.3. Conclusions

None of the RSV variants tested appeared to impact the sensitivity of the Quidel RT-PCR assay and five different rapid antigen tests commonly used for RSV diagnosis. One variant harboring N208S substitution was not detected by the Remel Xpect and Binax NOW tests when assessed at a 5-fold lower titer, which highlights the relative insensitivity of rapid antigen tests. There was no clear qualitative difference of 5 rapid antigen tests between WT and resistance variants, and a follow-up study showed no clear impact of the viral variants on the limits of detection of the assays.

18.4. Impact of Nirsevimab on RSV Diagnostic Assays

Study report: ID8897-0009.

Rapid antigen testing is the standard diagnostic for RSV infection in the clinic and depends on antibody-based detection of the RSV proteins (typically F protein) in patient samples. Prophylaxis with palivizumab can result in interference with rapid antigen RSV diagnostics targeting the F protein, because it targets an epitope that overlaps with the assay target epitope in the F protein, resulting in false-negative or equivocal results (Deming et al. 2013). The purpose of this study was to determine the potential for nirsevimab to interfere with commonly used rapid antigen RSV diagnostic tests.

18.4.1. Methodology

Five commonly used RSV rapid antigen tests kits were evaluated for detection of RSV A or RSV B in the presence or absence of nirsevimab or palivizumab, including BD Directigen™ EZ RSV, BinaxNOW™ RSV, Meridian TRU RSV®, Quidel QuickVue® RSV, and Remel™ Xpect™ RSV.

Prior to evaluating the potential for nirsevimab or palivizumab interference, the limit of detection of RSV A was established for each kit to select the appropriate concentration of virus to perform the analysis. Note that the Applicant did not appear to use the standard definition of LoD, i.e., the lowest concentration at which 95% of all replicates tested positive.

Based on the results from the RSV A titration, a titer of 5 x10^5 PFU/mL gave consistently positive results, and was selected for all assays, for both RSV A and RSV B. Samples containing RSV A or RSV B or PBS only in the presence or absence of 1 or 10 µg/mL nirsevimab or palivizumab were tested in each assay, following the manufacturer's instructions. The antibody concentrations were chosen based on values that would be expected to equal or exceed by at least 10-fold the levels of antibody predicted in nasal wash test samples (Robbie et al. 2013).

18.4.2. Results

RSV was detected by all test kits in the presence of nirsevimab up to concentrations of 10 µg/mL, although a slight decrease was seen in the case of the Remel Xpect kit in the presence of 10 µg/mL nirsevimab, but the result was still considered positive.

Consistent with previous reports (Deming et al. 2013), palivizumab at concentrations of 10 µg/mL, interfered with all assays for both RSV A and B detection. In particular, the Remel Xpect RSV and Binax NOW kits produced false negative results in the presence of 10 µg/mL of palivizumab. Palivizumab at 10 µg/mL also reduced the signals of the Quidel QuickVue, Meridian TRU and BD Directigen EZ kits, and in the case of the Meridian TRU kit, to the extent that results were equivocal. Palivizumab at 1 µg/mL also reduced the signal of the Binax NOW and Remel Xpect kits for detection of RSV A, and the Binax NOW, Meridian TRU, BD Directigen and Remel Xpect kits for RSV B.

Table 136. Impact of Nirsevimab or Palivizumab on RSV Detection[a]

Test Sample	Remel Xpect RSV	Quidel QuickVue RSV	Meridian TRU RSV	Binax NOW RSV	BD Directigen EZ RSV
RSV A+nirsevimab 1 µg/mL	+	+	+	+	+
RSV A+nirsevimab 10 µg/mL	+[a]	+	+	+	+
RSV A+palivizumab 1 µg/mL	+[a]	+	+	+[b]	+
RSV A+palivizumab 10 µg/mL	-	+[b]	+[b]	-	+[b]
RSV B+nirsevimab 1 µg/mL	+	+	+	+	+
RSV B+nirsevimab 10 µg/mL	+	+	+	+	+
RSV B+palivizumab 1 µg/mL	+[b]	+	+[b]	+[b]	+[b]
RSV B+palivizumab 10 µg/mL	-	+[b]	+[b]	-	+[b]

Source: pages 19-20, study report ID8897-0009
- = negative; + = positive
[a] Results for virus only controls, buffer controls and ant body only controls are not shown, but were as expected
[b] Intensity of positive test signal was less than the virus only test group
Abbreviation: RSV, respiratory syncytial virus

18.4.3. Conclusions

Nirsevimab did not appear to significantly reduce the signal in any of the evaluations of interference with diagnostic tests. This result was in contrast to palivizumab, which clearly interfered with multiple tests at both low and high concentrations.

The data do not indicate that a recommendation be made to avoid certain antigen tests in subjects dosed with nirsevimab, although the experiment only looked at qualitative effects and did not determine the limit of detection of each assay in the presence of antibody. Also, it is possible that antigenic tests using antibodies which target sites Ø and V may be impacted by nirsevimab. In a study of natural infection, most endogenous antibodies targeting site V were shown to compete with D25, the nirsevimab precursor (Gilman et al. 2016).

During the review process, the Applicant was requested to determine if there were other widely used rapid antigen tests which have not been evaluated for interference by nirsevimab. In response, they noted that based on data from RSVAlert®, a U.S.-based RSV surveillance study which they also sponsor (McGuiness et al. 2014), diagnostic testing using rapid antigen tests was relatively uncommon, with 97% of tests from 2021 to 2022 using an RT-PCR-based platform. These data concurred with reporting by the CDC (Centers for Disease Control and Prevention 2023b).

Of the 7 current FDA-cleared rapid antigen tests listed by the Applicant, four have been evaluated (listed above, not including BD Directigen EZ RSV), one is not expected to have interference because it targets the N protein (Quidel Sofia 2 test), one other did not appear to be widely used in the U.S. based on no reported use in the OUTSMART-RSV study (Integrated Biotechnology Quick Lab RSV test), and the last one will be assessed by the Applicant (BD Veritor™ System for Rapid Detection of RSV), with data to be reported by the end of 2023.

18.5. Bioanalytical Reports for Clinical Virology Assays

The Applicant submitted a number of bioanalytical reports, which include performance characteristics of assays used to detect and quantify RSV RNA in clinical trials, and to determine the RSV subtype, sequence and phenotype for resistance assessment. The features of key assays used in the clinical virology analyses are summarized in this section.

18.5.1. Diagnostic Testing for MA RSV LRTI

Respiratory secretions were tested in a central laboratory for RSV using the 510(k) U.S. FDA-cleared and Conformité Européenne (CE)-marked in vitro diagnostic Lyra® RSV+hMPV RT-PCR assay by Quidel Corporation, San Diego, CA. Viral RNA was extracted using the NucliSENS easyMAG® System or EMAG® automated extraction systems (BioMerieux, Durham, NC). Viral RNA was reverse transcribed and then amplified by PCR using primers/probes targeting the L and NS2 genes for detection of RSV (Section 18.2).

Details of the Quidel RT-PCR assay were provided in study report ID8897-cVAP-001 amend 1, and are summarized in Table 137. Validation reports for the assay from (b) (4) were also provided in study report ID8897-cVAP-001 amend 1, including verification report 21120.3812 and qualification reports 21120.5431 (assessment of limit of detection [LOD] for RSV A and B in transport medium following testing with Quidel assay) and 21120.5633 (assessment of RSV stability in transport medium after testing with Quidel assay). The Quidel 510k equivalence determination decision summary was also provided.

Table 137. Bioanalytical Assay Characteristics for Testing for RSV

Method	Sensitivity: LOD (TCID50/mL)	Sensitivity: LOD (copies/mL)[a]	Specificity	Precision (%CV)	Accuracy
Lyra® RSV + hMPV RT-PCR assay	RSV A: 0.629-1.89 RSV B: 0.75-2.25 hMPV: 1.05-26.5	RSV A: 0.000117 RSV B: 0.000269	RSV: 96.8-97.6% hMPV: 98.9-99.6%	RSV A: 3.16-4.69% (intra-assay), 3.85% (inter-assay) RSV B: 2.67-8.14% (intra-assay), 6.01% (inter-assay)	100%

Source: page 22, report ID8897-cVAP-001 amend 1
[a] There is currently no RSV RNA international standard, so RT-PCR data are typically reported as copies/mL
Abbreviations: %CV, percent coefficient of variation; LOD, limit of detection (defined as the lowest concentration at which 95% of all replicates tested positive); hMPV, human metapneumovirus; RSV, respiratory syncytial virus; RT-PCR, reverse transcriptase-polymerase chain reaction; TCID50, median tissue culture infectious dose

An annual review of primers and probes against available sequence data was conducted alongside the OUTSMART-RSV and INFORM-RSV surveillance programs to ensure that sensitivity of the Quidel assay was maintained for circulating RSV variants which may have mutations in the primer/probe binding sites. These studies are discussed in Section 18.2.

18.5.2. RSV Subtyping and Genotyping

RSV subtypes are generally identified by genetic sequencing of the RSV attachment glycoprotein (G) gene. RSV subtypes are further categorized into genotypes, including at least 11 genotypes for RSV A (GA1-GA7, SAA1, NA1-NA2, and ON1), and 23 genotypes for RSV B (GB1-GB4, SAB1-SAB3, SAB4, URU1, URU2, BAI - BAXII, and THB) (Tabatabai et al. 2014). The second hypervariable region (HVR2) of the RSV G gene is commonly used for identification of subtypes and genotypes.

The subtype and genotypic determination of RSV were performed directly on the nasal specimens that were collected from all subjects who were confirmed RSV-positive using the Lyra® RSV+hMPV real-time RT-PCR assay. RSV-positive nasal samples were assessed for subtype/genotype by sequencing of the RSV G gene using a validated Sanger sequencing assay (b) (4). The performance characteristics of the assay were provided in validation report 21120.4571 (included in study report ID8897-cVAP-001 amend 1).

Table 138 shows the sequencing primers used for this assay. The sensitivity of the assay (LOD) using standard laboratory strains is as follows:

- RSV A Long (VR-26): 363 copies/mL
- RSV A A2 (VR-1540): 238 copies/mL
- RSV B 9320 (VR-955): 1,020 copies/mL
- RSV B WV/14617/85 (VR-1400): 263 copies/mL

Table 138. RSV G Gene Amplification and Sequencing Primers

Process	Primer Name/Position	Oligonucleotide Sequence (5'-3')
RSV G Gene RT-PCR	RSV G 5184 Forward	AGTGTTCAAYTTYGTWCCYTGYA
	RSV G 5654 Reverse	TTTGCCCCAGAKTTDATTTYG
RSV G Gene Sequencing	RSV G 5184 Forward	AGTGTTCAAYTTYGTWCCYTGYA
	RSV G 5210 Forward	TATGYRGYAACAATCCAMCYTGC
	RSV G 5654 Reverse	TTTGCCCCAGAKTTDATTTYG

Source: page 25, report ID8897-cVAP-001 amend 1
"R" and "Y" in the nucleotide sequence refers to any purine and any pyrimidine, respectively
"W" refers to A-T weak bonds (2 H bonds)
"M" refers to adenine or cytosine
"K" refers to guanine or thymine
Abbreviation: RSV, respiratory syncytial virus

Sequences were compared with contemporary RSV A or B reference strains deposited in GenBank for classification and reported as RSV A, RSV B, RSV A and B, or not determined. Assignment of RSV A or B genotypes was performed by phylogenetic clustering of RSV G HVR2 sequences with a previously described 2014 reference database of RSV genotypes (Tabatabai et al. 2014).

18.5.3. Genotypic Resistance Characterization

The full-length F gene from RSV positive nasal samples was assessed by population-based Sanger sequencing and next-generation sequencing methods for polymorphism and minor variant detection, respectively.

RSV F nucleotide sequences were translated into amino acid sequences and aligned against reference sequences derived from year 2013 Netherlands RSV A/13-5275 (GenBank accession number KX858757.1) or year 2013 Netherlands RSV B/13-1273 (GenBank accession number KX858756.1), respectively. These reference strains were selected from year 2013 based on a high degree of sequence homology to most contemporary isolates, prior to initiation of the nirsevimab development program. Amino acid variations compared with the reference sequences were determined for the full-length F protein (AA 1-574), including the nirsevimab binding site (AA 62-69 and AA 196-212) and the palivizumab binding site (AA 262-275) (AstraZeneca 1998).

The performance characteristics of the Sanger sequencing genotyping assay for the F gene were provided in validation report 21120.2847 ([redacted] (b) (4); included in study report ID8897-cVAP-001 amend 1. A [redacted] (b) (4) qualification report for the assessment of RSV stability

in transport medium following testing with the Quidel RT-PCR assay and F gene genotyping assay was also provided (21120-5268). Table 139 shows the F gene amplification and sequencing primers used for this assay. The sensitivity (LOD) of the RSV F gene Sanger sequencing assay for standard laboratory strains of RSV was as follows:

- RSV A Long GA1 (VR-26): 1,000 copies/mL
- RSV A GA2: 1,000 copies/mL
- RSV B 9320 non-BA (VR-955): 4,000 copies/mL
- RSV B GB3: 1,000 copies/mL

Table 139. RSV F Gene Amplification and Sequencing Primers

Process	Primer Name/Position	Oligonucleotide Sequence (5'-3')
RSV F Gene RT-PCR	Target #1 RSV 5670 Forward	CRAAATHARMTCTGGGGCAAA
	Target #1 RSV 6637 Reverse	CCARCARGGWGTATCWATWACACCAT
	Target #2 RSV 6418 Forward	TCAATGATATGCCTATAACAAATGATC
	Target #2 RSV 7687 Reverse	CAAGCAATGACCTCKAATYTC
RSV F Gene Sequencing	Target #1 RSV 5670 Forward	CRAAATHARMTCTGGGGCAAA
	Target #1 RSV 6142 Forward	GAAGGRGAAGTGAACAARATCAAAA
	Target #1 RSV 6441 Reverse	CATTTGTTATAGGCATATCATTGA
	Target #1 RSV 6637 Reverse	CCARCARGGWGTATCWATWACACCAT
	Target #2 RSV 6418 Forward	TCAATGATATGCCTATAACAAATGATC
	Target #2 RSV 6907 Forward	GTGTCATGYTATGGYAAAACYAAAT
	Target #2 RSV 6907 Reverse	ATTTRGTTTTRCCATARCATGACAC
	Target #2 RSV 7323 Reverse	TCYTTRCTTARTGTRACTGGTGTG
	Target #2 RSV 7687 Reverse	CAAGCAATGACCTCKAATYTC

Source: page 28, report ID8897-cVAP-001 amend 1
"R" and "Y" in the nucleotide sequence refers to any purine and any pyrimidine, respectively
"W" refers to A-T weak bonds (2 H bonds)
"H" refers to any nucleotide base except guanine
"M" refers to adenine or cytosine
"K" refers to guanine or thymine
"V" refers to any nucleotide base except thymine
Abbreviation: RSV, respiratory syncytial virus

RSV F Gene Next-Generation Sequencing Assay

Genotypic analyses were performed using a validated NGS method to detect major (≥25% frequency within each sample) and minor (≥4% and ≥5% LLOQ frequency within each sample of RSV A and B, respectively) viral variants. For completeness, data up to the limit of detection (≥2% frequency) were also provided. Validation reports for establishing the performance characteristics of the NGS assay were provided by the Applicant: 21120-12049 for RSV A, 21120-12481 for RSV B.

The NGS analyses were performed by [redacted] (b) (4) using Illumina MiSeq technology generating 250 base pair paired-end reads. FASTQ files were analyzed in CLC Genomics Workbench to determine frequency of minor variants. RSV F nucleotide reads were mapped to Netherlands RSV A/13-5275 or Netherlands RSV B/13-1273

reference sequences, followed by variant calling and amino acid annotation. Results were exported from CLC Genomics Workbench as tab-delimited text files.

Variant frequency tables were generated to depict amino acid changes identified in the full-length RSV F protein (AA 1 to 574), including the nirsevimab (AA 62 to 69 and AA 196 to 212) and palivizumab (AA 262 to 275) binding sites, that differ from 2013 Netherlands reference strains (Netherlands RSV A/13-5275 or Netherlands RSV B/13-1273). RSV F protein sequence variations detected among clinical isolates were categorized as polymorphic if they were detected among surveillance RSV isolates since 2015 and nonpolymorphic if they had not been detected. Prevalence of all RSV F protein sequence variations were reported relative to consensus sequences of recent circulating RSV A and RSV B strains since February 2015.

An independent assessment of the NGS data submitted by the Applicant was performed for a total of 43 subjects from Trial 03 (NCT02878330), Trial 04 (NCT03979313), and Trial 05 (NCT03959488), who met the primary case definition of MA RSV lower respiratory tract infection (LRTI), with or without hospitalization. Several subjects were selected from each time period (through Day 150 or Day 151-360, etc.) in an attempt to have at least one subject from each treatment arm, RSV subtype, and hospitalization status where possible, for each time period.

18.5.4. Phenotypic Resistance Characterization

Amino acid changes identified in the full-length RSV F protein compared with the reference sequences were assessed by engineering into RSV A2-000 or RSV B9320-000 reference clone cDNAs through reverse genetics, as described in Sections 18.1.2 and 20.7. These reference clones include modifications to encode the contemporary E66 residue in RSV A2 and N197 in B9320 F proteins. Table 140 shows the F protein amino acid differences between the reference sequences used for sequence comparisons with those used in the recombinant RSV microneutralization assay.

Table 140. RSV F Protein Sequence Context Comparison

RSV/ Subtype	Reference Strain	RSV F Amino Acid Position										
RSV A		4	8	16	20	25	66[a]	67[a]	101	103	105	122
	rRSV A2	L	A	T	F	G	K	N	Q	T	N	A
	rRSV A2-000	L	A	T	F	G	E	N	Q	T	N	A
	NLD RSV A/13-5275	P	T	A	L	S	E	N	P	A	S	T
		124	152	276	384	540						
	rRSV A2	K	V	N	V	S						
	rRSV A2-000	K	V	N	V	S						
	NLD RSV A/13-5275	N	I	S	I	A						
RSV B		15	45	97	197[a]	294	529					
	rRSV B9320	L	F	T	S	K	T					
	rRSV B9320-000	L	F	T	N	E	T					
	NLD RSV B/13-1273	F	L	M	N	E	A					

Source: page 33, report ID8897-cVAP-001 amend 1
[a] Within nirsevimab binding sites (amino acids 62-69 and 196-212)
Abbreviation: RSV, respiratory syncytial virus

Substitutions were prioritized for phenotypic assessment based on whether they were identified up to Day 150 following nirsevimab dosing, and if they weren't identified in the RSV isolates from the placebo group. Substitutions identified in antigenic site Ø (AA 62-96 and AA 195-227; includes nirsevimab binding site AA 62-69 and AA 196-212) or antigenic site II (AA 254-277; includes palivizumab binding site AA 262-275), were assessed using reverse genetics, both individually and in the context in which they were observed.

Recombinant RSV was evaluated by microneutralization assay in HEp-2 cells, as described in Section 20.6.Neutralization activity (EC_{50} value) was compared with the rRSV A2-000 or rRSV B9320-000 reference strain EC_{50} values to determine the fold-change and impact of the substitutions on susceptibility to nirsevimab and palivizumab. Validation reports were provided in study report ID8897-cVAP-001 amend 1 for the microneutralization assay ((b) (4)-VAL146_RPT and (b) (4)-VAL146_RPT Amendment 1 [(b) (4)], and MS8897-(b) (4)-VAL146_memo-001, statistical power to detect EC_{50} fold-shift relative to reference virus).

18.5.5. Definition of Binding Site and Resistance-Associated Substitutions

Binding site substitutions for nirsevimab are defined as any RSV F protein polymorphism or minor variant amino acid change in the nirsevimab binding site (AA 62-69 and 196-212) of an RSV isolate relative to the corresponding RSV A F or B F reference sequence (Netherlands RSV A/13-5275 or Netherlands RSV B/13-1273).

Resistance-associated substitutions (RAS) for nirsevimab are defined as any polymorphism or minor variant amino acid change that has been associated with reduced susceptibility (≥5-fold change) of a clinical isolate or engineered rRSV variant to neutralization by nirsevimab (Table 141). Nirsevimab RAS have been located in the binding site, based on data from cell culture selection studies and historical circulating RSV strains (Section 20.7; (Zhu et al. 2017; Zhu et al. 2018)).

Table 141. RSV F Protein Residues That Comprise the Nirsevimab Binding Site and Their Association With Substitutions That Confer Reduced Susceptibility to Neutralization

RSV Subtype	Reference Strain	RSV F Subunit	RSV F Amino Acid Position								
			62	63	64	65	66	67	68	69	
RSV A	NLD RSV A/13-5275	F2	S	N	I	K[a]	E	N	K	C	
RSV B	NLD RSV B/13-1273	F2	S	N	I	K[a]	E	T	K	C	
			196	197	198	199	200	201	202	203	204
RSV A	NLD RSV A/13-5275	F1	K	N	Y	I	D[a]	K	Q[a]	L	L
RSV B	NLD RSV B/13-1273	F2	K	N	Y	I	N[a]	N	Q[a]	L	L

RSV Subtype	Reference Strain	RSV F Subunit	RSV F Amino Acid Position								
			205	206	207	208	209	210	211	212	
RSV A	NLD RSV A/13-5275	F1	P	I	V	N[a]	K[a]	Q	S	C	
RSV B	NLD RSV B/13-1273	F2	P	I	V	N[a]	Q[a]	Q	S	C	

Source: page 35, ID8897-cVAP-001 amend 1
[a] Amino acid residues with side chains making hydrogen bonds or salt bridges with nirsevimab.
Nirsevimab resistance-associated substitutions at positions 68, 201, and 208 identified among cell culture selected neutralization escape variants were located in the nirsevimab binding site (N67I+N208Y in RSV A and N208D, N208S, K68N+N201S, or K68N+N208S in RSV B). Additional nirsevimab resistance-associated substitutions were retrospectively identified among RSV isolates between 1956 and 2016 in the nirsevimab binding site (L203I, K65Q+K68N, or K68Q+S211N in RSV B).
Abbreviation: RSV, respiratory syncytial virus

Resistance-associated substitutions for palivizumab are defined as any polymorphism or minor variant amino acid change that has been associated with reduced susceptibility of a clinical isolate or engineered rRSV variant to neutralization by palivizumab (Table 142). Palivizumab RAS have been located in the binding site, based on data from cell culture selection studies and historical circulating RSV strains (Section 20.7; (Zhu et al. 2011; Zhu et al. 2012)).

Table 142. RSV F Protein Residues That Comprise the Palivizumab Binding Site and Their Association With Substitutions That Confer Reduced Susceptibility to Neutralization

RSV Subtype	Reference Strain	RSV F Subunit	RSV F Amino Acid Position								
			262	263	264	265	266	267	268	269	270
RSV A	NLD RSV A/13-5275	F1	N	D	M	P	I	T	N	D	Q
RSV B	NLD RSV B/13-1273	F1	N	D	M	P	I	T	N	D	Q
			271	272	273	274	275				
RSV A	NLD RSV A/13-5275	F1	K	K	L	M	S				
RSV B	NLD RSV B/13-1273	F2	K	K	L	M	S				

Source: page 36, ID8897-cVAP-001 amend 1
Palivizumab resistance-associated substitutions at positions 262, 268, and 272 identified among cell culture selected neutralization escape variants and clinical RSV isolates were located in the palivizumab binding site (N262D, K272E/M/N/Q/T, S275F/L in RSV A)
Abbreviation: RSV, respiratory syncytial virus

18.6. Clinical Virology Genotypic and Phenotypic Analyses

The Applicant submitted virology study reports for each of the supportive clinical trials (Trial 03, Trial 04, Trial 05, and Trial 08), which summarized genotypic and phenotypic resistance data. As stated in the clinical virology analysis plan, these analyses were conducted on all RT-PCR-confirmed RSV isolates from subjects with MA RSV LRTI (protocol or nonprotocol defined) or hospitalization due to RSV illness, and included:

- The percentage of subjects with primary and secondary efficacy events who had an RSV isolate containing nirsevimab resistance-associated substitutions by subtype and reporting period
- Cell culture neutralization susceptibility of recombinant RSV variants containing identified F protein sequence variations against nirsevimab and palivizumab
- The proportion of subjects containing F sequence variations by treatment group

Reference ID: 5208810

- The association between identified nirsevimab binding site substitutions and RSV disease severity among corresponding subjects who had an RSV LRTI event

In addition, the prevalence of all RSV F protein amino acid substitutions was reported relative to consensus sequences of recent circulating strains (since 2015), based on surveillance data (see Section 18.1).

To support the clinical study reports, clinical resistance datasets and raw NGS files were submitted, which were reviewed independently by the FDA. A spot check of the NGS raw data files gave results which were largely in agreement with those reported by the Applicant.

18.6.1. Clinical Virology Resistance Analyses for Trial 03

Trial 03 virology report.

Trial 03 was a phase 2b, randomized, double-blind, placebo-controlled trial to evaluate a single 50 mg IM dose of nirsevimab in healthy preterm infants who were born between 29 weeks 0 days and 34 weeks 6 days gestational age and entering their first RSV season. The primary objective was the reduction of MA RSV LRTI due to RT-PCR-confirmed RSV, compared to placebo. Details of the protocol and trial results are reviewed in Section 6.2.2.

Genotypic and phenotypic analyses were conducted on all subjects in Trial 03 who had MA RSV LRTI or hospitalization due to RSV illness. Because efficacy was estimated to be lower in infants weighing ≥5 kg in Trial 03 and a 100 mg dose was proposed for these infants to achieve efficacious exposures, a subgroup analysis in trial participants at the proposed dose (infants receiving 50 mg dose weighing <5 kg) was also conducted.

In the placebo and nirsevimab groups, there were 54/484 (11.2%) and 40/969 (4.1%) subjects, respectively, in the resistance analysis population. Table 143 summarizes the number of subjects in the resistance analysis population with evaluable sequence data available by RSV subtype, case definition and reporting period. There were similar numbers of events from RSV A and RSV B infections within each trial arm.

Two subjects in the placebo group had two separate RSV infections in the course of the trial and were counted twice: subject (b) (6), who had protocol-defined MA RSV LRTI hospitalization with RSV B on Day 65, and protocol-defined MA RSV LRTI with RSV A on Day 360, and subject (b) (6), who had nonprotocol defined MA RSV LRTI with RSV A on Day 38, and again with RSV B on Day 114.

Table 143. Number of Subjects in the Resistance Analysis Population for Trial 03 (All Subjects, ≥29 to ≤35 wGA) for Whom an Evaluable RSV NGS Sequence Was Available

	Through 150 Days Postdose		From Day 151 to 360 Postdose	
Case Definition,[a,b] RSV Subtype	**Placebo (N=484)**	**Nirsevimab (N=969)**	**Placebo (N=484)**	**Nirsevimab (N=969)**
Primary - MA RSV LRTI	44	25	4	6
RSV A	22	11	2	3
RSV B	22	14	2	3
Secondary - MA RSV LRTI hospitalization	20	8	2	1
RSV A	12	5	1	0
RSV B	8	3	1	1
Overall[a]	49	33	6	6[c]
RSV A	25	16	3	3
RSV B	24	17	3	3[c]

Source: Table 2, page 16, Trial 03 virology report
[a] Subjects were counted once for each efficacy category and each sampling period regardless of the number of events. Overall category sums the subjects meeting primary, secondary, or exploratory case definitions.
[b] Numbers of subjects infected with RSV A and B include only those for whom an evaluable viral sequence was available.
[c] One additional subject had a sample collected on Day 365.
Abbreviation: MA RSV LRTI, medically attended RSV lower respiratory tract infection; NGS, next generation sequencing; RSV, respiratory syncytial virus; wGA, weeks gestational age

18.6.2. Analysis of RSV A Resistance Analysis Population in Trial 03

The number of subjects with RSV A variants identified in Trial 03 are shown in Table 144 (through Day 150 postdose) and Table 145 (Day 151 to Day 360 postdose). Most concurrent substitutions were seen at ≥99% frequency, indicating that they were likely linked. There were no major (i.e., ≥25% frequency) or minor (i.e., ≥4% and <25% frequency) variants through Day 360 with substitutions in the nirsevimab binding site. Hence, all substitutions resided within or outside of the extracellular regions of RSV F. There were two subjects who had RSV A through Day 150 and more than one respiratory sample, with substitution(s) seen in the sample from the second visit, not reported in Table 144: (b) (6) (MA LRTI with hospitalization), with P4L substitution (99% frequency), and subject (b) (6) (MA LRTI), with V18A+Y33H (46% / 49%) and A149T (2.3%) substitutions.

A comparison of individual substitutions or specific amino acid positions did not identify any which were significantly increased in frequency in nirsevimab-treated subjects infected with RSV A, with or without hospitalization, compared with placebo subjects through Day 150. There were no individual substitutions or positions with changes seen in more than one nirsevimab-treated subject. All individual substitutions identified were seen at ≤3.7% prevalence in circulating isolates collected in surveillance studies.

Phenotypic data were reported for 6 substitutions occurring in the extracellular region of F protein through Day 360, with only S99N showing a change in susceptibility (5.3-fold) to nirsevimab. Two substitutions (G71S and K419E) were unsuccessfully rescued from recombinant RSV cDNA, and evaluation of the minor variant substitution, R49K, is ongoing.

Table 144. Number of Subjects in Trial 03 With MA RSV LRTI[a], Infected With RSV A Variants Harboring F Protein Substitutions Through Day 150 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=22 [12]	Nirsevimab N=11 [5]	Total N=33 [17]	EC_{50} Fold Change[b]	
					Nirsevimab	Palivizumab
No substitutions	-	14 [9]	4 [1]	18 [10]	-	-
Variants seen at ≥25% frequency[c]						
T13A	3.72	0	1 [1]	1 [1]	NA	NA
T13I+I14V	NA	1	0	1	NA / NA	1.4 / NA
A17V	0.03	0	1	1	NA	NA
A17V+F22I	NA	1 [1]	0	1 [1]	NA / NA	NA / NA
C21S+S99N[d]	NA	1	0	1	NA / 5.3[f]	NA / 2.1
A23T+G71S[d]	NA	1	0	1	NA / QNS	NA / QNS
A23T+A102S[d]	NA	1 [1]	1 [1]	2 [2]	NA / 1.4	NA / 0.9
S25N[d]+V127A	0.24	0	1	1	2.5 / NA	1.9 / NA
V76I[d]	NA	0	1	1	1.2	0.9
N116Y	NA	1	0	1	NA	NA
N120S	0.07	1	0	1	NA	NA
K123E+S213R[d]	NA	0	1 [1]	1 [1]	NA / 2.2	NA / 2.6
K419E[d]	0.03	1 [1]	1 [1]	2 [2]	QNS	QNS
Variants seen at ≥3% to <25% frequency						
R49K[d,e]	NA	1	0	1	NA	NA

Source: pages 28-38, Trial 03 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Data for individual substitutions
[c] Most substitutions detected at ≥99% frequency, except G71S (34%), S99N (35%), and A102S (43%, 68%)
[d] Occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
[e] Observed at < LLOQ of NGS assay for RSV A (4%)
[f] EC_{50} fold-change values: ≥5-fold change
Abbreviations: EC_{50}, 50% effective concentration; LLOQ, lower limit of quantification; MA RSV LRTI, medically attended RSV lower respiratory tract infection; N, number of subjects; NA, not available; NGS, next generation sequencing; QNS, quantity not sufficient (rRSV rescue failed)

Table 145. Number of Subjects in Trial 03 With MA RSV LRTI[a], Infected With RSV A Variants Harboring F Protein Substitutions From Day 151 to 360 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=2 [1][b]	Nirsevimab N=3 [0]	Total N=5 [1]	EC_{50} Fold Change[c]	
					Nirsevimab	Palivizumab
No substitutions	-	1	0	1	-	-
Variants seen at ≥25% frequency[d]						
I14V	0.07	0	1	1	NA	NA
A23T	3.23	0	1	1	NA	NA
A23T+*S190N*[e]	NA	0	1	1	NA / 2.2	NA / 2.6
R113K+Y117H	0.07	1 [1]	0	1 [1]	NA / NA	NA / NA

Source: pages 30-38, Trial 03 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Includes subject (b) (6) in placebo group, who also had RSV B infection at Day 65
[c] Data for individual substitutions
[d] No minor variants (≥4% to <25%) were seen in this subset; all substitutions were seen at ≥99% frequency.
[e] O outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: EC_{50}, 50% effective concentration; MA RSV LRTI, medically attended RSV lower respiratory tract infection; N, number of subjects; NA, not available

18.6.3. Analysis of RSV B Resistance Analysis Population in Trial 03

The number of subjects in the resistance analysis population for Trial 03 who were infected with RSV B variants are shown in Table 146 (through Day 150 postdose) and Table 148 (Day 151 to Day 360 postdose). Phenotypic data and numbers of subjects with individual substitutions through Day 150 are shown in Table 147. Most concurrent substitutions were seen at ≥99% frequency, indicating that they were likely linked.

As noted for surveillance studies (Section 18.1), compared with the reference isolate for RSV B from 2013 (Netherlands RSV B/13-1273), the three substitutions K191R, I206M and Q209R, became predominant in recent years, with F15L, A103V, L172Q and S173L substitutions also seen commonly since 2015. Hence, most variants identified at ≥25% frequency in clinical studies contained all or some of these substitutions. With respect to the nirsevimab binding site, the double substitution, I206M+Q209R, became dominant in 2017, and was seen in 66% of RSV B isolates circulating from 2015 to 2021 and in most variants seen at ≥25% frequency in Trial 03. While I206M substitution caused a 5-fold reduction in susceptibility to nirsevimab on its own, it was rarely seen in the absence of Q209R in surveillance studies, and the double substitution did not have reduced susceptibility to nirsevimab (Table 147).

Other variants which had substitutions in the nirsevimab binding site included one seen in a nirsevimab-treated subject through Day 150, with I64T+K68E+I206M+Q209R substitutions, and one with N208S substitution, also in a nirsevimab-treated subject through Day 150. The two variants harboring these substitutions were not seen in surveillance studies. The I64T, K68E and N208S substitutions all caused a substantial loss of susceptibility to nirsevimab, with fold-changes in EC_{50} values of >496, >283, and >387, respectively. Only I64T substitution impacted palivizumab susceptibility as well (5-fold change). The Applicant noted that the two subjects with I64T+K68E+I206M+Q209R and N208S substitutions were dosed below the proposed dose because they weighed >5 kg at the time of nirsevimab administration.

One other substitution in the nirsevimab binding site, N208K, was seen as a minor variant and below the LLOQ of the NGS assay (5% for RSV B). N208K substitution caused a >350-fold reduction in susceptibility to nirsevimab but retained susceptibility to palivizumab. N208K substitution was not observed in surveillance studies.

There were no variants which were clearly associated with nirsevimab-treated subjects compared with placebo subjects, or in hospitalized subjects compared with nonhospitalized subjects, although in general there were too few examples to draw firm conclusions. Most variants were identified in single subjects, in both nirsevimab and placebo groups. The two subjects with variants harboring I64T+K68E+I206M+Q209R and N208S substitutions were both hospitalized, but it is not known whether these variants were associated with the hospitalizations.

Overall, there were no individual substitutions or specific amino acid positions within or outside the nirsevimab binding sites which were significantly increased in frequency in nirsevimab-treated subjects compared with placebo, or in hospitalized subjects compared with nonhospitalized subjects. The 8 substitutions seen at ≥25% frequency through Day 360 in extracellular domains but outside the nirsevimab binding sites did not cause reduced susceptibility to nirsevimab (Table 147 and footnote to Table 148).

Table 146. Number of Subjects in Trial 03 With MA RSV LRTI[a], Infected With RSV B Variants Harboring F Protein Substitutions, Through Day 150 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=22 [8]	Nirsevimab N=14 [3]	Total N=36 [11]
Variants seen at ≥25% frequency[c]				
L4P+F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	1.46	1	0	1
F12L+F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**+*S276N*+*E463D*	4.11	1	1	2
F12L+F15L+**I64T**+**K68E**+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**+*S276N*+*K327R*+*E463D*+K574N	NA	0	1 [1]	1 [1]
F15I+*A103V*+*L172Q*+*S173L*	NA	1 [1]	0	1 [1]
F15L+A16T+*A103V*+*L172Q*+*S173L*	0.18	1 [1]	0	1 [1]
F15L+A16V+*A103V*+*L172Q*+*S173L*	1.18	1 [1]	0	1 [1]
F15L+*A103V*+T118I+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	0.04	1	0	1
F15L+*A103V*+V127I+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	0.07	0	1	1
F15L+*A103V*+*L172Q*+*S173L*	12.89	10 [3]	9 [1]	19 [4]
F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	40.71	3 [1]	1	4 [1]
F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**+I527M	NA	1	0	1
F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**+A543T	0.04	1	0	1
F15L+*A103V*+*L172Q*+*S173L*+**N208S**	NA	0	1 [1]	1 [1]
F15L+*A103V*+*L172Q*+*S173L*+*S276N*	0.11	1 [1]	0	1 [1]
Variants seen at ≥3% to <25% frequency				
L171M[b]	NA	1 [1]	0	1 [1]
N208K[b]	NA	0	1 [1]	1 [1]
L381F[b]	NA	1	0	1
Y457H	NA	1	0	1

Source: pages 29-34, Trial 03 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Observed at < LLOQ of NGS assay for RSV B (5%)
[c] Most substitutions detected at ≥99% frequency, except I64T (35%), K68E (32%), and N208S (81%)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Bold substitutions: occur within nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Abbreviations: MA RSV LRTI, medically attended RSV lower respiratory tract infection; N, number of subjects; NA, not available

Table 147. Phenotypic Data for Individual and Concurrent Substitutions Seen in RSV B Variants From Subjects in Trial 03 With MA RSV LRTI Through Day 150

Amino Acid Substitutions	Prevalence (%)	Placebo (N=22)	Nirsevimab (N=14)	Total (N=36)	EC_{50} Fold Change	
					Nirsevimab	Palivizumab
Variants seen at ≥25% frequency						
L4P	2.25	1	0	1	NA	NA
F12L	7.21	1	2	3	NA	NA
F15I	NA	1	0	1	NA	NA
F15L	99.57	21	14	35	1.0	1.0
A16T	1.21	1	0	1	NA	NA
A16V	1.57	1	0	1	NA	NA
I64T	NA	0	1	1	>496.3[b]	**5.2**
K68E	NA	0	1	1	>283.4[b]	2.1
A103V	98.5	22	14	36	0.9	1.8
T118I	0.07	1	0	1	NA	NA
V127I	0.07	0	1	1	NA	NA
L172Q	98.61	22	14	36	1.1	2.3
S173L	98.36	22	14	36	0.9	1.9
K191R	68.61	8	4	12	1.3	2.7
I206M	68.89	8	4	12	5.0[b]	2.0
N208S	NA	0	1	1	>386.6[b]	1.8
Q209R	68.18	8	4	12	0.5	3.1
S276N	7.14	2	2	4	2.0	2.5
K327R	0.11	0	1	1	0.9	1.9
E463D	7	1	2	3	1.4	2.1
I527M	0.04	1	0	1	NA	NA
A543T	0.07	1	0	1	NA	NA
K574N	0.07	0	1	1	NA	NA
I206M+Q209R	66	8	3	11	0.2	1.3
I64T+K68E+I206M+Q209R	NA	0	1	1	>447.1[b]	1.2

Amino Acid Substitutions	Prevalence (%)	Placebo (N=22)	Nirsevimab (N=14)	Total (N=36)	EC_{50} Fold Change	
					Nirsevimab	Palivizumab
Variants seen at ≥3% to <25% frequency						
L171M[a]	NA	1	0	1	NA	NA
N208K[a]	NA	0	1	1	>350.5[b]	2.0
L381F[a]	NA	1	0	1	NA	NA
Y457H	NA	1	0	1	QNS	QNS

Source: pages 29-37, Trial 03 virology report and FDA analysis
[a] Observed at < LLOQ of NGS assay for RSV B (5%)
[b] EC50 fold-change values: ≥5-fold change
Bold substitutions: occur within nirsevimab binding sites
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: EC50, 50% effective concentration; MA RSV LRTI, medically attended RSV lower respiratory tract infection; N, number of subjects; NA, not available; QNS, quantity not sufficient

Table 148. Number of Subjects in Trial 03 With MA RSV LRTI[a], Infected With RSV B Variants Harboring F Protein Substitutions, Day 151 to 360 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=2 [1]	Nirsevimab N=3 [1]	Total N=5 [2]
Variants seen at ≥25% frequency[b]				
F15L+A16V+*A103V*+*L172Q*+*S173L*	1.18	0	1	1
F15L+*Y33F*+*A103V*+*L172Q*+*S173L*	0.07	0	1	1
F15L+*A103V*+*L172Q*+*S173L*	12.89	1	1 [1]	2 [1]
F15L+*A103V*+V127I+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	0.07	1 [1]	0	1 [1]

Source: pages 30-32 Trial 03 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Phenotypic data for individual substitutions shown in Table 18.3.1.5, except for Y33F, which did not cause reduced susceptibility to nirsevimab or palivizumab (0.5-fold and 1.2-fold change, respectively)
Bold substitutions: occur within nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: MA RSV LRTI, medically attended RSV lower respiratory tract infection; N, number of subjects; NA, not available

Variants seen in subjects with nonprotocol-defined MA LRTI or unscheduled RSV events

Table 149 shows the substitutions seen at ≥25% frequency in subjects with nonprotocol-defined MA LRTI or unscheduled RSV events, for both RSV A and RSV B infections.

In general, individual variants were only seen in single subjects, with the exception of L119I+K209R substitutions seen in two nirsevimab-treated subjects infected with RSV A. However, only K209R substitution resides in the nirsevimab binding site, and did not cause reduced susceptibility to nirsevimab or palivizumab.

Table 149. Number of Subjects in Trial 03 With Nonprotocol-Defined MA RSV LRTI, Or Unscheduled RSV Events, Infected With RSV Variants Harboring F Protein Substitutions at ≥25% Frequency

Amino Acid Substitutions	Prevalence (%)	Placebo (n=4)	Nirsevimab (n=8)	Total (n=12)
RSV A, unscheduled events through 150 days postdose				
T13A	3.72	0	1	1
L119I+**K209R**[a]	NA	0	2	2
RSV A, nonprotocol defined RSV LRTI through 150 days postdose				
A103V[a]	0.07	0	1	1
G329R[a]	0.03	1	1	2
RSV B, unscheduled events through 150 days postdose				
F15L+*A103V+I129M+L172Q+S173L+K191R*+**I206M**+**Q209R**+*T518I*	NA	0	1	1
RSV B, unscheduled events 151 to 360 days postdose				
F12L+F15L+*A103V+L172Q+S173L+S190N+/ K191R*+**I206M**+**Q209R**+*S276N+E463D*	1.29	1	0	1
RSV B, nonprotocol defined RSV LRTI through 150 days postdose				
F15L+*A103V+L172Q+S173L*	12.89	0	1	1
F15L+*A103V+L172Q+S173L+K191R*+**I206M**+**Q209R**[b]	40.71	1	0	1
F15L+*A103V+L172Q+S173L+K191R*+**I206M**+**Q209R**+*V365A*	0.04	1	1	2

Source: pages 40-42, Trial 03 virology report and FDA analysis
[a] No change in susceptibility (<3-fold) to nirsevimab or palivizumab
[b] Also observed in one nirsevimab-treated subject at Day 366
Bold substitutions: occur within nirsevimab binding sites
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: MA RSV LRTI, medically attended RSV lower respiratory tract infection; N, number of subjects; NA, not available

18.6.4. Clinical Virology Resistance Analyses for Trial 04

Trial 04 virology report.

Trial 04 is an ongoing phase 3, randomized, double-blind, placebo-controlled trial to evaluate the safety and efficacy of nirsevimab in healthy late preterm and term infants who were born ≥35 weeks 0 days gestational age and entering their first RSV season. The primary objective is the reduction of MA RSV LRTI due to RT-PCR-confirmed RSV, compared to placebo. Details of the protocol and trial results are reviewed in Section 6.2.3.

The resistance analysis population for Trial 04 included all subjects who had MA RSV LRTI or hospitalization due to RSV illness and evaluable NGS data from the primary and safety cohorts combined. In the placebo and nirsevimab groups, there were 88/1003 (8.8%) and 60/2009 (3.0%) subjects, respectively, in the resistance analysis population. Table 150 summarizes the number of subjects in the resistance analysis population with evaluable sequence data available by RSV subtype, case definition and reporting period. Most concurrent substitutions were seen at ≥94% frequency, indicating that they were likely linked.

There were similar numbers of events from RSV A and RSV B infections within each trial arm. Of note, in the Primary Cohort there was a predominance of the RSV A subtype, and in the Safety Cohort RSV B was predominant, so that overall, the numbers of events based on subtype were similar. This difference was a result of different enrollment times of the Primary and Safety Cohorts which were coincident with predominance of RSV A and RSV B, respectively. For the Primary Cohort, 496 placebo and 994 nirsevimab subjects were enrolled between July 23, 2019, and November 30, 2019 (n=1,027; one subject enrolled in 2020) in the northern hemisphere, and between January 08, 2020, and March 15, 2020 (n=462) in the southern hemisphere (South Africa). For the Safety Cohort (n=507 placebo and 1,015 nirsevimab subjects), enrollment commenced on April 09, 2021, in the southern hemisphere.

Table 150. Number of Subjects in the Resistance Analysis Population for Trial 04 (Overall Population; Term and Preterm Infants ≥35 wGA), for Whom an Evaluable RSV NGS Sequence Was Available

	Through 150 Days Postdose		From Day 151 to 360 Postdose		From Day 361 to 511 Postdose	
Case Definition[a,b] **RSV Subtype**	**Placebo (N=1003)**	**Nirsevimab (N=2009)**	**Placebo (N=1003)**	**Nirsevimab (N=2009)**	**Placebo (N=1003)**	**Nirsevimab (N=2009)**
Primary - MA RSV LRTI	54	23	6	13	2	7
RSV A	25	13	6	7	1	4
RSV B	29	10	0	6	1	3
Secondary - MA RSV LRTI hospitalization	20	8	2	1	0	0
RSV A	8	6	2	1	0	0
RSV B	12	2	0	0	0	0

Case Definition[a,b] RSV Subtype	Through 150 Days Postdose		From Day 151 to 360 Postdose		From Day 361 to 511 Postdose	
	Placebo (N=1003)	Nirsevimab (N=2009)	Placebo (N=1003)	Nirsevimab (N=2009)	Placebo (N=1003)	Nirsevimab (N=2009)
Overall[a]	75	35	10	17	3	8
RSV A	39	24	10	11	2	5
RSV B	36	11	0	6	1	3

Source: Table 2, page 17, Trial 04 virology report
[a] Subjects were counted once for each efficacy category and each sampling period regardless of the number of events. Overall category sums the subjects meeting primary, secondary, or exploratory case definitions.
[b] Numbers of subjects infected with RSV A and B include only those for whom an evaluable viral sequence was available.
Abbreviations: MA RSV LRTI, medically attended RSV lower respiratory tract infection; N, number of subjects; RSV, respiratory syncytial virus; wGA, weeks gestational age

18.6.5. Analysis of RSV A Resistance Analysis Population in Trial 04

The number of subjects with RSV A variants identified in Trial 04 are shown in Table 151 (through Day 150 postdose), Table 152 (Day 151 to Day 360 postdose), and Table 153 (Day 361 to Day 511 postdose). There were no major (i.e., ≥25% frequency) variants through Day 511 with substitutions in the nirsevimab binding sites.

There was one minor variant (≥4% to <25% frequency) harboring substitutions in the nirsevimab binding site in a nirsevimab-treated subject with MA RSV LRTI from Day 360 to 511 (Table 152). Given the low frequency that these substitutions were observed, and that the variant was only seen in one subject, the importance of this finding is not clear. The Applicant indicated that they are in the process of evaluating D200N+K201N substitutions phenotypically. One other subject in the placebo group was seen with RSV A harboring K272E substitution at low frequency (<4%), which occurs in the palivizumab binding site (Table 151). This substitution occurs at a position associated with resistance to palivizumab and is also being evaluated for susceptibility to both nirsevimab and palivizumab.

Of the individual substitutions seen up to Day 511 occurring outside the nirsevimab binding sites but within the extracellular regions of F protein, none of the ones assessed showed reduced susceptibility of more than 2.4-fold to nirsevimab or palivizumab (Table 151, Table 152, Table 153). The Applicant indicated that the remaining substitutions are in the process of being assessed.

A comparison of individual substitutions or specific amino acid positions did not identify any which were increased in frequency in nirsevimab-treated subjects infected with RSV A, with or without hospitalization, compared with placebo subjects through Day 511. All individual substitutions identified were seen at ≤3.7% prevalence in circulating isolates collected in surveillance studies.

Table 151. Number of Subjects in Trial 04 With MA RSV LRTI[a], Infected With RSV A Variants Harboring F Protein Substitutions Through Day 150 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=23 [8]	Nirsevimab N=12 [6]	Total N=35 [14]	EC_{50} Fold Change[b]	
					Nirsevimab	Palivizumab
No substitutions	-	5 [1]	1 [0]	6 [1]	-	-
Variants seen at ≥25% frequency[c]						
L6H	0.1	1	0	1	NA	NA
T8I	0.31	0	1 [1]	1 [1]	NA	NA
T12I	2.61	3 [1]	2 [1}	5 [2]	NA	NA
T12I+V127A	NA	1 [1]	0	1 [1]	NA / NA	NA / NA
T12I+V127I+*S276N*	NA	1	0	1	NA / NA / 1.0	NA / NA / 1.0
T12I+*V406I*	0.03	1	0	1	NA / NA	NA / NA
T13A	3.72	2 [1]	1 [1]	3 [2]	NA	NA
L15F+L119F+T122A	NA	0	1	1	NA / NA / 1.0	NA / NA / 1.0
L15F+T122A+*K419E*	0.03	0	1 [1]	1 [1]	NA / 1.0 / NA	NA / 1.0 / NA
L20F	0.35	1	0	1	1.0	1.0
A23T	3.23	1	0	1	NA	NA
A103T+T122A	1.25	1 [1]	0	1 [1]	1.0 / 1.0	1.0 / 1.0
A103T+T122A+S255N	NA	0	1	1	1.0 / 1.0 / NA	1.0 / 1.0 / NA
A107T	0.73	0	2 [2]	2 [2]	2.1	2.3
F114S	0.63	1 [1]	0	1 [1]	NA	NA
F114S+*V144I*	NA	1	0	1	NA / 1.1	NA / 1.1
M115T+T122A	0.49	1	0	1	NA / 1.0	NA / 1.0
T122A+K123Q+*I384T*	2.75	0	1	1	1.0 / NA / 2.4	1.0 / NA / 1.3
T122A+*T245N*	NA	1	0	1	1.0 / NA	1.0 / NA
S276N	1.04	1 [1]	0	1 [1]	1.0	1.0
Q354R+D479N	NA	0	1	1	2.1 / NA	1.9 / NA
D486N	NA	1 [1]	0	1 [1]	NA	NA

Amino Acid Substitutions	Prevalence (%)	Placebo N=23 [8]	Nirsevimab N=12 [6]	Total N=35 [14]	EC_{50} Fold Change[b] Nirsevimab	EC_{50} Fold Change[b] Palivizumab
Variants seen at ≥2% to <25% frequency						
T8A+L20F+*I79M*[d]+*S105N*+N124K+*S213R*+*S276N*	NA	1	1	2	1.0 / 1.0 / NA / 1.0 / 1.0 / 2.2 / 1.0	1.0 / 1.0 / NA / 1.0 / 1.0 / 2.6 / 1.0
F22S	0.03	1	0	1	NA	NA
A89V[d] +*N165S*[d]	NA	1	0	1	NA / NA	NA / NA
K123R[d]	0.03	1 [1]	0	1 [1]	NA	NA
N126K	NA	1	0	1	NA	NA
N228S[d] +*V247L*[d]	NA	0	1 [1]	1 [1]	NA / NA	NA / NA
I384V[d] +*N515H*+A540S	NA	1	2 [1]	3 [1]	1.0 / NA / 1.0	1.0 / NA / 1.0
A543T	0.17	1	0	1	NA	NA

Source: pages 31-49, Trial 04 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Data for individual substitutions
[c] Most substitutions detected at ≥95% frequency, except L6H (47%) and D486N (61%)
[d] Observed at < LLOQ of NGS assay for RSV A (4%)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: EC50, 50% effective concentration; LLOQ, lower level of quantification; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available; NGS, next generation sequencing

Table 152. Number of Subjects in Trial 04 With MA RSV LRTI[a], Infected With RSV A Variants Harboring F Protein Substitutions From Day 151 to 360 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=6 [2]	Nirsevimab N=7 [1]	Total N=13 [3]	EC_{50} Fold Change[b] Nirsevimab	EC_{50} Fold Change[b] Palivizumab
Variants seen at ≥25% frequency[c]						
A23S+T122A	0.07	0	1	1	NA / 1.0	NA / 1.0
F114S	0.63	0	1	1	NA	NA
T122A+K123Q+*I384T*	2.75	5 [2]	5 [1]	10 [3]	1.0 / NA / 2.4	1.0 / NA / 1.3
T122A+K123Q+*I384T*+D562N	NA	1	0	1	1.0 / NA / 2.4 / NA	1.0 / NA / 1.3 / NA

Amino Acid Substitutions	Prevalence (%)	Placebo N=6 [2]	Nirsevimab N=7 [1]	Total N=13 [3]	EC_{50} Fold Change[b] Nirsevimab	EC_{50} Fold Change[b] Palivizumab
Variants seen at ≥2% to <25% frequency						
K272E[d]	NA	1	0	1	NA	NA
W341R	NA	0	1	1	NA	NA
A355V[d]	0.03	1	0	1	1.7	1.9
I384V[d] +*N515H*+A540S	NA	1	0	1	1.0 / NA / 1.0	1.0 / NA / 1.0

Source: Trial 04 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Data for individual substitutions
[c] Most substitutions detected at ≥98% frequency, except D562N (86%)
[d] Observed at < LLOQ of NGS assay for RSV A (4%)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Bold, italicized substitutions: occur in palivizumab binding site (amino acid residues 262-275)
Abbreviations: LLOQ, lower level of quantification; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available; NGS, next generation sequencing

Table 153. Number of Subjects in Trial 04 With MA RSV LRTI[a], Infected With RSV A Variants Harboring F Protein Substitutions From Day 361 to 511 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=1	Nirsevimab N=4	Total N=5	EC_{50} Fold Change[b] Nirsevimab	EC_{50} Fold Change[b] Palivizumab
Variants seen at ≥25% frequency[c]						
T12I+*S443T*	0.03	0	1	1	NA / NA	NA / NA
A23S+T122A	0.07	0	1	1	NA / 1.0	NA / 1.0
A23S+T122A+*E378D*	NA	1	0	1	NA / 1.0 / NA	NA / 1.0 / NA
A23S+T122A+K551R	NA	0	1	1	NA / 1.0 / NA	NA / 1.0 / NA
T122A+K123Q+*I384T*	2.75	0	1	1	1.0 / NA / 2.4	1.0 / NA / 1.3
Variants seen at ≥2% to <25% frequency						
K42R+**N67T**+A74T+L78F+*S169N*+**D200N**+**K201N**+**K209fs** +**K209fs**+*S213R*+*N228S*+*V247L*[d]	NA	0	1	1	S213R: 2.2 Others: NA	S213R: 2.6 Others: NA

Source: pages 35-48, Trial 04 virology report and FDA analysis
[a] There were no subjects in this subset with MA RSV LRTI and hospitalization
[b] Data for individual substitutions
[c] All substitutions detected at ≥94% frequency
[d] All substitutions seen at < LLOQ of NGS assay for RSV A (4%), except N228S (4.6%) and V247L (5.3%)
Bold substitutions: occur within of nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: EC50, 50% effective concentration; fs, frameshift; LLOQ, lower level of quantification; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available; NGS, next generation sequencing

18.6.6. Analysis of RSV B Resistance Analysis Population in Trial 04

The number of subjects in the resistance analysis population for Trial 04 who were infected with RSV B variants are shown in Table 154 (through Day 150 postdose), Table 156 (Day 151 to Day 360 postdose), and Table 157 (Day 361 to Day 511 postdose). Phenotypic data and numbers of subjects with individual substitutions through Day 150 are shown in Table 155.

As noted for surveillance studies (Section 18.1), compared with the reference isolate for RSV B from 2013 (Netherlands RSV B/13-1273), the three substitutions K191R, I206M and Q209R, became predominant in recent years, with F15L, A103V, L172Q and S173L substitutions also seen commonly since 2015. Hence, most variants identified at ≥25% frequency in clinical studies contained all or some of these substitutions. The double substitution in the nirsevimab binding site, I206M+Q209R, became dominant in 2017, and was seen in 66% of RSV B isolates circulating from 2015 to 2021 and in most variants seen at ≥25% frequency in Trial 04. The double substitution did not have reduced susceptibility (<5-fold) to nirsevimab in a recombinant RSV assay (Table 155), although I206M substitution alone caused a 5-fold reduction in susceptibility to nirsevimab but was rarely seen in the absence of Q209R in surveillance studies.

Other variants which had substitutions in the nirsevimab binding site included ones harboring I206M+Q209R+S211N substitutions, and ones with L204S+I206M+Q209R+S211N substitutions. The I206M+Q209R+S211N substitutions were seen through Day 150 and in the Day 151 to 360 period, and at 1.1% frequency in surveillance studies but more frequently in 2021 (28.6%), at a time when Trial 04 was in progress. These substitutions together did not confer reduced susceptibility (<5-fold) to nirsevimab or palivizumab. The L204S+I206M+Q209R+S211N substitutions seen only through Day 150 have not been detected concurrently in surveillance studies, and the Applicant is in the process of evaluating them together phenotypically, as well as the L204S substitution on its own.

One subject harbored a variant with K272R substitution, which occurs in the palivizumab binding site, at a position associated with resistance to palivizumab. This substitution was seen rarely in surveillance studies and is in the process of being evaluated phenotypically.

Additional substitutions in the nirsevimab binding sites seen at low frequency (<25%) through Day 150 only, included I64T+K68E, K65E+N200Y, and N208I, in one nirsevimab-treated subject each (). None of these three subjects were hospitalized for RSV. Of these substitutions, I64T and K68E conferred reduced susceptibility to nirsevimab individually (>496-fold and >283-fold, respectively) and together (>280-fold) (Table 155). The other substitutions, K65E, N200Y and N208I, are in the process of being evaluated phenotypically; notably, K65E was only seen at < LLOQ (5%) frequency of the NGS assay, so may not be important.

There were no variants which were clearly associated with nirsevimab-treatment or hospitalization. Most variants were identified in single subjects, in both nirsevimab and placebo groups, so limited conclusions can be drawn regarding individual substitutions. Overall, there were no individual substitutions or specific amino acid positions within or outside the nirsevimab binding sites which were significantly increased in frequency in nirsevimab-treated subjects compared with placebo, or in hospitalized subjects compared with nonhospitalized subjects.

Of the individual substitutions with available phenotypic data seen in the extracellular domain of F protein but outside the nirsevimab binding sites (A103V, L172Q, S173L, K191R, S330T) none had reduced susceptibility (<5-fold) to nirsevimab (Table 155); the Applicant is in the process of assessing the remaining substitutions seen in the extracellular domain, including the ones seen at low frequency (D356N, I475M, Y477H, seen through Day 150, and K87R seen from Day 151 to Day 360 postdose).

Table 154. Number of Subjects in Trial 04 With MA RSV LRTI[a], Infected With RSV B Variants Harboring F Protein Substitutions Through Day 150 Postdose

Amino Acid Substitutions	Prevalence (%)	Placebo N=29 [12]	Nirsevimab N=10 [2]	Total N=39 [14]
Variants seen at ≥25% frequency[b]				
I11L+F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	NA	1	0	1
F12I+F15L+T91N+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	NA	1	0	1
F12I+F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+***K272R***+*S389P*	NA	0	1	1
F12I+F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	0.04	5 [2]	3	8 [2]
F12I+F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**L204S**+**I206M**+**Q209R**+**S211N**+*S389P*	NA	0	1	1
F12I+F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*+*S436P*	NA	1 [1]	0	1 [1]
F15L+A16V+*A103V*+*L172Q*+*S173L*	1.18	1 [1]	0	1 [1]
F15L+N18S+L22P+*A103V*+A111V+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**+*K327E*+A529V	NA	1 [1]	0	1 [1]
F15L+*A103V*+N116S+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	0.18	1	0	1
F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*V239I*+*S389P*	NA	1 [1]	0	1 [1]
F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*V365I*+*S389P*	NA	1	0	1
F15L+*A103V*+*L172Q*+*S173fs*[c]+*T174fs*[c]+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	NA	0	2 [1]	2 [1]
F15L+*A103V*+*L172Q*+*S173fs*[c]+*T174fs*[c]+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*+*T522A*	NA	1 [1]	0	1 [1]
F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*P376S*+*S389P*	NA	1	0	1
F15L+*A103V*+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	0.61	8 [5]	2	10 [5]
F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	40.71	3	1 [1]	4 [1]
A103V+*L172Q*+*S173L*+*S190N*+*K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	NA	2	0	2
A103V+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**[d]	0.04	1	0	1

Amino Acid Substitutions	Prevalence (%)	Placebo N=29 [12]	Nirsevimab N=10 [2]	Total N=39 [14]
Variants seen at ≥4% to <25% frequency				
I64T+K68E+*Y117H*[e]	NA	0	1	1
K65E[e]**+N200Y**	NA	0	1	1
N208I	NA	0	1	1
D356N[e]	NA	1 [1]	0	1 [1]
I475M	NA	1	0	1
Y477H	NA	1	0	1

Source: page 32-50, Trial 04 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Phenotypic data for individual substitutions shown in Table 18.4.1.6. Most substitutions detected at ≥98%, except S436P (85%)
[c] Applicant indicated that S173fs and T174fs were listed as frameshifts by the sequencing vendor, but further analysis of FASTQ data showed an insertion and deletion within the same codon resulting in S173L substitution
[d] Only variant assessed phenotypically; no change (<5-fold) in susceptibility to nirsevimab or palivizumab
[e] Observed at < LLOQ of NGS assay for RSV B (5%)
Bold substitutions: occur within of nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Bold, italicized substitutions: occur in palivizumab binding site (amino acid residues 262-275)
Abbreviations: fs, frameshift; LLOQ, lower level of quantification; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available; NGS, next generation sequencing

Table 155. Phenotypic Data and Prevalence of Individual and Concurrent Substitutions Seen in RSV B Variants From Subjects in Trial 04 With MA RSV LRTI Through Day 150

Amino Acid Substitutions	Prevalence (%)	Placebo (N=29)	Nirsevimab (N=10)	Total (N=39)	EC_{50} Fold Change	
					Nirsevimab	Palivizumab
Variants seen at ≥25% frequency						
I11L	NA	1	0	1	NA	NA
F12I	0.07	7	5	12	NA	NA
F15L	99.57	26	10	36	1.0	1.0
A16V	1.57	1	0	1	NA	NA
N18S	0.21	1	0	1	NA	NA
L22P	1.68	1	0	1	NA	NA
T91N	NA	1	0	1	NA	NA
A103V	98.5	29	10	39	0.9	1.8
A111V	0.07	1	0	1	NA	NA
N116S	0.21	1	0	1	NA	NA
L172Q	98.61	29	10	39	1.1	2.3

Amino Acid Substitutions	Prevalence (%)	Placebo (N=29)	Nirsevimab (N=10)	Total (N=39)	EC_{50} Fold Change	
					Nirsevimab	Palivizumab
S173fs[a]	NA	1	2	3	NA	NA
S173L	98.36	28	8	36	0.9	1.9
T174fs[a]	NA	1	2	3	NA	NA
S190N	2.79	22	9	31	NA	NA
K191R	68.61	28	10	38	1.3	2.7
L204S	NA	0	1	1	NA	NA
I206M	68.89	28	10	38	5.0	2.0
Q209R	68.18	28	10	38	0.5	3.1
S211N	1.14	22	9	31	1.2	1.9
V239I	0.14	1	0	1	NA	NA
K272R	0.04	0	1	1	NA	NA
K327E	0.04	1	0	1	NA	NA
S330T	NA	0	1	1	0.8	1.9
V365I	NA	1	0	1	NA	NA
P376S	NA	1	0	1	NA	NA
S389P	1.07	22	9	31	NA	NA
S436P	NA	1	0	1	NA	NA
T522A	0.07	1	0	1	NA	NA
A529V	0.93	1	0	1	NA	NA
I206M+Q209R	66	6	1	7	0.2	1.3
I206M+Q209R+S211N	1.14[b]	22	8	30	0.5	3.7
L204S+I206M+Q209R+S211N	NA	0	1	1	NA	NA

Amino Acid Substitutions	Prevalence (%)	Placebo (N=29)	Nirsevimab (N=10)	Total (N=39)	EC_{50} Fold Change	
					Nirsevimab	Palivizumab
Variants seen at ≥4% to <25% frequency						
I64T	NA	0	1	1	>496.3	5.2
K65E[c]	NA	0	1	1	NA	NA
K68E	NA	0	1	1	>283.4	2.1
Y117H[c]	0.14	0	1	1	NA	NA
N200Y	NA	0	1	1	NA	NA
N208I	NA	0	1	1	NA	NA
D356N[c]	0.11	1	0	1	NA	NA
I475M	NA	1	0	1	NA	NA
Y477H	0.07	1	0	1	NA	NA
I64T+K68E	NA	0	1	1	>280.4	2.0

Source: pages 22, 38-50, Trial 04 virology report and FDA analysis
[a] Applicant indicated that S173fs and T174fs were listed as frameshifts by the sequencing vendor, but further analysis of FASTQ data showed an insertion and deletion within the same codon resulting in S173L substitution
[b] I206M+Q209R+S211N was observed in 28.57% of sequences in the 2021 calendar year in the combined INFORM/OUTSMART global surveillance studiesc
[c] Observed at < LLOQ of NGS assay for RSV B (5%)
Bold substitutions: occur within nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Bold, italicized substitutions: occur in palivizumab binding site (amino acid residues 262-275)
Abbreviations: EC_{50}, 50% effective concentration; LLOQ, lower level of quantification; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available; NGS, next generation sequencing

Table 156. Number of Subjects in Trial 04 With MA RSV LRTI[a], Infected With RSV B Variants Harboring F Protein Substitutions From Day 151 to 360 Postdose

Amino Acid Substitutions[b]	Prevalence (%)	Placebo N=0	Nirsevimab N=6	Total N=6
Variants seen at ≥25% frequency[c]				
F15L+*A103V*	NA	0	1	1
F15L+*A103V+L172Q+S173L+S190N+K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	0.61	0	1	1
F15L+*A103V+L172Q+S173L+K191R*+**I206M**+**Q209R**	40.71	0	2	2
F15L+*A103V+L172Q+S173L+K191R*+**I206M**+**Q209R**+*S330T*	NA	0	1	1
F15L+*A103V+S389P*	NA	0	1	1

Amino Acid Substitutions[b]	Prevalence (%)	Placebo N=0	Nirsevimab N=6	Total N=6
Variants seen at ≥5% to <25% frequency				
K87R	NA	0	1	1

Source: pages 35-48, Trial 04 virology report and FDA analysis
[a] There were no subjects in this subset with MA RSV LRTI and hospitalization
[b] Phenotypic data for individual substitutions, except K87R (NA), shown in Table 18.4.1.6
[c] All substitutions detected at ≥99% frequency
Bold substitutions: occur within of nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available

Table 157. Number of Subjects in Trial 04 With MA RSV LRTI[a], Infected With RSV B Variants Harboring F Protein Substitutions From Day 361 to 511 Postdose

Amino Acid Substitutions[b]	Prevalence (%)	Placebo N=1	Nirsevimab N=3	Total N=4
Variants seen at ≥25% frequency[c]				
F15L+*A103V+N120S+L172Q+S173L+K191R*+**I206M+Q209R**	NA	0	1	1
F15L+*A103V+L172Q+S173L+K191R*+**I206M+Q209R**	40.71	0	2	2
F15L+*A103V+L172Q+S173L+K191R*+**I206M+Q209R+S330T**	NA	1	0	1

Source: pages 36-52, Trial 04 virology report and FDA analysis
[a] There were no subjects in this subset with MA RSV LRTI and hospitalization
[b] Phenotypic data for individual substitutions, except N120S (NA), shown in Table 18.4.1.6; no substitutions were seen at <25% frequency
[c] All substitutions were detected at ≥99% frequency
Bold substitutions: occur within of nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available

Variants seen in subjects with nonprotocol-defined MA LRTI or unscheduled RSV events Table 158 shows the substitutions seen at ≥25% frequency in subjects with nonprotocol-defined MA LRTI or unscheduled RSV events, for both RSV A and RSV B infections.

In general, individual variants were only seen in single subjects, with the exception of an RSV B variant harboring I206M+Q209R+S211N substitutions in the nirsevimab binding site, seen in 2 placebo subjects with nonprotocol-defined RSV LRTI. Hence, overall, there is no clear evidence of particular variants, individual substitutions or amino acid positions being associated with nirsevimab treatment.

Table 158. Number of Subjects in Trial 04 With Nonprotocol-Defined MA RSV LRTI, or Unscheduled RSV Events, Infected With RSV Variants Harboring F Protein Substitutions at ≥25% Frequency

Amino Acid Substitutions[a]	Prevalence (%)	Placebo	Nirsevimab	Total
RSV A, nonprotocol defined RSV LRTI through 150 days postdose				
Number of subjects	-	7	5	12
No change	NA	4	0	4
T12I	2.61	1	1	2
L15F+*S24F*+T122A	NA	0	1	1
A103T	0.21	1	0	1
N116S	0.1	1	0	1
T122A+T125A	0.17	0	1	1
S276N	1.04	0	1	1
A355V	0.03	0	1	1
RSV A, unscheduled RSV events through 150 days postdose				
Number of subjects	-	7	5	12
No change	NA	1	1	2
T12I	2.61	0	1	1
T12I+*S330T*+A552T	NA	1	0	1
T13A	3.72	1	0	1
T13A+*N325Y*	NA	1	0	1
L15F+T122A+*S362L*	NA	1	0	1
A103T+T122A	1.25	1	0	1
A103T+T122A+A540V	NA	0	1	1
S276N	1.04	0	1	1
K419N	NA	1	0	1
A518V	0.42	0	1	1
RSV A, nonprotocol defined RSV LRTI Day 151 to 360 postdose				
Number of subjects	-	2	2	4
T12I+L20F	NA	1	0	1
I57V+L111I+T122A	NA	0	1	1
T122A+K123Q+*I384T*	2.75	1	1	2
RSV A, unscheduled RSV events Day 151 to 360 postdose				
Number of subjects	-	1	1	2
L111I+T122A+*E378D*	NA	0	1	1
T122A+K123Q+*I384T*	2.75	1	0	1

Amino Acid Substitutions[a]	Prevalence (%)	Placebo	Nirsevimab	Total
RSV A, nonprotocol defined RSV LRTI Day 361 to 511 postdose				
Number of subjects	-	1	0	1
T122A+K123Q+*I384T*	2.75	1	0	1
RSV A, unscheduled events Day 361 to 511 postdose				
Number of subjects	-	0	1	1
T12I+*S105N+E497D*	NA	0	1	1
RSV B, nonprotocol defined RSV LRTI through 150 days postdose[b]				
Number of subjects		5	0	5
F12I+F15L+*A103V+L172Q+S173L+S190N+K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	0.04	2	0	2
F15L+*A103V+L172Q+S173L+S190N+K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	0.61	1	0	1
F15L+*A103V+L172Q+S173L+S190N+K191R*+**I206M**+**Q209R**+**S211N**+*S389P+N437I*	NA	1	0	1
F15L+*A103V+L172Q+S173L+K191R*+**I206M**+**Q209R**+**S330A**	NA	1	0	1
RSV B, unscheduled events through 150 days postdose[b]				
Number of subjects	-	2	1	3
F12I+F15L+*A103V+L172Q+S173L+S190N+K191R*+**I206M**+**Q209R**+**S211N**+S389P	0.04	1	0	1
F15L+A19T+*A103V+L172Q+S173L+S190N+K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	NA	0	1	1
F15L+*N99T+A103V+L172Q+S173fs*[c]*+T174fs*[c]*+S190N+K191R*+**I206M**+**Q209R**+**S211N**+*S389P*	NA	1	0	1

Source: pages 55-60, Trial 04 virology report and FDA analysis
[a] Available phenotypic data for individual substitutions shown in Table 18.4.1.2 (RSV A) and Table 18.4.1.6 (RSV B)
[b] No nonprotocol defined RSV LRTI or unscheduled events seen for RSV B from Day 151 to 360 or Day 361 to 511 postdose
[c] Applicant indicated that S173fs and T174fs were listed as frameshifts by the sequencing vendor, but further analysis of FASTQ data showed an insertion and deletion within the same codon resulting in S173L substitution.
Bold substitutions: occur within of nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available

18.6.7. Clinical Virology Resistance Analyses for Trial 05

A link to the Trial 05 Virology Report can be found here: Trial 05 virology report.

Trial 05 is an ongoing phase 2/3, randomized, double-blind, palivizumab-controlled trial to evaluate the safety, PK, ADA response and descriptive efficacy of nirsevimab in high-risk infants eligible to receive palivizumab when entering their first or second RSV season. The primary objective is to evaluate the safety and tolerability of nirsevimab compared to palivizumab when administered to preterm infants entering their first RSV season and children with chronic lung disease (CLD) or chronic heart disease (CHD) entering their first and second RSV season. Details of the protocol and trial results are reviewed in Section 6.2.4.

The resistance analysis population for Trial 05 included all subjects who had MA RSV LRTI or hospitalization due to RSV illness, and evaluable NGS data. In the palivizumab and nirsevimab groups for Season 1, there were 10/309 (3.2%) and 12/616 (1.9%) subjects, respectively, in the resistance analysis population. In the resistance analysis population for Season 2, there were 0/42 (0.0%) subjects in the palivizumab/palivizumab (i.e., Season 1/Season 2 treatment) group, 1/40 (2.5%) subjects in the palivizumab/nirsevimab group, and 1/180 (0.6%) subjects in the nirsevimab/nirsevimab group. Table 159 summarizes the number of subjects in the resistance analysis population with evaluable sequence data available by RSV subtype, case definition and reporting period (including both Season 1 and Season 2).

Table 159. Number of Subjects in the Resistance Analysis Population for Trial 05 (Overall Population), for Whom an Evaluable RSV Sequence Was Available

	Season 1[c]				Season 2[d]		
	Through 150 Days Postdose		From Day 151 to 360 Postdose		Through 360 Days Postdose		
Case Definition[a,b] RSV Subtype	**Palivizumab (N=309)**	**Nirsevimab (N=616)**	**Palivizumab (N=309)**	**Nirsevimab (N=616)**	**Palivizumab / Palivizumab (N=42)**	**Palivizumab / Nirsevimab (N=40)**	**Nirsevimab / Nirsevimab (N=180)**
Primary - MA RSV LRTI	3	4	4	7	0	1	0
RSV A	1	4	3	3	0	1	0
RSV B	2	0	1	4	0	0	0
Secondary - MA RSV LRTI hospitalization	2	2	1	3	0	0	0
RSV A	0	2	1	1	0	0	0
RSV B	2	0	0	2	0	0	0
Overall[a]	5	4	5	8	0	1	1
RSV A	3	4	3	3	0	1	0
RSV B	2	0	2	5	0	0	1

Source: page 19, Trial 05 virology report
[a] Subjects were counted once for each efficacy category and each sampling period regardless of the number of events. Overall category sums the subjects meeting primary, secondary, and exploratory case definitions.
[b] Numbers of subjects infected with RSV A and B include only those for whom an evaluable viral sequence was available.
[c] Preterm and CLD/CHD Cohorts
[d] ≥12 and ≤24 months with CLD/CHD who received nirsevimab or palivizumab in Season 1
Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects

18.6.8. Analysis of RSV A Resistance Analysis Population in Trial 05

The number of subjects in Trial 05 with MA RSV LRTI and RSV A variants identified are shown in Table 160 (through Day 150 postdose, Season 1), and Table 161 (Day 151 to Day 360 postdose, Season 1). Only one subject treated with palivizumab/nirsevimab was seen in Season 2 with RSV A (Table 159) and harbored a variant with T12I substitution (outside extracellular region; phenotypic analysis ongoing).

There were no major (i.e., ≥25% frequency) variants through Day 360, Season 1, with substitutions in the nirsevimab binding sites. One subject treated with palivizumab was infected through Day 150 with a variant harboring S275L substitution, which is associated with palivizumab resistance (AstraZeneca 1998), and is in the process of being assessed for susceptibility to nirsevimab. One subject treated with palivizumab was infected through Day 150 with a variant harboring S275L substitution, which is associated with palivizumab resistance (AstraZeneca 1998), and is in the process of being assessed for susceptibility to nirsevimab. A minor variant with a mix of K272M and K272T substitutions was also seen in one palivizumab-treated subject; both these substitutions showed loss of susceptibility to palivizumab, but not to nirsevimab.

The few other variants seen through Day 360, Season 1, with substitutions outside the nirsevimab and palivizumab binding sites, were only seen in one or two subjects each and so not clearly associated with either palivizumab or nirsevimab treatment or with hospitalization. The two substitutions with phenotypic data occurring outside nirsevimab binding sites but in extracellular regions, A103T and I384T, did not have reduced susceptibility (<5-fold) to nirsevimab or palivizumab (note that A103T is present in the RSV reference strain, and is assigned a fold-change of 1.0). Other substitutions are being assessed phenotypically, including the two substitutions, A147V and W341R, which occur in the extracellular regions and were seen in minor variants from Day 151 to Day 360 (Table 161).

Table 160. Number of Subjects in Trial 05 With MA RSV LRTI[a], Infected With RSV A Variants Harboring F Protein Substitutions Through Day 150 Postdose, Season 1

Amino Acid Substitutions	Prevalence (%)	Palivizumab N=1 [0]	Nirsevimab N=4 [2]	Total N=5 [2]	EC_{50} Fold Change[a,b]	
					Nirsevimab	Palivizumab
Variants seen at ≥25% frequency						
A103T+T122A+N126Y[c]	NA	0	1 [1]	1 [1]	1.0 / 1.0 / NA	1.0 / 1.0 / NA
E110G	NA	0	2	2	NA	NA
M115T+T122A[c]	0.49	0	1 [1]	1 [1]	NA / 1.0	NA / 1.0
S275L	NA	1	0	1	NA	NA
Variants seen at ≥4% to <25% frequency						
K272M*+*K272T	NA	1	0	1	2.7 / 1.1	>179.9 />213.9

Source: pages 32-43, Trial 05 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Data for individual substitutions
[c] Subjects with MA RSV LRTI and hospitalization
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Bold, italicized substitutions: occur in palivizumab binding site (amino acid residues 262-275)
Abbreviations: EC_{50}, 50% effective concentration; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available

Table 161. Number of Subjects in Trial 05 With MA RSV LRTI[a], Infected With RSV A Variants Harboring F Protein Substitutions From Day 151 to 360 Postdose, Season 1

Amino Acid Substitutions	Prevalence (%)	Palivizumab N=3 [1]	Nirsevimab N=3 [1]	Total N=6 [2]	EC_{50} Fold Change[a,b]	
					Nirsevimab	Palivizumab
No change	-	0	1 [1]	1 [1]	-	-
Variants seen at ≥25% frequency						
A103T+T122A	1.25	0	2	2	1.0 / 1.0	1.0 / 1.0
M115T+T122A	0.49	1 [1]	0	1 [1]	NA / 1.0	NA / 1.0
T122A+K123Q+*I384T*	2.75	1	0	1	1.0 / NA / 2.4	1.0 / NA / 1.3
S377N	0.31	1	0	1	NA	NA

Amino Acid Substitutions	Prevalence (%)	Palivizumab N=3 [1]	Nirsevimab N=3 [1]	Total N=6 [2]	EC_{50} Fold Change[a,b]	
					Nirsevimab	Palivizumab
Variants seen at ≥2% to <25% frequency						
A147V[c]	NA	1	0	1	NA	NA
W341R	NA	0	1	1	NA	NA

Source: pages 41-44, Trial 05 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Data for individual substitutions
[c] Observed at < LLOQ of NGS assay for RSV A (4%)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: EC_{50}, 50% effective concentration; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available

18.6.9. Analysis of RSV B Resistance Analysis Population in Trial 05

The numbers of subjects in the resistance analysis population for Trial 05 who were infected with RSV B variants are shown in Table 162 (through Day 150 postdose, Season 1), and Table 163 (Day 151 to Day 360 postdose, Season 1). Available phenotypic data for individual substitutions are shown in the previous section (Table 155).

There were no RSV B infections in nirsevimab-treated subjects through Day 150. Two variants were seen in palivizumab-treated subjects at >25% frequency through Day 150, both of whom were hospitalized. Both variants harbored the I206M+Q209R double substitution. In addition, a K272T substitution in the palivizumab binding site was seen at low frequency (<LLOQ of NGS assay) in a palivizumab-treated subject.

In the Day 151 to Day 360 time period, 5 variants were seen at >25% frequency, 4 of which were in nirsevimab-treated subjects. The I206M+Q209R substitutions were concurrent with S211N in four of these variants, occurring in one palivizumab-treated and 3 nirsevimab-treated subjects; these three substitutions together had 29% prevalence in 2021, but do not confer reduced susceptibility (<5-fold) to nirsevimab or palivizumab (Table 155). Notably, the variant with only F15L and A103V substitutions according to NGS data was shown to have additional substitutions with Sanger sequencing: F15L+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P; it is not clear why there was a difference with the assay readouts in this case.

Overall, there were too few subjects with variants to draw conclusions with respect to association with nirsevimab treatment or hospitalization.

Table 162. Number of Subjects in Trial 05 With MA RSV LRTI[a], Infected With RSV B Variants Harboring F Protein Substitutions Through Day 150 Postdose, Season 1

Amino Acid Substitutions[b]	Prevalence (%)	Palivizumab N=2 [2]	Nirsevimab N=0	Total N=2 [2]
Variants seen at ≥25% frequency				
L4P+F12L+F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**+I525V	NA	1 [1]	0	1 [1]
S9I+F15L+*A103V*+*L172Q*+*S173L*+*K191R*+**I206M**+**Q209R**	0.04	1 [1]	0	1 [1]
Variants seen at ≥3% to <25% frequency				
K272T[c]	NA	1 [1]	0	1 [1]
E294V[c]	NA	1 [1]	0	1 [1]

Source: pages 33-43, Trial 05 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Available phenotypic data for individual substitutions, other than L4P, S9I, F12L, K272T, E294V and I525V (all NA), shown in Table 18.4.1.6
[c] Observed at < LLOQ of NGS assay for RSV B (5%)
Bold substitutions: occur within of nirsevimab binding sites (amino acid residues 62-69 and 196-212)
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Bold, italicized substitutions: occur in palivizumab binding site (amino acid residues 262-275)
Abbreviations: LLOQ, lower limit of quantification; MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available; NGS, next generation sequencing

Table 163. Number of Subjects in Trial 05 With MA RSV LRTI[a], Infected With RSV B Variants Harboring F Protein Substitutions From Day 151 to 360 Postdose, Season 1

Amino Acid Substitutions[b]	Prevalence (%)	Palivizumab N=1	Nirsevimab N=4 [2]	Total N=5 [2]
Variants seen at ≥25% frequency[c]				
F12I+F15L+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P	0.04	1	0	1
F15L+R42K+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P	0.25	0	1 [1]	1 [1]
F15L+A103V	NA	0	1	1
F15L+A103V+L172Q+S173fs[d]+T174fs[d]+S190N+K191R+I206M+Q209R+S211N+S389P	NA	0	1	1
F15L+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P	0.61	0	1 [1]	1 [1]

Source: pages 34-44, Trial 05 virology report and FDA analysis
[a] Number of subjects with MA RSV LRTI and hospitalization shown in square brackets
[b] Available phenotypic data for individual substitutions, other than R42K (NA), shown in Table 18.4.1.6
[c] No substitutions were seen at <25% frequency
Applicant indicated that S173fs and T174fs were listed as frameshifts by the sequencing vendor, but further analysis of FASTQ data showed an insertion and deletion within the same codon resulting in S173L substitution
Italicized substitutions: occur outside of nirsevimab binding sites (amino acid residues 62-69 and 196-212) but within extracellular domain of F protein (amino acid residues 24-109 and 137-524)
Abbreviations: MA RSV LRTI, medically attended respiratory syncytial virus lower respiratory tract infection; N, number of subjects; NA, not available

Variants Seen in Subjects With Nonprotocol-Defined MA LRTI or Unscheduled RSV Events

No subjects with nonprotocol defined RSV LRTI (RSV A or RSV B) through 150 days postdose, Season 1, or with RSV A from Days 151 to 360 postdose, Season 1. One subject with nonprotocol defined RSV LRTI and RSV B from Days 151 to 360 postdose, Season 1, with a major variant (≥25%): F15L+A103V+L172Q+S173L+S190N+K191R+**I206M**+**Q209R**+**S211N**+S389P (no phenotypic data for variant). There was one subject with an unscheduled RSV event and RSV A through 150 days postdose, Season 1, with a major variant (≥25%): M526I (no phenotypic data), and one subject with an unscheduled RSV event and RSV B from Days 151 to 360 postdose, Season 1: F15L+A103V+L172Q+S173L+P312H (no phenotypic data for variant).

In Season 2, one subject treated with nirsevimab in Season 1 and Season 2 met an exploratory case definition of RSV infection and harbored a variant with F15L+A103V+L172Q+S173fs+T174fs+S190N+K191R+**I206M**+**Q209R**+**S211N**+S389P substitutions.

18.6.10. Clinical Virology Resistance Analyses for Trial 08

Trial 08 virology report.

Trial 08 is an ongoing single-dose trial assessing the safety and tolerability, pharmacokinetics, occurrence of ADA, and descriptive efficacy of nirsevimab in immunocompromised children ≤24 months of age at the time of dose administration, who are entering their first or second RSV season. Details of the protocol are shown in Table 3. For this trial, 60 subjects were in the ITT population, with 36 treated with nirsevimab (50/100 mg) in the first year of life, and 24 treated with nirsevimab (200 mg) in the second year of life. In the resistance analysis population, there were a total of three subjects with NGS data, of whom 2 were treated with nirsevimab in the first year of life, and one with nirsevimab in the second year of life.

No subjects were identified who were infected with RSV A. The 3 remaining subjects were infected with RSV B and met exploratory case definitions. One subject had a nonprotocol defined RSV LRTI event through Day 150 postdose with a major variant (≥25%): F15L+R42K+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P (no phenotypic data for variant). The other two subjects had an RSV LRTI event from Day 151 to 360, both with major variants (≥25%): F15L+A103V+N116S+L172Q+S173L+K191R+I206M+Q209R, and F15L+A103V+L172Q+S173L+K191R+I206M+Q209R, respectively (no phenotypic data for these variants).

18.7. Pooled Analysis of Variant Sequences in Clinical Trials of Nirsevimab

An analysis was conducted of all RSV A and RSV B variants, F protein substitutions, and amino acid positions with changes seen in pooled clinical trials of nirsevimab (i.e., combined resistance analysis populations from Trial 03, Trial 04, Trial 05, Trial 08). The goal was to identify variants or substitutions/positions to recommend for prioritizing in phenotypic analyses.

The Applicant prioritized substitutions for phenotypic testing based on whether they were identified up to Day 150 following nirsevimab dosing, and if they weren't identified in the RSV isolates from the placebo group. Substitutions occurring in the external regions of F protein (amino acids 62-69 and 196-212) were prioritized over ones outside of these regions. Many of the substitutions identified in the external regions are in the process of being evaluated phenotypically. The FDA analysis was inclusive of all breakthrough infections across all clinical trials, regardless of the time period when they occurred, the dose of nirsevimab administered, and the frequency at which individual substitutions were detected by NGS. For identifying individual and concurrent substitutions to recommend for prioritizing, the following criteria were used:

- Variant or individual substitution with no phenotypic data, seen in ≥2 nirsevimab-treated subjects, or ≥1 subject if variant has substitution in or adjacent to nirsevimab binding site or individual substitution/position occurs in or adjacent to binding site

18.7.1. RSV A F Protein Variants and Substitutions in Pooled Clinical Trials of Nirsevimab

In a pooled analysis of RSV A F protein sequences, there were 31 unique variants which were identified across trials in nirsevimab-treated subjects (n=66) harboring at least 2 concurrent substitutions in the F protein compared with the reference sequence (Table 164). Many of the individual substitutions have been evaluated phenotypically by the Applicant (shown in bold font in Table 164, and listed in Table 165).

Overall, there was no clear evidence that particular variants were more likely to be detected in nirsevimab-treated subjects compared with placebo, although there was one variant with L119I+K209R substitutions seen in 2 nirsevimab-treated subjects and no placebo subjects, and another variant with T122A+K123Q+I384T substitutions seen in 8 nirsevimab-treated subjects and 5 placebo subjects. The K209R substitution occurs in the nirsevimab binding site but does not confer reduced susceptibility (<5-fold) to nirsevimab when tested individually.

Table 164. RSV A Variants With Concurrent F Protein Substitutions Seen in Nirsevimab-Treated Subjects in Pooled Clinical Trials of Nirsevimab[a]

Nirsevimab N=66	Placebo N=79	F Protein Amino Acid Substitution								
1	0	T12I	S443T							
1	0	A23S	T122A[b]							
1	1	A23T	**A102S**							
1	0	A23T	**S190N**							
1	0	**S25N**	V127A							
1	0	D84N	G329R							
1	2	A103T[b]	T122A[b]							
1	1	M115T	T122A[b]							
2	0	L119I	**K209R[c]**							
1	0	K123E	**S213R**							
1	0	S276N[b]	*T400I*							
1	0	Q354R	D479N							
1	0	T8I	*N228S*	*V247L*						
1	0	T12I	S105N[b]	E497D						
1	0	L15F	S24F	T122A[b]						
1	0	L15F	T122A[b]	K419E						
1	0	A23S	T122A[b]	W341R						
1	0	I57V	L111I	T122A[b]						
1	0	A103T[b]	T122A[b]	N126Y						
1	0	A103T[b]	T122A[b]	S255N						
1	0	A103T[b]	T122A[b]	W341R						
1	0	A103T[b]	T122A[b]	A540V						
1	0	L111I	T122A[b]	E378D						
8	5	T122A[b]	K123Q	**I384T**						
1	0	T13A	*I384V[b]*	N515H	A540S[b]					
1	0	*I384V[b]*	N515H	A518V	A540S[b]					
1	0	T12I	*P112L*	S330T	V360A	A552T				
1	0	L15F	L119F	T122A[b]	*I384V[b]*	N515H	A540S[b]			
1	2	T8A[b]	L20F[b]	*I79M*	S105N[b]	N124K[b]	***S213R***	S276N[b]		
1	0	T8A[b]	L20F[b]	*I79M*	S105N[b]	T122A[b]	N124K[b]	T125A	**S213R**	S276N[b]
1	0	A23S	*K42R*	*N67T[c]*	*A74T*	*L78F*	T122A[b]	*S169N*	*D200N[c]*	*K201N[c]*
		K209fs	*K209fs*	***S213R***	N228S	V247L	K551R			

Source: FDA analysis
[a] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions.
[b] Harbored by reference used for recombinant RSV neutralization assay
[c] Substitution within nirsevimab binding sites (F protein amino acids 62-69 and 196-212)
Bold substitutions: Phenotypic data for individual substitutions available (excluding those in reference); see Table 18.7.2
Italicized substitutions: Detected at < LLOQ for NGS assay (4% for RSV A). The linkage of these substitutions to others detected concurrently is not clear.
Abbreviations: LLOQ, lower limit of quantification; N, number of subjects; NGS, next generation sequencing; RSV, respiratory syncytial virus

Table 165. Individual RSV A F Protein Substitutions With Phenotypic Data in Pooled Clinical Trials of Nirsevimab

Substitution	Number of Subjects[a]		EC_{50} Fold Change	
	Nirsevimab N=66	Placebo N=79	Nirsevimab	Palivizumab
P4L[b]	0	1	1.0	1.0
T8A[b]	2	2	1.0	1.0
L20F[b]	2	4	1.0	1.0
S25N	1	0	2.5	1.9
Y33H	0	1	1.4	1.8
V76I	1	0	1.2	0.9
S99N	0	1	**5.3**	2.1
A102S	1	1	1.4	0.9
A103T[b]	5	3	1.0	1.0
A103V	1	0	2.2	1.5
S105N[b]	3	2	1.0	1.0
A107T	2	0	2.1	2.3
T122A[b]	24	15	1.0	1.0
N124K[b]	2	2	1.0	1.0
V144I	0	1	1.1	1.1
S190N	1	0	2.2	2.6
K209R[d]	2	0	0.9	1.0
S213R	4	2	2.6	2.6
K272M[c]	0	0	2.7	**>179.9**
K272T[c]	0	0	1.1	**>213.9**
S276N[b]	4	5	1.0	1.0
N325Y	0	1	1.8	1.5
G329R	1	1	2.1	1.3
Q354R	1	0	2.1	1.9
A355V	1	1	1.7	1.9
E378D	1	1	2.2	1.4
I384T	8	9	2.4	1.3
I384V[b]	3	2	1.0	1.0
A518V	1	0	1.0	1.2
A540S[b]	3	3	1.0	1.0

Source: FDA analysis
[a] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions and regardless of nirsevimab dose
[b] Harbored by reference used for recombinant RSV neutralization assay
[c] Seen in one palivizumab-treated subject each
[d] Substitution within nirsevimab binding sites (F protein amino acids 62-69 and 196-212)
Bold: ≥5-fold change
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

Table 166 lists the individual RSV A substitutions occurring in extracellular regions of F protein which were identified across clinical trials, and the numbers of subjects in whom they were detected. There were 40 substitutions in total, mostly occurring in one nirsevimab-treated or placebo subjects, and there was no evidence that particular substitutions or amino acid positions were associated with nirsevimab treatment.

Table 167 lists the individual RSV A substitutions occurring outside the extracellular regions of F protein which were seen in at least two subjects total for nirsevimab and placebo groups. There were 20 individual substitutions, with no evidence that any were associated with nirsevimab

treatment. The T122A substitution was seen in 24/66 (36%) nirsevimab-treated subjects and 15/79 (19%) placebo subjects, but this is a common substitution seen in 78% of RSV A sequences in 2021 and is included in the neutralization assay reference sequence.

Table 166. RSV A Substitutions in F Protein Extracellular Domains[a], Not Assessed Phenotypically, in Pooled Clinical Trials of Nirsevimab

	# Subjects[b]			# Subjects[b]	
Substitution	**Nirsevimab N=66**	**Placebo N=79**	**Substitution**	**Nirsevimab N=66**	**Placebo N=79**
S24F	1	0	N262D	0	1
K42R	1	0	K272E	0	1
R49K	0	1	S275L[d]	0	0
I57V	1	0	S330T	1	1
N67T[c]	1	0	W341R	2	0
G71S	0	1	V360A	1	0
A74T	1	1	S362L	0	1
L78F	1	0	S377N	0	0
I79M	2	2	T397I	0	1
D84N	1	0	T400I	1	0
A89V	0	1	V406I	0	1
A147V[d]	0	0	K419E[e]	2	1
A149T	0	1	K419N	0	1
N165S	0	1	S443T	1	0
S169N	1	0	V450M	0	1
D200N[c]	1	0	I474T	1	0
K201N[c]	1	0	D479N	1	0
N228S	2	0	D486N	0	1
V247L	2	0	E497D	1	0
S255N	1	0	N515H	3	3

Source: FDA analysis
[a] Extracellular domains of F protein include amino acid residues 24-109 and 137-524
[b] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions.
[c] Substitution within nirsevimab binding sites (F protein amino acids 62-69 and 196-212)
[d] Seen in 1 palivizumab-treated subject each in Trial 05 trial
[e] Recombinant RSV rescue failed
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

Table 167. RSV A Substitutions Outside F Protein Extracellular Domains[a], in ≥2 Subjects, in Pooled Clinical Trials of Nirsevimab

	# Subjects[b]			# Subjects[b]	
Substitution	**Nirsevimab N=66**	**Placebo N=79**	**Substitution**	**Nirsevimab N=66**	**Placebo N=79**
T8A[c]	2	2	L111I	2	0
T12I	7	9	F114S	1	2
T13A	2	4	M115T	1	1
I14V	1	1	L119I	2	0
L15F	3	1	**T122A**[c]	24	15
A17V	1	1	K123Q	8	9
L20F[c]	2	4	**N124K**[c]	2	2

Substitution	# Subjects[b] Nirsevimab N=66	# Subjects[b] Placebo N=79	Substitution	# Subjects[b] Nirsevimab N=66	# Subjects[b] Placebo N=79
A23S	3	1	V127A	1	1
A23T	3	3	**A540S**[c]	1	3
E110G	2	0	A552T	1	1

Source: FDA analysis
[a] Extracellular domain of F protein includes amino acid residues 24-109 and 137-524
[b] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions
[c] Harbored by reference used for recombinant RSV neutralization assay
Bold = phenotypic data available (see Table 18.7.2)
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

18.7.2. RSV B F Protein Variants and Substitutions in Pooled Clinical Trials of Nirsevimab

In a pooled analysis of RSV B F protein sequences, there were 5 unique variants which were identified across trials in nirsevimab-treated subjects (n=50) harboring at least 2 concurrent substitutions in the F protein compared with the reference sequence and seen in at least 2 nirsevimab-treated subjects (Table 168). There were also 19 unique variants seen in at least one nirsevimab-treated subject (Table 169). Some of the concurrent substitutions with phenotypic data are also shown in Table 168, and the individual substitutions which have been evaluated phenotypically are listed in Table 170.

Overall, there was no clear evidence that particular variants or concurrent substitutions were more likely to be detected in nirsevimab-treated subjects compared with placebo. There were a few variants harboring resistance-associated substitutions, including I64T, K68E, N208K, and N208S, and N208I which has not been evaluated (Table 169); each variant was only seen in one nirsevimab-treated subject. Notably, substitutions at positions K68 and N208 were observed in cell culture resistance selection studies (Section 20.7).

Table 168. RSV B Variants With Concurrent F Protein Substitutions in Pooled Clinical Trials of Nirsevimab With Phenotypic Data or Seen in ≥2 Nirsevimab-Treated Subjects

Dosing Group		F Protein Amino Acid Substitution													EC50 Fold Change	
Nirsevimab N=50	**Placebo N=64**	**F15L**	**R42K**	**I64T**	**K68E**	**A103V**	**L172Q**	**S173L**	**S190N**	**K191R**	**I206M**	**Q209R**	**S211N**	**S389P**	**Nirsevimab**	**Palivizumab**
Subjects with variants harboring concurrent substitutions tested in neutralization assay[a]																
33	48										I206M	Q209R			0.2	1.3
17	28										I206M	Q209R	S211N		0.5	3.7
2	0			I64T	K68E						I206M	Q209R			>447	1.2
0	1[b]					A103V	L172Q	S173L		K191R	I206M	Q209R			0.3	2.5
Variants seen in ≥2 nirsevimab-treated subjects[c]																
11	10	F15L				A103V	L172Q	S173L							NA	NA
8	5	F15L				A103V	L172Q	S173L		K191R	I206M	Q209R			NA	NA
8[d]	9	F15L				A103V	L172Q	S173L	S190N	K191R	I206M	Q209R	S211N	S389P	NA	NA
2	0	F15L	R42K			A103V	L172Q	S173L	S190N	K191R	I206M	Q209R	S211N	S389P	NA	NA
2	0	F15L				A103V									NA	NA

Source: FDA analysis
[a] Total number of subjects with concurrent substitutions indicated (i.e., regardless of other substitutions in variants)
[b] Substitutions were assessed concurrently in clinical isolates (Section 20.6)
[c] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions. For clarity, substitutions are arranged to align the most common ones.
[d] Includes 4 subjects with L173fs and L174fs, assumed to be S173L
Bold value: ≥5-fold change
Abbreviations: fs, frameshift; N, number of subjects; NA, not available; RSV, respiratory syncytial virus

Table 169. RSV B Variants Seen in One Nirsevimab-Treated Subject With Concurrent F Protein Substitutions in Pooled Clinical Trials of Nirsevimab[a]

Nirsevimab N=50	Placebo N=64	F Protein Amino Acid Substitution																		
1	0		F15L	K87R		A103V												S389P		
1	1		F15L	A16V		A103V		L172Q	S173L											
1	0		F15L	Y33F		A103V		L172Q	S173L											
1	0		F15L			A103V		L172Q	S173L					N208S[b]						
1	1		F15L			A103V	N116S	L172Q	S173L		K191R		I206M[b]		Q209R[b]					
1	0		F15L			A103V	N120S	L172Q	S173L		K191R		I206M[b]		Q209R[b]					
1	1		F15L			A103V	V127I	L172Q	S173L		K191R		I206M[b]		Q209R[b]					
1	1		F15L			A103V		L172Q	S173L		K191R		I206M[b]		Q209R[b]		V365A			
1	1		F15L			A103V		L172Q	S173L		K191R		I206M[b]		Q209R[b]		S330T			
1	0		F15L			A103V	I129M	L172Q	S173L		K191R		I206M[b]		Q209R[b]					T518I

Nirsevimab N=50	Placebo N=64	F Protein Amino Acid Substitution																		
1	1	F12L	F15L			A103V		L172Q	S173L		K191R		I206M[b]		Q209R[b]		S276N			E463D	
1	7	F12I	F15L			A103V		L172Q	S173L	S190N	K191R		I206M[b]		Q209R[b]	S211N[b]			S389P		
1	0		F15L	A19T		A103V		L172Q	S173L	S190N	K191R		I206M[b]		Q209R[b]	S211N[b]			S389P		
1	0		F15L			A103V		L172Q	S173L	S190N	K191R		I206M[b]	N208I[b]	Q209R[b]	S211N[b]			S389P		
1	0	F12I	F15L			A103V		L172Q	S173L	S190N	K191R	L204S[b]	I206M[b]		Q209R[b]	S211N[b]			S389P		
1	0	F12I	F15L			A103V		L172Q	S173L	S190N	K191R		I206M[b]		Q209R[b]	S211N[b]	K272R		S389P		
1	0	F12I	F15L	K65E[b]		A103V		L172Q	S173L	S190N	K191R	N200Y[b]	I206M[b]		Q209R[b]	S211N[b]			S389P		
1	0	F12I	F15L	I64T[b]	K68E[b]	A103V	Y117H	L172Q	S173L	S190N	K191R		I206M[b]		Q209R[b]	S211N[b]			S389P		
1	0	F12L	F15L	I64T[b]	K68E[b]	A103V		L172Q	S173L		K191R		I206M[b]	N208K[b]	Q209R		S276N	K327R		E463D	K574N

Source: FDA analysis
[a] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions. For clarity, the most commonly occurring substitutions are aligned
[b] Substitution within nirsevimab binding sites (F protein amino acids 62-69 and 196-212); substitutions at K68 and N208 were observed in cell culture resistance selection studies (Section 20.7).
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

Table 170. Individual RSV B Substitutions With Phenotypic Data in Pooled Clinical Trials of Nirsevimab

	Number of Subjects[a]		EC_{50} Fold Change	
Substitution	Nirsevimab N=50	Placebo N=64	Nirsevimab	Palivizumab
F15L[b]	50	60	1.0	1.0
Y33F	1	0	0.5	1.2
I64T	2	0	>496	5.2
K68E	2	0	>283	2.1
A103V	50	64	0.9	1.8
L172Q	47	64	1.1	2.3
S173L	47[c]	64[c]	0.9	1.9
K191R	33	48	1.3	2.7
I206M	33	48	5.0	2.0
N208K	1	0	>350	2.0
N208S	1	0	>387	1.8
Q209R	33	48	0.5	3.1
S211N	17	28	1.2	1.9
S276N	2	3	2.0	2.5
P312H[d]	0	0	1.6	2.6
K327R	1	0	0.9	1.9
S330T	1	1	0.8	1.9
V365A	1	1	0.8	1.0
E463D	2	2	1.4	2.1
T518I	1	0	3.0	4.4

Source: FDA analysis
[a] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions and regardless of nirsevimab dose
[b] Harbored by reference used for recombinant RSV neutralization assay
[c] Assumes subjects in nirsevimab (n=4) and placebo (n=2) groups with S173fs+T174fs had S173L substitution
[d] Seen in one palivizumab-treated subject
Bold values: ≥5-fold change
Abbreviations: EC_{50}, 50% effective concentration; N, number of subjects; RSV, respiratory syncytial virus

Table 171 lists the individual RSV B substitutions occurring in extracellular regions of F protein which were identified across clinical trials, and the numbers of subjects in whom they were detected. There were 27 substitutions in total, mostly occurring in one nirsevimab-treated or placebo subjects, and there was no evidence that particular substitutions or amino acid positions were associated with nirsevimab treatment.

Table 172 lists the individual RSV B substitutions occurring outside the extracellular regions of F protein which were seen in at least two subjects total for nirsevimab and placebo groups. There were 6 individual substitutions, with no evidence that any were associated with nirsevimab treatment.

Table 171. RSV B F Protein Substitutions in F Protein Extracellular Domains[a], Not Assessed Phenotypically Against Nirsevimab, in Pooled Clinical Trials of Nirsevimab

	Number of Subjects[b]			Number of Subjects[b]			Number of Subjects[b]	
Substitution	Nirsevimab N=50	Placebo N=64	Substitution	Nirsevimab N=50	Placebo N=64	Substitution	Nirsevimab N=50	Placebo N=64
R42K	2	0	N208I[c]	1	0	P376S	0	1
K65E[c]	1	0	V239I	0	1	L381F	0	1
K87R	1	0	K272R	1	0	S389P[d]	18	28
T91N	0	1	K272T[d]	0	0	S436P	0	1
N99T	0	1	E294V[d]	0	0	N437I	0	1
L171M	0	1	K327E	0	1	Y457H[e]	0	1
S190N[d]	17	29	S330A	0	1	I475M	0	1
N200Y[c]	1	0	D356N	0	1	Y477H	0	1
L204S[c]	1	0	V365I	0	1	T522A	0	1

Source: FDA analysis
[a] Extracellular domains of F protein include amino acid residues 24-109 and 137-524
[b] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions.
[c] Substitution within nirsevimab binding sites (F protein amino acids 62-69 and 196-212)
[d] Seen in one palivizumab-treated subject each
[e] Recombinant RSV rescue failed
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

Table 172. RSV B Substitutions Outside Extracellular Regions[a], in ≥2 Subjects, Not Assessed Phenotypically, in Pooled Clinical Trials of Nirsevimab

	Number of Subjects[b]	
Substitution	Nirsevimab N=50	Placebo N=64
L4P[c]	0	1
F12I[c]	5	10
F12L[c]	2	2
A16V	1	2
N116S	1	1
V127I	1	1

Source: FDA analysis
[a] Extracellular domain of F protein includes amino acid residues 24-109 and 137-524
[b] Inclusive of all subjects meeting primary, secondary, and exploratory case definitions.
[c] Seen in one palivizumab-treated subject each
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

18.7.3. F Protein Variants and Substitutions Recommended for Phenotypic Evaluation

Based on the pooled analysis of RSV A and RSV B F protein sequences across clinical trials of nirsevimab, individual substitutions and variants with concurrent substitutions were selected for further analysis (Table 173). Individual substitutions were selected if they occurred within or adjacent to the nirsevimab or palivizumab epitope in at least one nirsevimab-treated subject, or if they occurred in the extracellular domain outside of the mAb epitopes in at least two nirsevimab-treated subjects. Concurrent substitutions were seen in variants from nirsevimab-treated subjects and were included to assess the impact of context on substitutions which do not necessarily cause loss of susceptibility on their own. Only concurrent substitutions which were likely linked based on frequency data were included.

Table 173. Individual and Concurrent Substitutions Recommended for Prioritizing in Phenotypic Analyses

Number of Subjects[a]		F Protein Amino Acid Substitution						
Nirsevimab	Placebo							
RSV A								
1	0	N67T						
2	2	I79M						
2	0	E110G						
2	0	L111I						
2	0	L119I						
8	9	K123Q						
1	0	D200N						
1	0	K201N						
2	0	N228S						
2	0	V247L						
2	0	W341R						
2	1	K419E						
3	3	N515H						

Number of Subjects[a]		F Protein Amino Acid Substitution						
Nirsevimab	Placebo							
2	0	L119I	K209R					
8	5	T122A	K123Q	I384T				
1	2	T8A	L20F	I79M	S105N	N124K	S213R	S276N
RSV B								
2	0	R42K						
1	0	K65E						
1	0	K65E	N200Y					
17	29	S190N						
1	0	N200Y						
1	0	L204S						
1	0	N208I						
1	0	K272R						
18	28	S389P						
11	10	F15L	A103V	L172Q	S173L			
8	6	F15L	A103V	L172Q	S173L	K191R	I206M	Q209R
8	9	F15L	A103V	L172Q	S173L	S190N	K191R	I206M
		Q209R	S211N	S389P				
2	0	F15L	R42K	A103V	L172Q	S173L	S190N	K191R
		I206M	Q209R	S211N	S389P			
1	0	F15L	A103V	L172Q	S173L	S190N	K191R	I206M
		N208I	Q209R	S211N	S389P			
1	0	F12I	F15L	A103V	L172Q	S173L	S190N	K191R
		L204S	I206M	Q209R	S211N	S389P		
1	0	F12I	F15L	K65E	A103V	L172Q	S173L	S190N
		K191R	N200Y	I206M	Q209R	S211N	S389P	

Source: FDA analysis
[a] Inclusive of all subjects meeting primary, secondary, or exploratory case definitions.
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

19. Clinical Microbiology

Not applicable.

20. Mechanism of Action/Drug Resistance

20.1. Mechanism of Action

Nirsevimab is a recombinant human immunoglobulin G1 kappa (IgG1κ) monoclonal antibody (mAb) that binds the prefusion conformation of the respiratory syncytial virus (RSV) fusion (F) protein. The precursor to nirsevimab, D25, is an anti-RSV F mAb isolated directly from human memory B cells (Kwakkenbos et al. 2010). D25 was optimized for RSV neutralization activity using parsimonious mutagenesis, resulting in 5 amino acid substitutions in the complementarity-determining regions (CDR), generating the mAb 1G7. The Fc region of 1G7 was then modified with three amino acid substitutions, M252Y/S254T/T256E ("YTE"; (Dall'Acqua et al. 2002)), in

Reference ID: 5208810

the heavy chain C_{H2} region, generating nirsevimab. The YTE substitutions increase the binding between the Fc region of IgG and FcRn extending the serum half-life in humans ((Robbie et al. 2013; Griffin et al. 2017)). However, substitutions such as YTE in the Fc region may also impact FcγR binding and effector functions differently, including reduction of antibody-dependent cell-mediated cytotoxicity (ADCC) (Dall'Acqua et al. 2006), and complement-dependent cytotoxicity (CDC) (Lee et al. 2019).

Co-crystallography studies of D25 with RSV F protein (described below) showed that D25 binds to a quaternary epitope within antigenic site Ø, at the membrane-distal apex of the RSV F glycoprotein (McLellan et al. 2013)). This interaction locks the F protein in its prefusion state, preventing the irreversible conformational change to the more stable postfusion conformation, which brings viral and host membranes together prior to viral entry.

The Applicant conducted competition studies (described below) to show that nirsevimab/1G7 bind to the same antigenic site Ø as D25, and not to other F binding sites, including the binding sites targeted by palivizumab (site II) and mAbs 131-2A (site I) and 133-1H (site IV). In addition, biolayer interferometry showed that 1G7 bound to prefusion RSV A and RSV B F proteins with dissociation constants (K_D) of 0.12nM and 1.22nM, respectively.

20.2. Characterization of the Nirsevimab Binding Site

Study reports: ID8897-0010, ID8897-0013.

The co-crystal structure of D25 bound to RSV F protein in the prefusion conformation was initially published in (McLellan et al. 2013). The Applicant conducted additional studies of nirsevimab Fab complexed with RSV A and RSV B F proteins to further define the binding site and identify F protein contact residues (study report ID8897-0010; (Zhu et al. 2017)). The conservation of amino acid residues in the nirsevimab binding site was assessed using sequences from GenBank and the Applicant's internal databases.

Methods

For determining the binding region and potential contact residues of nirsevimab, RSV F protein was expressed as a disulfide stabilized-cavity filling (DS-Cav) version for RSV A2 (RSV-A2 DS-Cav) and RSV B9320 (RSV B9320 DS-Cav). Purified recombinant DS-Cav proteins were complexed with an excess of nirsevimab Fab, and the complexes used for crystallization. The crystal structures of nirsevimab Fab bound to RSV A2 DS-Cav and RSV B9320 DS-Cav were solved at 3.3Å resolution and 4.3Å resolution, respectively. The nirsevimab/RSV A2 co-crystal was comprised of 3 Fabs and 3 monomers of F protein, whereas the co-crystal of the nirsevimab/RSV B9320 was comprised of only 2 Fabs with the 3 monomers of F protein. Contact residues were determined using PDBePISA (Protein interfaces, surfaces and assemblies service [PISA] at the European Bioinformatics Institute).

The level of conservation in RSV F of the nirsevimab contact residues was evaluated in an alignment of sequences encoding the full-length mature F protein (at least nucleotide bases from 78 to 1572 as counted from the start of the full-length coding DNA sequence), derived from

clinical and laboratory isolates obtained from GenBank® and the Applicant's internal databases (final set of sequences obtained by September 21, 2016). A number of partial F protein sequences covering the nirsevimab binding site (at least nucleotide bases from 105 to 870 and 50% coverage of the full-length sequence) were also included. The clinical isolates RSV A-NLD-13-005275 (GenBank® accession number KX858757) and RSV B-NLD-13-001273 (GenBank® accession number KX858756) were used as references because their F protein sequences were similar to the consensus F sequence. The percent conservation by identity of the amino acid at any individual site was calculated for each subtype (1,525 RSV A sequences and 860 RSV B sequences).

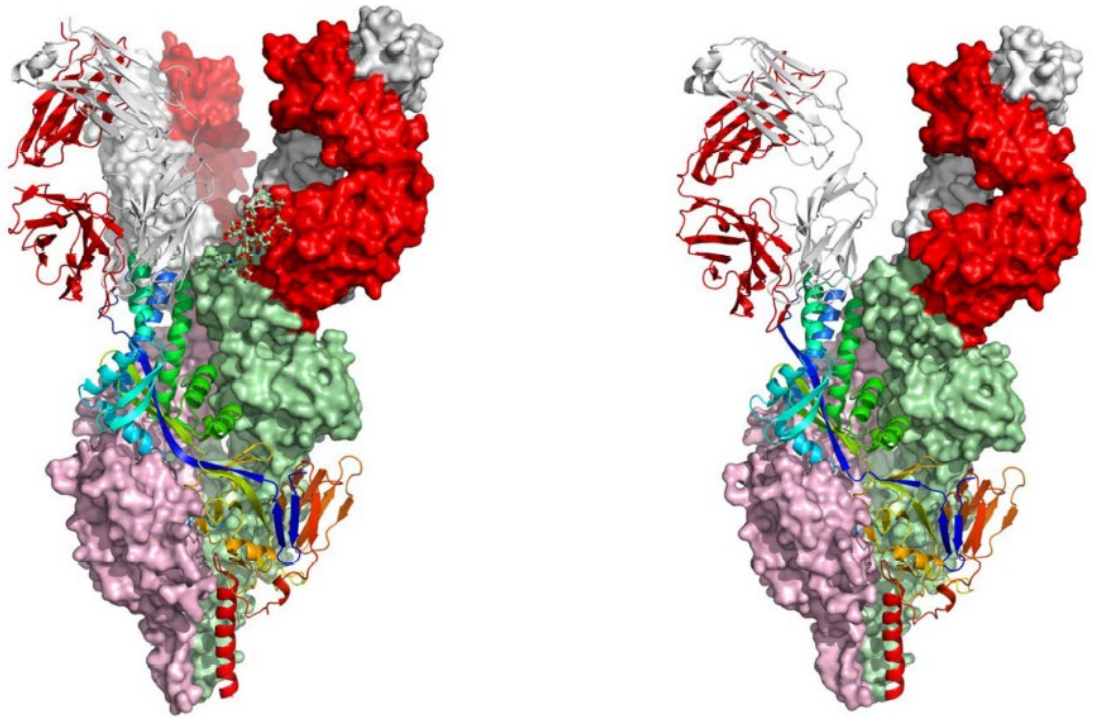
Results

Crystallography

The crystal structures of nirsevimab Fab bound to recombinant RSV A2 DS-Cav and recombinant RSV B DS-Cav are shown in Figure 66. From the co-crystals, the contact residues were determined, as well as the amino acids located within RSV A and RSV B F proteins which were adjacent to these contact residues and with at least one atom within a 5 Å radius around any atom in the nirsevimab Fab (Table 20.2.1). Overall, from the RSV A and RSV B co-crystals, the binding site was determined as comprising 25 amino acids with the potential to contact nirsevimab. These contact resides were localized in two regions spanning amino acids 62 to 69 in the F2 subunit and amino acid residues 196 to 212 in the F1 subunit of RSV F.

Figure 66. Nirsevimab Fab Complexes With RSV F Proteins

RSV A2 DS-Cav/Nirsevimab Fab **RSV B9320 DS-Cav/Nirsevimab Fab**



Source: page 11, study report ID8897-0010
Ribbon diagrams of a single subunit-Fab complex against the space filling of the 2 remaining monomers for the DS-Cav-Fab structures. The nirsevimab heavy chain is colored in red and the light chain in white.
Abbreviations: DS-Cav, disulfide stabilized-cavity filling versions of F protein; RSV, respiratory syncytial virus

Sequence Conservation

A total of 2,385 F protein sequences were selected from GenBank® and internal databases, including 1,525 RSV A and 860 RSV B sequences. Of the RSV A and RSV B sequences, 76 and 56, respectively, were partial (>260 amino acids).

Of the 25 amino acid positions identified from crystallography studies as potential nirsevimab contact residues, a total of 24 of 25 positions in RSV A and 22 of 25 in RSV B were >99% conserved (Table 174). The remaining four positions (S63 in RSV A, K65, N201 and Q209 in RSV B) were at least 94% conserved. The neutralization activity of nirsevimab/1G7 against polymorphic changes identified across these positions is discussed in Section 20.6. All positions identified with substitutions in variants selected in cell culture (N208 in RSV A and K68, N201 and N208 in RSV B; Section 20.7) were >98% conserved.

From surveillance studies, since 2015, most amino acid positions in the nirsevimab binding site have remained highly conserved (>99%) at all 25 positions in RSV A, and 22 of the 25 positions in RSV B (Section 18.1). For the three positions in RSV B with lower conservation, position 211 was >98% conserved and the other two (206 and 209) were >31% conserved, as a consequence of the emergence and spread of variants harboring I206M/Q209R substitutions. Clinical isolates harboring I206M/Q209R substitutions did not show reduced susceptibility to neutralization by nirsevimab (Section 20.6).

Table 174. RSV F Protein Amino Acid Residues Contacting Nirsevimab and Their Conservation

F Subunit	Amino Acid Position	RSV A, N=1,525		RSV B, N=860	
		Amino Acid	Conservation (%)	Amino Acid	Conservation (%)
F2	62	S	100.0	S	100.0
	63[a]	N	97.2	N	100.0
	64	I	100.0	I	100.0
	65	K[c]	99.9	K[c]	97.7
	66	E	99.3	E	99.4
	67	N	100.0	T	99.1
	68	K	99.9	K	99.8
	69	C	99.9	C	100.0

F Subunit	Amino Acid Position	RSV A, N=1,525		RSV B, N=860	
		Amino Acid	Conservation (%)	Amino Acid	Conservation (%)
F1	196[b]	K	100.0	K	100.0
	197	N	99.9	N	99.4
	198	Y	100.0	Y	100.0
	199	I	99.9	I	100.0
	200	D[c]	99.9	N[c]	100.0
	201	K	100.0	N	98.1
	202	Q[c]	100.0	Q[c]	99.8
	203	L	99.9	L	99.9
	204	L	100.0	L	100.0
	205	P	100.0	P	100.0
	206[d]	I	99.3	I	99.9
	207	V	99.9	V	99.9
	208	N[c]	100.0	N[c]	100.0
	209[d]	K[c]	99.9	Q[c]	94.4
	210	Q	100.0	Q	100.0
	211	S	99.9	S	99.0
	212	C	100.0	C	100.0

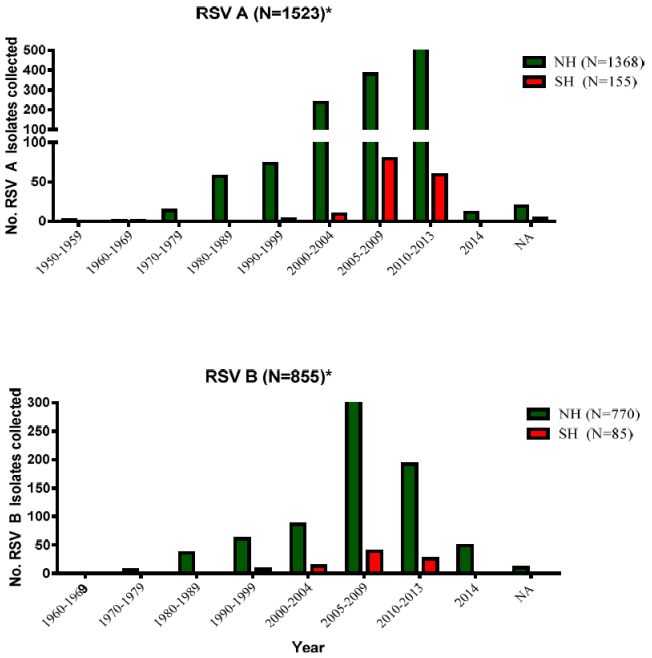
Source: page 12, study report ID8897-0013
[a] 1 RSV A F protein sequence containing amino acid changes at positions from 63 to 66 was excluded due to potential sequencing error
[b] 8 RSV A and 14 RSV B sequences derived from isolates in Vietnam in 2010 that contain X (ambiguous nucleotide) at position 196 were excluded in the conservation calculation for this residue
[c] Residues with side chains that make hydrogen bonds or salt bridges
[d] Since 2015, positions 206 and 209 are only >31% conserved because of the prevalence of variants harboring I206M/Q209R substitutions
Bold residues: Identified in cell culture selection studies as important for nirsevimab neutralization (Section 20.7)
Abbreviations: N, number of subjects; RSV, respiratory syncytial virus

Geographic and Temporal Distribution of the Sequences in F Protein Databases

An analysis was conducted of the geographic location and temporal background information for more than 98% of the sequences in the F protein databases. The sequences represent RSV isolates collected from 30 countries in the northern hemisphere and 7 countries in the southern hemisphere between 1956 and 2014, and mostly from 2000 to 2013 (Figure 67). Approximately 53% and 49% of RSV A and B sequences, respectively, were derived from isolates collected in the USA.

For the sequences containing natural polymorphic changes in the nirsevimab binding region of RSV A and RSV B in the F protein databases (Section 20.6), there was no clear trend showing that strains containing any particular polymorphisms became prevalent over the time that isolates were collected. More recent information on these polymorphisms is presented in Section 18.1; notably, variants harboring I206M/Q209R substitutions have become more prevalent since 2015, as noted in the previous section.

Figure 67. Geographic Origins and Collection Year of RSV F Sequences in the F Protein Databases



Source: page 15, study report ID8897-0013
* 2 RSV A and 5 RSV B F protein sequences were excluded because of lack of information about geographic origins and collection year
Abbreviations: NH, northern hemisphere; N, number of subjects; RSV, respiratory syncytial virus; SH, southern hemisphere

Conclusions

Co-crystallization studies identified 25 amino acid positions spanning a discontinuous binding site on the RSV F protein as contact residues for nirsevimab Fab, residing in the F2 subunit (residues 62 to 29) or the F1 subunit (residues 196 to 212). The sequence conservation in sequences from isolates collected from 1956 to 2014 across all amino acid contact residues was at least 94%, with the majority (24/25 in RSV A and 22/25 in RSV B) having >99% conservation. Since 2015, nirsevimab contact positions in RSV A have remained highly conserved (>99%), while two positions in RSV B sequences have become >31% conserved with the emergence and spread of variants with I206M/Q209R substitutions. Clinical isolates harboring I206M/Q209R substitutions did not show reduced susceptibility to neutralization by nirsevimab (Section 20.6).

20.3. Binding Activity of Nirsevimab to RSV F Protein

Study report: ID8897-0011-amend-1.

The binding kinetics of 1G7 (nirsevimab without YTE modification) were determined against recombinant RSV A and RSV B prefusion proteins. The impact on binding of resistance-associated substitutions were also assessed in this study. These data were also published in (Zhu et al. 2017).

Methods

RSV A2 and B9320 prefusion stabilized gene variants were expressed in 293F cells using transiently transfected pcDNA3.1(+) based plasmids harboring F protein residues 1-513 with DS-Cav1 stabilization substitutions, a C-terminal T4 fribritin trimerization domain, thrombin cleavage site, hexa-histidine tag, and a terminal AviTag. Proteins were purified from culture supernatants by affinity chromatography and gel filtration.

Binding kinetics of 1G7 against RSV A2 and B9320 trimeric prefusion proteins were measured on an Octet QK384 (Forte Biosciences). Trimeric F protein and F protein variants (20 µg/mL) were loaded onto biosensors for 300 sec, which were then equilibrated in kinetics buffer for 60 sec, followed by mAb association (250-62.5nM) for 60 sec and binding dissociation for 180 sec. Data analyses and curve fitting were performed using Octet data analysis software.

Results

The binding kinetics of 1G7 to immobilized RSV-A2 or B9320 DS Cav1 prefusion protein variants which contained the resistance-associated substitutions are shown in Table 175. The binding affinity (K_D value) of 1G7 to RSV A2 and RSV B9320 prefusion F protein was 0.12nM and 1.22nM, respectively.

1G7 exhibited reduced binding affinity to RSV A F protein harboring N208Y and N67I/N208Y substitutions, with K_D values reduced approximately 5- and 56-fold, respectively, compared with the parental F protein. Reduced affinity was also seen for 1G7 binding to RSV B F protein harboring K68N and N201S substitutions, with K_D values reduced by 12- and 29-fold, respectively. The reduced binding of antibody to these F variants was largely because of a faster dissociation rate constant, k_{off}.

No binding activity under the assay conditions was detected to the RSV B F proteins containing the N208D, N208S and double substitutions K68N/N201S and K68N/N208S that are associated with a greater reduction in susceptibility (see Section 20.7). There appeared to be a reasonable correlation between antibody binding kinetics and virus neutralization, indicating that one mechanism of viral resistance may be to acquire substitutions that weaken or prevent antibody binding.

Table 175. The Binding Kinetics of 1G7 to RSV DS Cav1 Prefusion Protein Variants

RSV Subtype	Amino Acid Substitutions in RSV F DS-Cav1	1G7 (Non-YTE Version of Nirsevimab)			
		K_D (nM)	k_a (M^{-1} s^{-1})	k_d (s^{-1})	Fold Change Relative to Parental F in k_d
RSV A2	None	0.12	$3.11 \times 10^5 \pm 8.32 \times 10^3$	$3.74 \times 10^{-5} \pm 4.09 \times 10^{-5}$	1
	N67I	0.098	$2.92 \times 10^5 \pm 8.62 \times 10^3$	$2.86 \times 10^{-5} \pm 4.45 \times 10^{-5}$	0.8
	N208Y	0.552	$4.48 \times 10^5 \pm 1.48 \times 10^4$	$2.47 \times 10^{-4} \pm 5.24 \times 10^{-5}$	4.6
	N67I, N208Y	6.67	$9.08 \times 10^5 \pm 8.97 \times 10^4$	$6.05 \times 10^{-3} \pm 2.16 \times 10^{-4}$	55.6
RSV B9320	None	1.22	$4.69 \times 10^5 \pm 1.52 \times 10^4$	$5.71 \times 10^{-4} \pm 5.27 \times 10^{-5}$	1
	K68N	14.4	$3.70 \times 10^5 \pm 1.44 \times 10^4$	$5.32 \times 10^{-3} \pm 8.64 \times 10^{-5}$	11.8
	N201S	35.4	$3.26X \times 0^5 \pm 1.74 \times 10^4$	$1.16 \times 10^{-2} \pm 1.79 \times 10^{-4}$	29
	N208D	< Measurable Limit			
	N208S	< Measurable Limit			
	K68N, N201S	< Measurable Limit			
	K68N, N208S	< Measurable Limit			

Source: page 20, study report ID8897-0011-amend-1
Abbreviations: NH, northern hemisphere; N, number of subjects; RSV, respiratory syncytial virus; SH, southern hemisphere

Conclusions

The 1G7 antibody bound with approximately 10-fold higher affinity to prefusion F protein from subtype RSV A2 compared with RSV B9320 (0.12nM versus 1.22nM, respectively). This is not consistent with the neutralization activity for the two strains, which are similar (EC_{50} values =13pM and 11pM, respectively; (Section 20.6). However, in general, reduction of binding activity to RSV A and RSV B F protein harboring resistance-associated substitutions correlated with the loss of neutralization activity (Section 20.7), indicating that resistance may be mediated through acquisition of substitutions which impact mAb binding.

20.4. Competition Studies With Nirsevimab and Other mAbs Targeting the RSV F Protein

Study report: ID8897-0008.

To confirm that 1G7/nirsevimab and D25 target the same binding site on RSV F, and that nirsevimab does not interfere with the binding of mAbs used for detection in neutralization assays, the Applicant conducted competition studies with biotinylated 1G7 (as a surrogate for nirsevimab) and mAbs targeting previously described antigenic sites on RSV F.

Methods

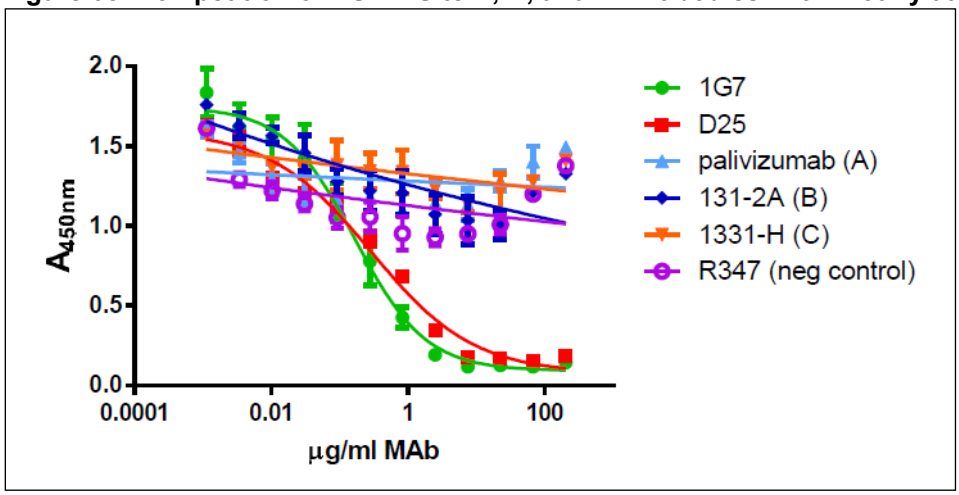
Biotinylated 1G7 and mouse mAbs targeting previously characterized RSV F mAb binding sites were assessed in competition assays. The mAbs included palivizumab (site A, positions 255-276), 131-2A (site B, position 389) and 1331H (site C, positions 422-438) (Beeler and van Wyke Coelingh 1989), with D25 and unlabeled 1G7 as controls.

HEp-2 cells were plated at a density of 5×10^4 cells per well in 96-well plates, and infected with RSV at a multiplicity of infection (MOI) of 1.0. After 24 hours, plates were fixed with paraformaldehyde, washed and dried. Plates were blocked with 3% bovine serum albumin, then competitor mAb in blocking buffer added followed by biotinylated 1G7. The competition mAbs were added at final concentrations of 1 ng/mL to 200 µg/mL, in the presence of 60 ng/mL of biotinylated 1G7 (the half-maximal binding concentration). Plates were incubated for 2 hours at room temperature, washed, and streptavidin-HRP added for 15-20 minutes at room temperature. Plates were washed and 3,3′,5,5′-tetramethylbenzidine (TMB) added for 10 to 15 minutes, then absorbance read at 450 nm.

Results

None of the competitor mAbs (i.e., palivizumab, 131-2A, and 1331H) targeting the previously characterized RSV F binding sites interfered with the binding of biotinylated 1G7 to RSV-infected HEp-2 cells (Figure 68). As expected, both D25 and 1G7 competed with biotinylated 1G7 for binding to RSV-infected HEp-2 cells. These results indicate that 1G7 (and therefore nirsevimab) and D25 bind to the same, or at least overlapping, antigenic site(s) on RSV F. In addition, these data indicate that the nirsevimab/1G7 binding site does not overlap with previously described mAb binding sites targeted by palivizumab, 131-2A, or 1331H.

Figure 68. Competition of RSV F Site A, B, and C Antibodies With Biotinylated1G7



Source: page 13, study report ID8897-0008
Different concentrations of competitor mAbs were incubated with RSV-infected HEp-2 cells in the presence of biotinylated 1G7. Binding of biotinylated 1G7 was quantified by measuring absorbance at 450 nm after incubation with streptavidin-HRP and an ABTS substrate.
Abbreviations: RSV, respiratory syncytial virus

Conclusions

Competition studies of binding using 1G7 and other mAbs targeting RSV F protein showed that 1G7 and D25 mAbs competed for binding and therefore likely targeted the same site, whereas palivizumab and mAbs targeting other sites on F protein did not interfere with 1G7 binding. These data also indicate that nirsevimab/1G7 does not interfere with the Applicant's 1331H-based microneutralization assay (see Section 20.6). The Applicant concluded that this study also demonstrates that nirsevimab will not interfere with standard RSV diagnostic assays based on antibody-mediated detection of RSV F that utilize commercially available antibodies targeting

sites A, B, or C on RSV F; however, the lack of interference should be verified for any immunoassays which do not identify the target epitope.

20.5. Evaluation of Nirsevimab Effector Function

20.5.1. Nirsevimab FcRn Binding Activity

Study report: ID8897-0005.

The Fc domain of nirsevimab contains the YTE substitutions which have been shown to increase binding to human neonatal receptor (FcRn) and extend the serum half-life in humans (Robbie et al. 2013). To determine the effect of the YTE substitutions in nirsevimab, the Applicant evaluated the dissociation constants (K_D) for nirsevimab interaction with human and cynomolgus monkey FcRn.

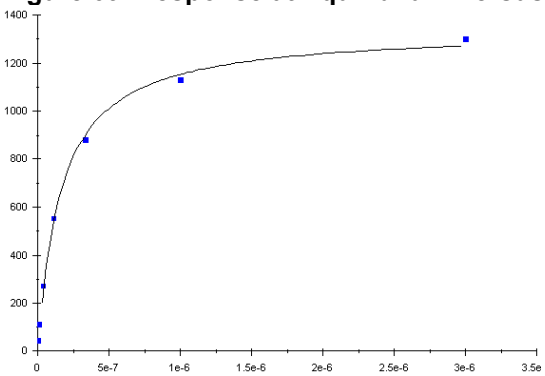
Methods

The binding affinity of nirsevimab to recombinant FcRn proteins was determined using surface plasmon resonance (SPR) on a BIAcore 3000 instrument. Nirsevimab IgG was diluted to approximately 250nM (37.5 µg/mL) and used to prepare a high-density IgG surface on a CM5 sensor chip according to the instrument manufacturer's protocol. FcRn proteins were produced as described in (Dall'Acqua et al. 2002; Dall'Acqua et al. 2006). FcRn proteins were serially 3-fold diluted from 3,000 to 4.11nM in instrument buffer (50mM sodium phosphate buffer, pH 6, containing 150mM NaCl, and 0.05% (v/v) Tween 20 [T20]), and dilutions were individually injected over immobilized nirsevimab IgG to record binding data over 50 minutes, and then bound FcRn removed with ten 60 second pulses of 50mM sodium phosphate buffer, pH 7.4, containing 150mM NaCl, and 0.05% (v/v) T20. From the binding data, individual data sets were averaged during steady-state binding (Req) at each concentration (C) of FcRn, and then fit to a 1:1 binding model (Req versus C plot) using the vendor's BIAevaluation software, v. 1.1, to determine the K_D values.

Results

A sensorgram overlay for each FcRn concentration series was plotted (not shown), and the averaged, individual binding responses at equilibrium were plotted against concentration. Figure 69 shows the resulting Req versus C isotherms for nirsevimab binding to human FcRn, which was used to determine the K_D value. From these experiments, the K_D values for nirsevimab IgG binding to human and cynomolgus FcRn was determined to be 161nM and 253nM, respectively.

Figure 69. Response at Equilibrium Versus Concentration: huFcRn Binding to Nirsevimab IgG



Source: page 13, study report ID8897-0005
Maximal responses at equilibrium from the sensorgram (not shown) were plotted as a function of concentration of FcRn.

Conclusions

Nirsevimab was demonstrated to have similar binding activity to human and cynomolgus FcRn, with K_D values of 161nM and 253nM, respectively. The Applicant did not include a non-YTE version of nirsevimab for comparison in these experiments, but previous studies with motavizumab showed an approximately 10-fold increase in FcRn binding at pH 6.0 for motavizumab-YTE compared with the non-YTE version (from 2249nM to 210nM; (Dall'Acqua et al. 2006)). This increase binding of motavizumab-YTE resulted in a 2- to 4-fold increase in serum half-life in humans (Robbie et al. 2013); nirsevimab has an extended mean half-life of 85 to 117 days in healthy adults (Griffin et al. 2017).

20.5.2. Nirsevimab FcγR Binding and Role in Antiviral Activity

Study report: ID8897-0031.

The potential of nirsevimab for Fc effector activity was evaluated by assessing binding activity to different recombinant human Fcγ receptors. The contribution of effector activity was evaluated in cell culture and the cotton rat model of RSV infection by comparing nirsevimab with 1G7 (unmodified Fc region) and 1G7-TM (Fc region modified to reduce effector function).

Methods

FcγR Binding Assay

Surface plasmon resonance (SPR) was used to determine the binding affinity of nirsevimab to different recombinant human Fcγ receptors immobilized via histidine tags on sensor chips: FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA-158V (CD16). Each FcγR was captured to anti-histidine tag antibody on the experimental flow cell, then nirsevimab flowed over the FcγR or reference cells with a single injection. The binding affinity constants (K_D values) were determined for each receptor.

Reference ID: 5208810

Neutralization Assay

The neutralization activities against RSV A2 of nirsevimab, IG7, and IG7-TM were determined in HEp-2 cells using methodology as described in Section 20.6.

Cotton Rat Challenge Model

The prophylaxis activities of IG7 and IG7-TM in the cotton rat model of RSV infection were determined using similar methodology as described in Section 20.8. The 1G7 mAb was tested rather than nirsevimab because YTE substitutions decrease exposure in rodents (Dall'Acqua et al. 2002). Weight-based doses of 2.0, 1.0, or 0.5 mg/kg of each antibody, including negative control anti-HIV mAb R347 dosed at 2.0 mg/kg, were administered to 4- to 6-week-old female cotton rats (groups of 6 to 8) by intramuscular injection. Blood was collected 24 hours later, and animals were intranasally challenged with RSV A2 (amount not indicated). Lungs and nasal turbinates were harvested 4 days postchallenge and homogenized for determination of viral titers by plaque assay.

Tissue homogenates were diluted in HEp-2 medium and added to HEp-2 cells seeded 24 hours prior at 2.5×10^5 cells/well in 24-well plates. After 1 hour of incubation, inoculum was removed and replaced with cell culture medium containing 0.75% methylcellulose, and cultures incubated for 5 days at 37°C. Cells were then fixed with methanol and blocked with 5% milk, then anti-RSV goat polyclonal antibody added for 1 hour at room temperature. After washing, horseradish peroxidase (HRP)-conjugated rabbit anti-goat antibody was added for 1 hour at room temperature, followed by washing and addition of 3-amino-9-ethylcarbazole (AEC) substrate for 30 minutes. The limit of detection for lung and nasal turbinates for this assay was 30.0 pfu/g of tissue.

To determine serum concentrations of mAbs, a standard IgG ELISA was conducted using goat anti-human IgG (H+L) as capture antibody, and an HRP-coupled goat anti-human IgG detection antibody.

Results

FcγR Binding Assay

Using SPR, the binding affinity of nirsevimab to various Fcγ receptors was determined. Nirsevimab showed binding to each receptor tested, with K_D values for FcγRI, FcγRIIA, FcγRIIB, or FcγRIII A158V of 8.94×10^{-9}, 1.87×10^{-5}, 5.30×10^{-4}, and 1.67×10^{-5} M, respectively. In the absence of control data, it is not clear how to interpret these data or determine whether the YTE substitutions impacted binding. In studies by (Dall'Acqua et al. 2006), the introduction of YTE substitutions into an anti-integrin-$\alpha_v\beta_3$ antibody, MEDI-522, reduced the binding affinity to FcγRIIIA by approximately 2-fold, to 6.82×10^{-5} M, and the ADCC activity was reduced by 100-fold compared with the parental antibody.

Cell Culture Neutralization Activity

Against the RSV A2 strain, the mean neutralization activities (EC_{50} values) of nirsevimab, 1G7 and 1G7-TM were 2.2 ng/mL, 2.0 ng/mL and 2.0 ng/mL, respectively. These data are similar and consistent with data reported for nirsevimab and 1G7 in other neutralization studies (see Section 20.6).

Cotton Rat Challenge Model

In the cotton rat model of RSV infection, administration of 1G7 or 1G7-TM one day prior to challenge with RSV A2 caused a reduction of RSV replication in lungs and nasal turbinates, which was dose-dependent (Table 176, Figure 70). Treatment with the highest dose (2 mg/kg) of 1G7 or 1G7-TM resulted in a >3.5 $\log_{10}$ reduction in mean viral titers in the lung and nasal turbinates of infected cotton rats when compared to the control mAb R347. There was no significant difference in viral titer in the lungs or nasal turbinates between animals treated with 1G7 and those with 1G7-TM at each mAb dose administered. Median serum antibody concentration was similar between 1G7 and 1G7-TM at each dose (data not shown).

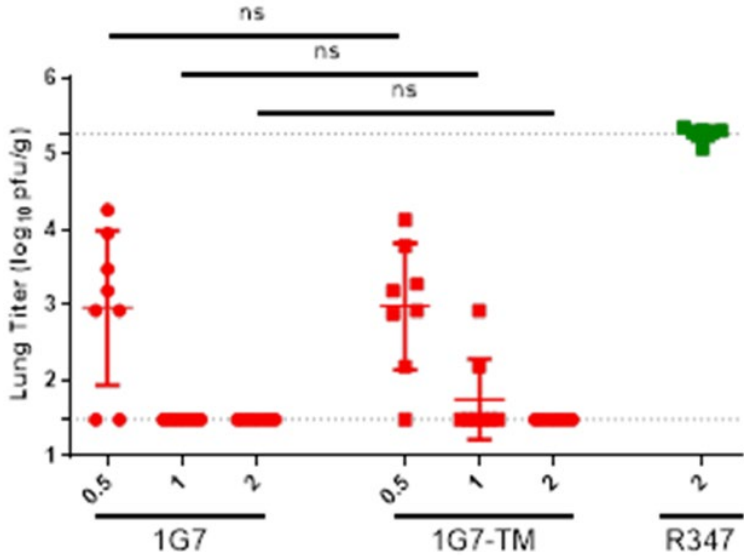
Table 176. RSV Infectious Titers in Lung and Nasal Turbinates

	Mean Lung RSV Titer ±SD (pfu/g)			Mean Nasal Turbinate RSV Titer ±SD (pfu/g)		
mAb	**0.5 mg/kg**	**1 mg/kg**	**2 mg/kg**	**0.5 mg/kg**	**1 mg/kg**	**2 mg/kg**
1G7	909.9±10.6	30.0±1.0	30.0±1.0	1,256.0±4.9	66.7±4.5	39.4±2.3
1G7-TM	950.6±7	56.0±3.4	30.0±1.0	1,932.0±3.1	109.6±5.0	54.7±3.4
R347	ND	ND	182,810±1.3	ND	ND	187,499.5±1.2

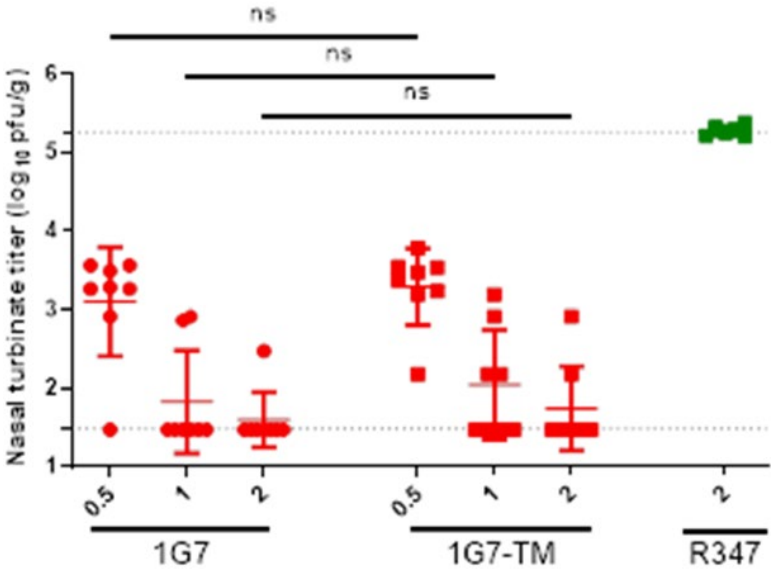
Source: pages 18-19, study report ID8897-0031
Lung or nasal turbinate viral titers on Day 4 postinfection from animals treated one day prior to infection with the indicated dose of 1G7, 1G7-TM, or R347 (negative control mAb). Values are the mean and SD of 6-8 animals per group. Limit of detection is 30 pfu/g.
ND = not done. pfu/g = RSV plaque forming unit per gram of tissue.
Abbreviations: RSV, respiratory syncytial virus; SD, standard deviation

Figure 70. RSV Titers (Log_{10} pfu/g) in Lung and Nasal Turbinates in Cotton Rat Challenge Model

Lung Titers



Nasal Turbinate Titers



Source: pages 17-18, study report ID8897-0031
RSV titers on day 4 postinfection from animals treated one day prior with the indicated dose of 1G7, 1G7-TM, or R347 (negative control mAb). Limit of detection =30 pfu/g. RSV plaque forming units per gram of tissue (pfu/g) values are the mean and SD of 6-8 animals per group. Significance determined by one-way ANOVA.
Abbreviations: ANOVA, analysis of variance; ns, not significant; RSV, respiratory syncytial virus

Conclusions

SPR affinity data indicated that nirsevimab is able to bind different human Fcγ receptors and therefore has the potential for Fc effector function. However, in the absence of control data, it is not known whether the binding affinities are biologically relevant. In cell culture, there was no clear difference in neutralization activity (EC_{50} value) between 1G7 and the 1G7-TM mAb with reduced effector function.

To determine if effector function impacted antiviral activity, a cotton rat model of RSV infection was used, and a similar reduction in RSV titers in lung and nasal turbinates was seen in animals treated prophylactically with 1G7 and those treated with 1G7-TM. However, it is not clear

whether 1G7 binds cotton rat Fcγ receptors with the same affinity as seen for human Fcγ receptors, so overall, the relevance of these findings is not clear.

20.5.3. Effector Function of Nirsevimab in Cell Culture

Study report: ID8897-0033-amend-1.

To characterize the effector function of nirsevimab, the Applicant conducted several cell culture-based immunomodulatory assays, including antibody-dependent cellular phagocytosis (ADCP), antibody-dependent complement deposition (ADCD), antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent NK cell activation (ADNKA), and antibody-dependent cell-mediated cytotoxicity (ADCC).

Methods

Antibody-Dependent Cellular Phagocytosis

Recombinant RSV A2 prefusion trimer was coupled to carboxylate-modified beads (Invitrogen, F8823/F8815) using a two-step carbodiimide reaction. Coupled beads were blocked with 5% BSA, washed and resuspended in 0.1% BSA prior to use.

ADCP was assessed using antigen-functionalized fluorescent beads which were incubated with diluted mAb for 2 hours at 37°C to form immune complexes. Monocytes (THP-1 cells at 2.5×10^4 cells/well) were added to the immune complexes and plates incubated for 16 hours at 37°C. Cells were fixed with 4% PFA for 15 minutes and fluorescence acquired with a Stratedigm S1000EON. The phagocytic score was calculated from the formula: (percentage of $FITC^+$ cells x geometric mean fluorescent intensity [gMFI] of $FITC^+$ cells) / 1,000.

Antibody-Dependent Neutrophil Phagocytosis

Bead-based immune complexes were generated as described above for ADCP. Primary neutrophils were isolated from human peripheral whole blood using EasySep™ neutrophil isolation kit and the manufacturer's instructions (StemCell Technologies).

Neutrophils were added to the immune complexes at 2.5×10^4 cells/well and incubated for 30 minutes at 37°C. Cells were stained for the neutrophil marker CD66b (Biolegend, 305112), washed with PBS, then fixed for 15 minutes in 4% PFA. Fluorescence was acquired with a Stratedigm S1000EON.

Antibody-Dependent Complement Deposition

Immune complexes were generated using antigen coupled beads as described above for ADCP. Lyophilized guinea pig complement (Sigma, G9774) was diluted 1:60 in gelatin veronal buffer (Sigma, G6514), and added to the immune complexes. Following incubation for 60 minutes at 37°C, plates were incubated with fluorescein-conjugated goat anti-guinea pig complement C3 (MP Biomedicals, 0855385) for 20 minutes in the dark, and fluorescence acquired with a Stratedigm S1000EON.

Antibody-Dependent NK Cell Activation

NK cells were isolated from leukopaks using the EasySep™ NK cell enrichment kit (StemCell Technologies). ELISA plates were coated with 300 ng/well recombinant RSV A2 Pre-F trimer and incubated for 2 hours at room temperature. Plates were blocked with 5% BSA and sample mAb added to the plates for 2 hours at 37°C to form immune complexes. NK cells were added to the plates at 5×10^4 cells/well in R10 medium supplemented with anti-CD107a PE-Cy5 (BD Biosciences, 55802), GolgiPlug (BD Biosciences, 555029) and GolgiStop (Biolegend, 420701). Plates were incubated for 5 hours at 37°C then NK cells stained with anti-CD56 PE-Cy7, anti-CD16 APC-Cy7 and anti-CD3 Pacific Blue (BD Biosciences, 557747, 557758, 558124, respectively). Cells were fixed with Fix&Perm™ cell permeabilization kit (Life Technologies, GAS001S100/GAS002S100), then stained for intracellular markers with anti-MIP-1β PE and anti-IFNγ FITC (BD Biosciences, 550078, 340449) and fluorescence acquired on a Stratedigm S1000EON.

Antibody-Dependent Cell-Mediated Cytotoxicity

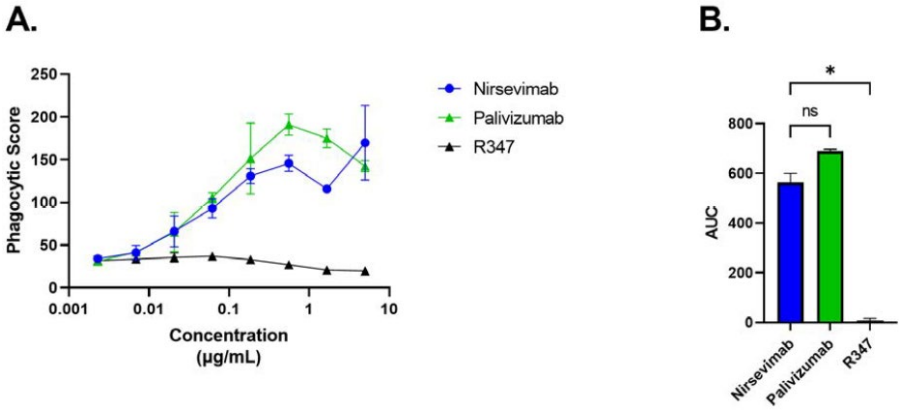
Recombinant RSV A2 prefusion trimer was streptavidin-conjugated using Abcam Lightning-Link® Streptavidin Conjugation kit (Abcam, ab102921). Target cells were biotinylated (EZ-Link™ Sulfo-NHS-LC-Biotin) and stained with one of two dyes: half with CellTrace™ Violet and half with CellTrace™ Far Red (ThermoFisher, C34564, C34557). The cells stained with CellTrace™ Violet were pulsed with streptavidin-conjugated RSV A2 prefusion trimer and the cells stained with CellTrace™ Far Red were not pulsed. Diluted mAb was added to the target cells, then primary NK cells added at a 20:1 effector:target ratio and incubated for 4 hours. Fluorescence was acquired with a Stratedigm S1000EON, and ADCC activity calculated using the formula: percent specific lysis = $(\%dead_{[pulsed]} - \%dead_{[not\ pulsed]}) / (100-\%dead_{[not\ pulsed]})$.

Results

Antibody-Dependent Cellular Phagocytosis

Using flow cytometry, nirsevimab was shown to mediate phagocytosis of RSV prefusion-conjugated fluorescent beads by human THP-1 monocytes (Figure 71). Nirsevimab showed similar phagocytic scores (including proportion and intensity of fluorescent-positive THP-1 cells) as seen for palivizumab over the range of concentrations tested, which was statistically greater than seen with the negative control R347 where no measurable ADCP activity was detected.

Figure 71. Nirsevimab ADCP Activity

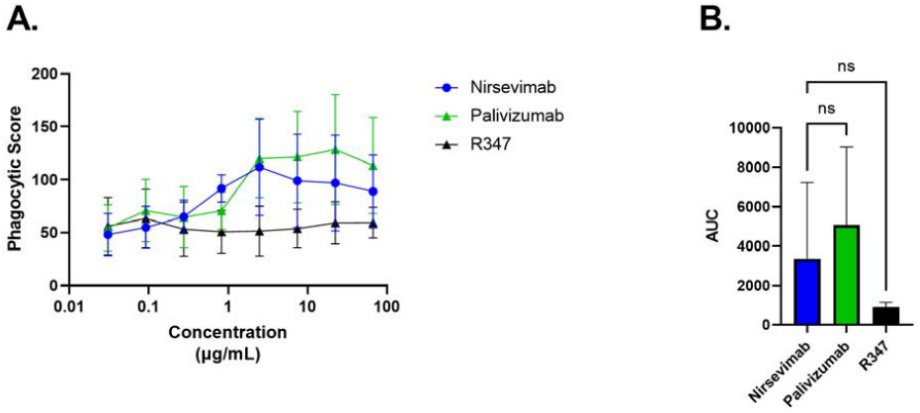


Source: page 16, study report ID8897-0033-amend-1
Nirsevimab, palivizumab or R347 was evaluated for ADCP activity in THP-1 cells at a range of 5 µg/mL to 2.28624 ng/mL. Samples were tested in duplicate, and the average phagocytic score (±SD) at the indicated dilution is reported. (B) Area Under the Curve (AUC) analysis for phagocytic score of each mAb tested across eight dilutions shown in (A). The mean AUC value (±SD) is reported with an ordinary one-way ANOVA with post hoc Dunnett multiple comparisons test to compare nirsevimab to palivizumab and R347 (* = p≤0.05; ns = not significant).
Abbreviations: ADCP, antibody-dependent cellular phagocytosis; ANOVA, analysis of variance; AUC, area under the time-concentration curve; SD, standard deviation

Antibody-Dependent Neutrophil Phagocytosis

Using flow cytometry, nirsevimab was shown to mediate phagocytosis of RSV prefusion-conjugated fluorescent beads by primary human neutrophils from 4 different donors (Figure 72). Nirsevimab showed similar phagocytic scores as for palivizumab over the range of concentrations tested. The nirsevimab-mediated ADNP activity was higher than seen for the negative control antibody R347, but the difference was not significant.

Figure 72. Nirsevimab ADNP Activity



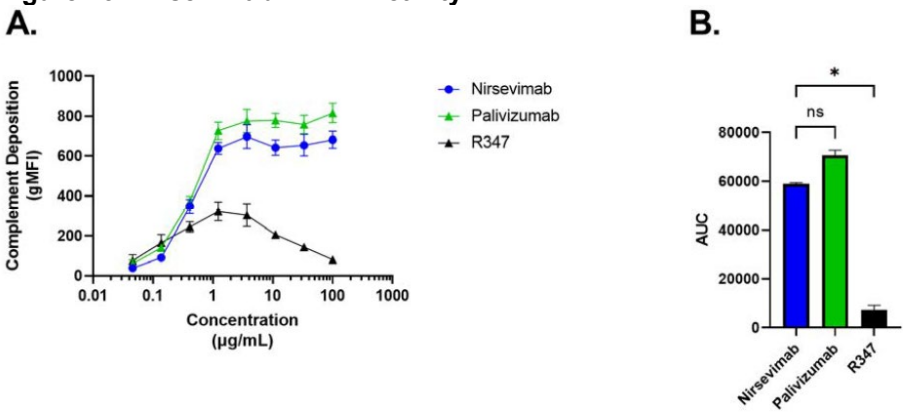
Source: page 17, study report ID8897-0033-amend-1
Nirsevimab, palivizumab or R347 was evaluated for ADNP activity in primary neutrophils from four donors at a range of 67 µg/mL to 30.64 ng/mL. Samples were tested in quadruplicate using cells from four donors, and the average phagocytic score (±SD) at the indicated dilution is reported. (B) Area Under the Curve (AUC) analysis for phagocytic score of each mAb tested across eight dilutions shown in (A). The mean AUC value (±SD) is reported with an ordinary one-way ANOVA with post hoc Dunnett multiple comparisons test to compare nirsevimab to palivizumab and R347
Abbreviations: ADNP, antibody-dependent neutrophil phagocytosis; ANOVA, analysis of variance; AUC, area under the time-concentration curve; ns, not significant; SD, standard deviation

Reference ID: 5208810

Antibody-Dependent Complement Deposition

A cell culture-based ADCD assay was used to quantify the ability of nirsevimab to induce the recruitment of complement component C3 on the surface of RSV prefusion-conjugated beads. Flow cytometry was used to detect complement deposition on the immune complex by a fluorescently conjugated anti-C3 antibody (Figure 73). Nirsevimab mediated ADCD at similar levels as palivizumab, and at significantly higher levels than seen for the negative control antibody R347, which had no detectable ADCD in this assay.

Figure 73. Nirsevimab ADCD Activity

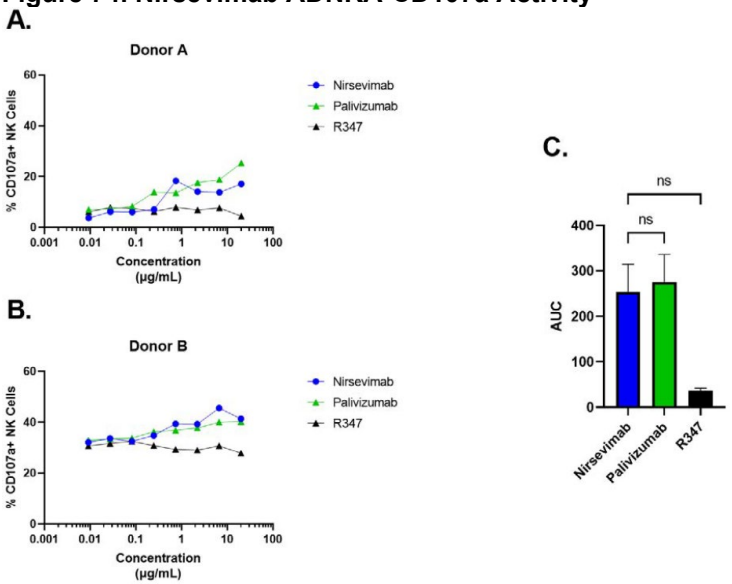


Source: page 18, study report ID8897-0033-amend-1
Nirsevimab, palivizumab or R347 was evaluated for ADCD activity in THP-1 cells at a range of 100 µg/mL to 45.72 ng/mL. Samples were tested in duplicate, and the average complement deposition (gMFI ± SD) at the indicated dilution is reported. (B) Area Under the Curve (AUC) analysis for complement deposition of each mAb tested across eight dilutions shown in (A). The mean AUC value (±SD) is reported with an ordinary one-way ANOVA with post hoc Dunnett multiple comparisons test to compare nirsevimab to palivizumab and R347 (* = p≤0.05)
Abbreviations: ADCD, antibody-dependent complement deposition; ANOVA, analysis of variance; AUC, area under the time-concentration curve; ns, not significant; SD, standard deviation

Antibody-Dependent NK Cell Activation

Using primary NK cells from two donors, a cell-based assay assessed the ability of nirsevimab to induce three NK cell functions: CD107a expression as a surrogate for antibody-dependent NK cell degranulation, IFN-γ production, and MIP-1β production. Fluorescently labelled intracellular straining and flow cytometry were used to measure ADNKA activity following activation of the primary NK cells with nirsevimab-prefusion immune complexes. Figure 74 shows the CD107a expression for both donors; similar results were seen for IFN-γ and MIP-1β production (data not shown). Nirsevimab mediated ADNKA at levels similar to those seen for palivizumab, and higher than seen for the negative control antibody R347, but the difference with the control was not significant.

Figure 74. Nirsevimab ADNKA-CD107a Activity



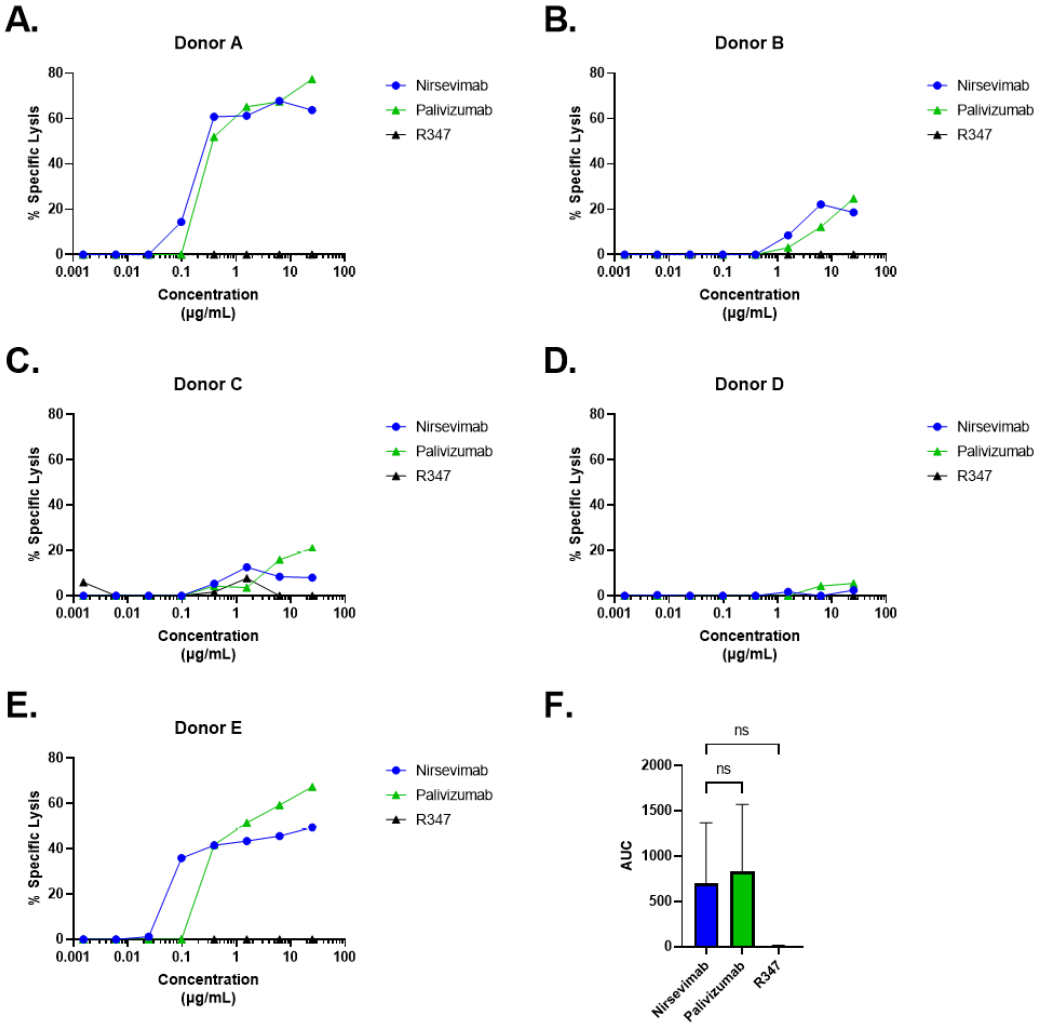
Source: page 19, study report ID8897-0033-amend-1
(A, B) Nirsevimab, palivizumab or R347 was evaluated for ADNKA-CD107a activity at a range of 20 µg/mL to 9.1449 ng/mL. Samples were tested in duplicate using primary NK cells from two donors, and the percent of CD107a+ NK cells from each respective donor at the indicated dilution is reported. (C) Area Under the Curve (AUC) analysis for phagocytic score of each mAb tested across eight dilutions shown in (A) and (B). The mean AUC value (±SD) is reported with an ordinary one-way ANOVA with post hoc Dunnett multiple comparisons test to compare nirsevimab to palivizumab and R347.
Abbreviations: ADNKA, antibody-dependent NK cell activation; ANOVA, analysis of variance; AUC, area under the time-concentration curve; ns, not significant; SD, standard deviation

Antibody-Dependent Cell-Mediated Cytotoxicity

Flow cytometry was used to assess target cell lysis by NK cells from five separate donors in three experiments. Antibody-mediated specific lysis was determined by calculating the ratio of affected RSV prefusion conjugated cells to unconjugated cells. ADCC activity was induced by nirsevimab for all donors, at levels similar to palivizumab, and at higher, but not significant, levels compared with the negative control antibody R347 (Figure 75). There was considerable donor to donor variability which may account for the nirsevimab-induced ADCC activity not reaching significance compared with the negative control.

Reference ID: 5208810

Figure 75. Nirsevimab ADCC Activity



Source: page 22, study report ID8897-0033-amend-1
(A-E) Nirsevimab, palivizumab or R347 was evaluated for ADCC activity at a range of 25 µg/mL to 1.526 ng/mL. Samples were tested using primary NK cells from five different donors, and the percent specific lysis of the target cells from each respective donor at the indicated dilution is reported. (F) Area Under the Curve (AUC) analysis for % specific lysis activity of each mAb tested across eight dilutions shown in (A-E). The mean AUC value (±SD) is reported with an ordinary one-way ANOVA with post hoc Dunnett multiple comparisons test to compare nirsevimab to palivizumab and R347 (ns = not significant).
Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; ANOVA, analysis of variance; AUC, area under the time-concentration curve; ns, not significant; SD, standard deviation

Conclusions

In a series of cell-based assays, the effector function of nirsevimab was characterized. In assays for ADCP and ADCD, nirsevimab demonstrated significant activity compared with the negative control antibody R347, and similar activity as seen for palivizumab. In addition, ADNP, ANDKA and ADCC activities were seen for nirsevimab at similar levels as palivizumab, although were not significantly different from the negative control. While it is important to characterize these effector activities, it is not clear what role they may play in the context of prophylaxis, or for moderating disease in nirsevimab-treated subjects who subsequently become infected with RSV.

20.6. Antiviral Activity in Cell Culture

Study reports: ID8897-0001, ID8897-0002, ID8897-0011-amend-1, ID8897-0013, ID8897O-1516, ID8897O-1617.

The antiviral activity of nirsevimab/1G7 was determined by microneutralization assays in HEp-2 cells, using laboratory strains and clinical isolates of RSV A and RSV B.

Methods

For the microneutralization assays using laboratory strains of RSV, 2-fold serial dilutions of mAb were added to 384-well plates. RSV was diluted into HEp-2 cell culture medium to a concentration of 80 or 150 pfu/well for RSV A2 and B9320, respectively, and added to the plates containing culture medium and antibody. Plates were then incubated for 1.5 hours at 37°C. HEp-2 cells were added ($7.5x10^3$ cells; multiplicity of infection [MOI] of approximately 0.01 for RSV A2 and 0.02 for RSV B9320) to each well and the plates incubated at 37°C for 3 days for RSV A2 or 4 days for RSV B9320. Cells were fixed in acetone and viral replication quantified by colorimetric readout of a horseradish peroxidase conjugated anti-RSV F mAb mixed with TMB peroxidase as substrate. The conjugated anti-RSV F mAb, 1331H, targets the C site of RSV F, and can bind to infected cells in the presence of 1G7 (see Section 20.4).

For evaluating clinical isolates, similar methodology was used as for laboratory strains, with some modifications. All virus isolates were diluted to a tissue culture half-maximal infectious dose ($TCID_{50}$) of 25 to 3,000 in culture media, and on a separate plate virus dilutions were back titrated to determine the actual $TCID_{50}$ value used in the assay. A panel of 54 RSV A and 41 RSV B clinical isolates was assessed initially, which were obtained during the 2004 to 2008 RSV seasons from sites within Australia, Israel, Italy, Netherlands, and the U.S. The isolates were used at a cell culture passage number of less than 4. As controls, palivizumab (positive control) and R347 (negative control) were used.

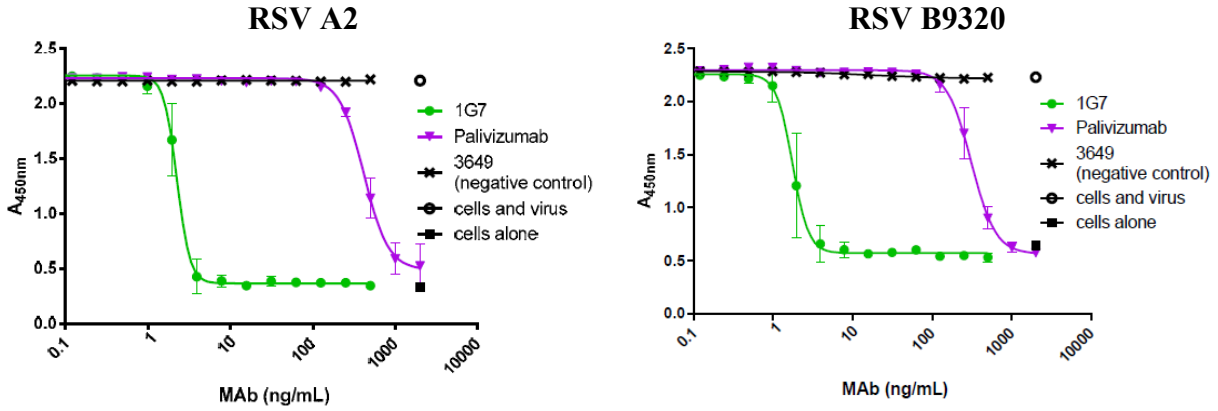
Additional RSV clinical isolates from 2011 to 2013 were collected from patient nasopharyngeal lavage and expanded in HEp-2 cell culture at [redacted] (b) (4), using similar methodology. Also, RSV isolates collected as part of the OUTSMART program (2015 to 2016 and 2016 to 2017 seasons), were evaluated using the microneutralization assay.

Results

Neutralization of Laboratory Strains of RSV

The 1G7 mAb neutralized RSV A2 and B9320 with mean EC_{50} values of 13pM (1.9 ng/mL) and 11 pm (1.6 ng/mL), respectively. In comparison, palivizumab neutralized RSV A2 and B9320 with mean EC_{50} values of 2.3nM (347 ng/mL) and 1.6nM (242 ng/mL), respectively, indicating that 1G7 was approximately 180-fold and 150-fold more potent, respectively, than palivizumab against these strains. Dose-response curves from representative experiments are shown in Figure 76.

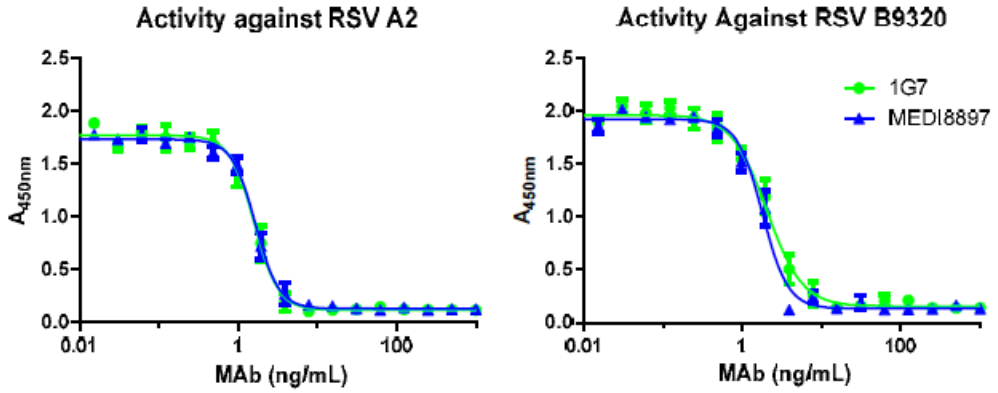
Figure 76. Antiviral Activity of 1G7 Against RSV A2 and RSV B9320



Source: pages 15-16, study report ID8897-0001
Viral replication was monitored by measuring the expression of RSV F protein in infected cells using an ELISA and is presented as absorbance units measured at 450 nm. EC_{50} values were calculated and represent the concentration of mAb required for a 50% reduction in absorbance measured at 450 nm.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; RSV, respiratory syncytial virus

The microneutralization assay was repeated using 1G7 and nirsevimab (Figure 77). 1G7 neutralized RSV A2 and RSV B9320 with EC_{50} values of 11pM (1.7 ng/mL) and 14pM (2.1 ng/mL), respectively. Nirsevimab neutralized RSV A2 and RSV B9320 with EC_{50} values of 11pM (1.6 ng/mL) and 12pM (1.8 ng/mL), respectively. These data indicate that the YTE substitutions in the Fc domain of nirsevimab do not affect the antiviral activity in this assay.

Figure 77. Antiviral Activity of Nirsevimab Against RSV A2 and RSV B9320



Source: page 17, study report ID8897-0001
Viral replication was monitored by measuring the expression of RSV F protein in infected cells using an ELISA and is presented as absorbance units measured at 450 nm. EC50 values were calculated and represent the concentration of mAb required for a 50% reduction in absorbance measured at 450 nm.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; RSV, respiratory syncytial virus

Neutralization of RSV Clinical Isolates

In the microneutralization assay using a panel of clinical isolates from 2004 to 2008, 1G7 inhibited RSV A isolates with a median EC_{50} value of 21pM (3.15 ng/mL; n=54, range: 3pM [0.48 ng/mL] to 100pM [15 ng/mL]) and RSV B isolates with a median EC_{50} value of 20pM (3.0 ng/mL; n=41, range: 5pM [0.8 ng/mL] to 398pM [59.7 ng/mL]). EC_{50} values for individual isolates are listed in Table 177.

The range of EC_{50} values was larger for RSV B isolates because of a single outlier, for which 1G7 had an EC_{50} value of 398pM (59.7 ng/mL), approximately 20-fold higher than the median value for the isolates tested. This outlier harbored polymorphisms within both the F_2 (K65Q) and F_1 (S211N) regions of the nirsevimab/1G7 epitope. Evaluation of the antiviral activity of 1G7 against a clinical isolate expressing K65Q and against a recombinant virus expressing S211N indicated that the combination of K65Q/S211N was required for the observed reduction in susceptibility (see Section 20.7). However, it is unclear if the genetic backgrounds of the three viruses are identical, given that two of the three viruses were clinical isolates, and one was a recombinant virus.

Table 177. Neutralization Activity of 1G7 Against RSV A and RSV B Clinical Isolates

Isolate Name	Season	Location	EC_{50} (ng/mL)	Sequence Variation[a]	$TCID_{50}$ Assayed
RSV A isolates					
CP117 A3	2006	USA	3.2	NA	1000
CP117 A6	2006	USA	2.5	NA	299
CP117 A14	2006	USA	2.3	NA	178
CP117 A15	2006	USA	3.8	NA	106
CP117 A17	2006	USA	1.6	NA	63
CP117 A18	2006	USA	4	NA	211
CP117 A24	2005	USA	3.6	NA	355
CP117 A29	2006	USA	4.5	I206V, S213R	299
CP117 A33	2006	USA	8.5	NA	841
CP117 A49	2007	USA	1.8	NA	422
CP117 A51	2007	USA	1.5	NA	211
CP117 A58	2005	USA	1.5	NA	501
CRL CP096 A12	2006	USA RI	3.7	NA	1000
CRL CP096 A14	2006	USA RI	0.48	NA	299
CRL CP096 A15	2006	USA RI	1.6	NA	1189
CRL CP096 A22	2006	USA CO	4.5	NA	501
CRL CP096 A26	2006	USA CO	3.2	I206T	150
CRL CP096 A27	2005	USA TX	2.7	NA	2371
CRL CP096 A30	2005	USA NY	3.1	NA	2371
CRL CP096 A31	2006	USA NY	1.6	NA	596
CRL CP096 A33	2006	USA NY	3.1	NA	299
CRL CP096 A35	2006	USA NY	2.2	NA	1189
CRL CP096 A38	2006	Italy	7.9	NA	2371
CRL CP096 A43	2006	Italy	12	NA	2371
CRL CP096 A44	2006	Italy	10.6	NA	2371
CRL CP096 A45	2006	Italy	6.1	NA	596
CRL CP096 A46	2006	Italy	15	NA	2371
CRL CP096 A47	2004	Italy	14.9	NA	501
CRL CP096 A56	2005	Australia	8.9	NA	596
CP096 CA20	2004	USA NY	4.3	S211G	501
CRL NLD A37	2006	Netherlands	2.7	NA	1000
CRL NLD A39	2006	Netherlands	3.6	NA	1995
CRL NLD A40	2007	Netherlands	5.8	NA	2818
CRL NLD A41	2007	Netherlands	4	NA	1679
CRL NLD A44	2007	Netherlands	4.8	NA	708
CRL NLD A45	2007	Netherlands	1.2	NA	63

Isolate Name	Season	Location	EC_{50} (ng/mL)	Sequence Variation[a]	$TCID_{50}$ Assayed
CRL NLD A49	2007	Netherlands	3.1	NA	211
CRL NLD A54	2007	Netherlands	1.8	NA	211
CRL NLD A55	2007	Netherlands	3.2	NA	841
CRL NLD A57	2008	Netherlands	1.3	NA	150
CRL NLD A58	2008	Netherlands	3.2	NA	211
CRL NLD A59	2008	Netherlands	2.8	NA	422
CRL NLD A63	2008	Netherlands	1.2	NA	75
CRL NLD A64	2008	Netherlands	0.85	NA	89
CRL NLD A66	2008	Netherlands	0.6	NA	106
CRL NLD A67	2008	Netherlands	0.6	NA	106
CRL NLD A68	2008	Netherlands	1.1	NA	596
CRL NLD A69	2008	Netherlands	0.84	NA	596
CRL ISR B28	2004	Israel	10.4	NA	422
CRL ISR B101	2007	Israel	3.7	NA	705
CRL ISR B121	2007	Israel	8.2	NA	2818
CRL NLD A60	2008	Netherlands	2.1	NA	32
CRL NLD A61	2008	Netherlands	0.94	NA	32
CRL ISR 071	2005	Israel	4.8	N63S	38
RSV B isolates					
CP117 B15	2006	USA	8.3	NA	596
CP117 B18	2005	USA	2.1	NA	63
CP117 B73	2006	USA	2.9	NA	126
CP117 B77	2005	USA	3.3	NA	355
CP096 B15	2004	USA NY	8.1	Q209K	1000
CRL CP096 B5	2005	USA PA	1.1	NA	355
CRL CP096 B11	2005	USA RI	1.9	NA	1413
CRL CP096 B16	2006	USA RI	3.7	NA	1189
CRL CP096 B19	2006	USA CO	4.9	NA	178
CRL CP096 B25	2006	USA CO	0.87	NA	1000
CRL CP096 B36	2006	USA NY	6.6	NA	2371
CRL CP096 B59	2006	Australia	9.4	NA	2818
CRL NLD B1	2005	Netherlands	1.8	NA	126
CRL NLD B9	2005	Netherlands	6	NA	1000
CRL NLD B17	2005	Netherlands	4.2	NA	53
CRL NLD B27	2005	Netherlands	13.8	NA	841
CRL NLD B38	2006	Netherlands	4.3	NA	355
CRL NLD B48	2007	Netherlands	2	NA	299
CRL NLD B50	2007	Netherlands	2.6	NA	178
CRL NLD B53	2007	Netherlands	3.7	NA	150
CRL NLD B62	2008	Netherlands	2.1	NA	63
CRL NLD B65	2008	Netherlands	8.5	NA	89
CRL NLD B70	2008	Netherlands	1.4	NA	53
CRL ISR B12	2004	Israel	8.3	NA	106
CRL ISR B19	2004	Israel	5.3	NA	53
CRL ISR B50	2004	Israel	1.4	NA	63
CRL ISR B57	2005	Israel	3	NA	106
CRL ISR B62	2005	Israel	6.3	NA	1189
CRL ISR B73	2005	Israel	5.4	NA	596
CRL ISR B56	2005	Israel	1.2	NA	32

Isolate Name	Season	Location	EC_{50} (ng/mL)	Sequence Variation[a]	$TCID_{50}$ Assayed
CRL NLD B42	2007	Netherlands	2.9	NA	32
CRL NLD B46	2007	Netherlands	0.8	NA	38
CRL NLD B47	2007	Netherlands	2.1	NA	27
CRL NLD B51	2007	Netherlands	1.9	NA	38
CRL NLD B05	2005	Netherlands	2	NA	27
CRL NLD B18	2005	Netherlands	3.8	NA	45
CP117 B42	2006	USA	1.2	E66D	38
CP117 B76	2005	USA	1.4	NA	32
CRL V B27	Unk	USA TN	5.3	T67I	1995
CRL NLD 007[b]	2005	Netherlands	59.7	K65Q, S211N	150
CP110-B012	2005	Brazil	2.3	K65Q	178

Source: pages 12-15, study report ID8897-0002
[a] Sequence variation in RSV F from consensus (epitope regions)
[b] RSV B isolate seen as outlier
Abbreviations: EC_{50}, half-maximal effective concentration; NA, not applicable; RSV, respiratory syncytial virus; $TCID_{50}$, tissue culture half-maximal infectious dose; Unk, unknown

Additional clinical isolates from the 2011 and 2012/2013 seasons, and subsequently from 2015/2016 and 2016/2017 seasons as part of the OUTSMART surveillance program (Section 18.1), were assessed by microneutralization using 1G7 or nirsevimab. For the 2011 to 2013 seasons (Table 178), there were 5 RSV A isolates and 2 RSV B isolates, and all showed sensitivities to nirsevimab neutralization which were similar to those seen with the panel collected from 2003 to 2008, including one isolate which contained a polymorphic D200N substitution in the nirsevimab binding region. The median EC_{50} value for these 7 isolates was 21pM (3.1 ng/mL), ranging from 7pM (1.1 ng/mL) to 46pM (6.9 ng/mL).

For the 2015/2016 and 2016/2017 season isolates (Table 179), there were a total of 11 RSV A and 6 RSV B isolates, and one additional isolate from 2013 of each subtype as a reference. Compared with the reference isolate, all isolates were susceptible to nirsevimab, with EC_{50} values within 3-fold. The median EC_{50} value for all isolates (n=17, excluding the two references) was 25pM (3.8 ng/mL), ranging from 2pM (0.3 ng/mL) to 59pM (8.8 ng/mL), similar to values seen for isolates from prior years.

Table 178. Neutralization Activity of Nirsevimab Against RSV A and RSV B Clinical Isolates Collected From 2011 to 2013

RSV Subtype	Isolate Name	Season	Geographic Location	EC_{50} (ng/mL)
RSV A	RSVA/12-000782	2012	Netherlands	2.4
	RSVA/13-001348	2013	Netherlands	3.1
	RSVA/13-005589	2013	Netherlands	2.0
	RSVA/12-048821[a]	2012	Netherlands	5.3
	RSVA/12-049788	2012	Netherlands	6.9
RSV B	RSVB/11-014548	2011	Netherlands	3.8
	RSVB/11-051327	2011	Netherlands	1.1

Source: page 18, study report ID8897-0011-amend-1
[a] Contains a polymorphic change (D200N) in the nirsevimab binding region
RSV-A isolates were tested against 1G7, RSV-B isolates were tested against nirsevimab
Abbreviations: EC_{50}, half-maximal effective concentration; RSV, respiratory syncytial virus

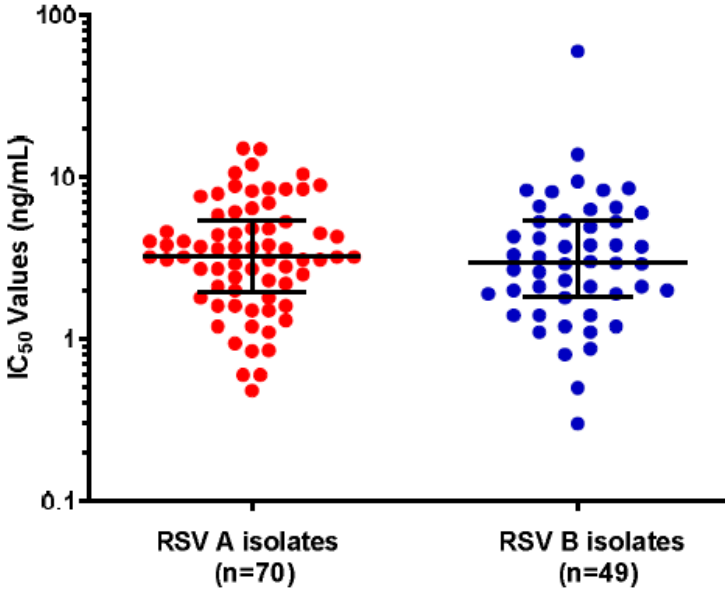
Table 179. Neutralization Activity of Nirsevimab Against RSV A and RSV B Clinical Isolates Collected From 2013 to 2017

		RSV F Protein				Nirsevimab	
RSV Subtype	Isolate Name	Region in F Subunit	Amino Acid Changes[a]	Geographic Location	Season	EC_{50} (ng/mL)	Fold Reduction[d]
RSV A	RSVA/13-005275	–	None	Netherlands	2013	1.0	2.2
	20000230655	F2	P101L	USA, WI	2015	7.6	1.9
	20000230691	–	None	USA, WI	2015/2016	4.6	1.2
	20000230702	–	None	USA, WI	2015/2016	3.8	1.0
	20000230737	–	None	USA, WI	2015/2016	8.4	2.2
	5013-004[b]	F1	I152M	USA, NE	2015/2016	2.9	0.7
	5013-006	–	None	USA, NE	2015/2016	8.4	2.2
	5013-018	–	None	USA, NE	2015/2016	6.4	1.6
	5013-024	–	None	USA, NE	2015/2016	8.8	2.3
	5013-030[b]	F2	P101S	USA, NE	2015/2016	4.4	1.1
	Osmt17-0433	F2	P101L	USA, MA	2016/2017	2.7	1.2
	CM-B03-64	NA	NA	China	2014	3.7	1.8
RSV B	RSV B/13-001273	–	None	Netherlands	2013	1.0	1.5
	5013-012	F2 and F1	A103V/L172Q/S173L/P312H/S377N	USA, NE	2015/2016	6.5	1.5
	Osmt17-0210-B046[c]	F2 and F1	A103V/L172Q/S173L/K191R/I206M/Q209R	USA, PA	2016/2017	0.5	0.3
	Osmt17-0989-B047[c]			USA, NE	2016/2017	0.3	0.2
	XY-B01-08	NA	NA	China	2014	2.9	1.6
	XY-B01-09	NA	NA	China	2014	2.7	1.5
	XY-B01-13	NA	NA	China	2014	3.2	1.7

Source: study reports ID8897O-1516-amend-2 (page 29), ID8897O-1617 (page 35), and MDMN-20140127 (page 21)
[a] RSV F protein substitutions among 2015-2016 RSV strains compared to year 2013 RSV A/13-005275 and RSV B/13-001273 reference strains, respectively
[b] Putative cell culture adaptive substitutions P101S and I152M were observed in F protein derived from 5013-030 and 5013-004 isolates, respectively, in the RSV F protein compared to the sequence directly obtained from nasal samples
[c] Putative cell-culture adaptive substitution, S405L was observed in in the RSV F protein derived from Osmt17-0210-B046 and Osmt17-0989-B047 isolates compared to the sequence directly obtained from nasal samples
[d] Fold change in EC50 value of mAb compared to wild type reference strain tested in parallel on the same plate unless otherwise indicated
Abbreviations: EC_{50}, half-maximal effective concentration; NA, not available (not included in study reports); RSV, respiratory syncytial virus

Overall, for all clinical isolates evaluated, the median EC_{50} value for nirsevimab/1G7 against RSV A isolates (n=70) was 21pM (3.2 ng/mL), ranging from 3pM (0.48 ng/mL) to 100pM (15.0 ng/mL). The median EC_{50} value for RSV B isolates (n=49) was 19pM (2.9 ng/mL), ranging from 2pM (0.3 ng/mL) to 398pM (59.7 ng/mL). The individual EC_{50} values are plotted in Figure 78.

Figure 78. Distribution of EC_{50} Values for RSV A and RSV B Clinical Isolates



Source: page 17, pharmacology written summary
1G7 EC50 values were determined against a panel of 70 RSV A and 49 RSV B clinical isolates collected between 2003 and 2017. The EC50 value of each individual isolate is plotted as a function of its subtype. Medians and the interquartile range are indicated for each data set.
Abbreviations: EC_{50}, half-maximal effective concentration; RSV, respiratory syncytial virus

Conclusions

Laboratory isolates of RSV A and RSV B were neutralized by 1G7 with EC_{50} values of 13pM and 11pM, respectively, which were approximately 180- and 150-fold lower, respectively, than for palivizumab. A geographically and temporally (2003 to 2017) diverse panel of RSV A and RSV B isolates were susceptible to neutralization by nirsevimab/1G7 with similar median EC_{50} values of 21 and 19pM, respectively, and a single RSV B outlier which was approximately 20-fold less susceptible to 1G7. The loss of susceptibility for this outlier was likely a consequence of two substitutions, K65Q/S211N, in the epitope region of nirsevimab/1G7. A more comprehensive evaluation of polymorphisms is described in Section 20.7, and the temporal and consensus genetic variability of F protein sequences and nirsevimab resistance-associated substitutions in recently circulating isolates is discussed in Section 18.1.

The activity of nirsevimab in human airway epithelial (HAE) cells has also been assessed, using RSV A2 expressing green fluorescent protein (GFP; (Kinder et al. 2020)). Following preincubation for 1 hour prior to infection, nirsevimab and palivizumab completely blocked RSV A2-GFP entry at concentrations of 3.3nM (0.5 µg/mL) and 68nM (10 µg/mL), respectively. In addition, HAE infected with RSV A2-GFP and treated with mAbs 6 hours later showed a dose-dependent reduction in GFP signal 48 to 72 hours later. Hence, nirsevimab appeared to block

Reference ID: 5208810

viral entry and cell-cell spread in a 3D HAE cell culture model, with 10- and 20-fold higher activity than palivizumab. Whether the activity and differences seen with palivizumab in this model, or the cotton rat model of infection (6-fold higher activity for nirsevimab against RSV A2; Section 20.8), are more predictive than HEp-2 cell culture of potential clinical activities, is not clear.

20.7. Nirsevimab Resistance in Cell Culture

20.7.1. Nirsevimab Neutralization of RSV Variants With Polymorphic Changes in the Nirsevimab Binding Site

Study report: ID8897-0011-amend-1.

The ability of nirsevimab to neutralize RSV variants harboring polymorphic substitutions in the nirsevimab binding region was assessed. Polymorphisms were identified from GenBank® and the Applicant's internal databases (Section 20.2) and were evaluated using a microneutralization assay as single or double substitutions in recombinant RSV or using the clinical isolate containing the polymorphism of interest.

Methods

Generation of Recombinant RSV

RSV F protein amino acid substitutions were engineered as nucleotide mutations in the F genes of full-length RSV A2 or RSV B9320 plasmid vectors by site-directed mutagenesis, using standard methodology. Recombinant virus was generated using an approach described by (Hotard et al. 2012). Essentially, BSR-T7 cells plated one day prior at 1.2×10^6 cells/well in 6-well plates were transfected with 1.6 µg of the full-length RSV plasmid carrying the F mutation of interest and 0.4 µg of N, P and M2.1, and 0.2 µg of L helper plasmids, using 8 µL of lipofectamine 2000 and following the manufacturer's guidelines. Transfected BSR/T7 cells were passaged every 2 to 3 days until cytopathic effect was seen. Virus was harvested from supernatants and propagated by passaging in HEp-2 cells. Recombinant viruses were confirmed to have the substitutions of interest through population sequencing.

Microneutralization Assay

Neutralization activity of nirsevimab against RSV variants was conducted as described in Section 20.6.

Results

For RSV A and RSV B, a total of 16 polymorphisms in each subtype were identified from GenBank® and internal databases spanning the years 1956 to 2014 (Table 180). For RSV A, 14 variant viruses were available for phenotypic testing, and all were neutralized by nirsevimab/1G7, with EC_{50} values within 3-fold of that of the recombinant RSV A2 reference virus. For RSV B, 15 variants were available for testing, and 9 of these were neutralized by

nirsevimab/1G7 with EC_{50} values within 3-fold of the RSV B9320 reference virus. Three RSV B variants with K65Q, K65T or Q202R substitutions, had approximately 4-fold reduced susceptibility. One variant with K65Q/S211M substitutions had reduced susceptibility compared with recombinant RSV of 36-fold, and the remaining two RSV B variants, with K65Q/K68N and K203I substitutions, had reduced susceptibility of 1,239- and 3,005-fold, respectively.

For the RSV B polymorphisms, which had been identified using a panel of 860 F sequences, substitutions at position 65 causing minor (4-fold) or moderate (36-fold) reductions in susceptibility were seen in 11 (1.3%; K65Q), 2 (0.2%; K65T), 2 (0.2%; Q202R), and 6 (0.7%; K65Q/S211N) sequences. The substitutions in the two variants with substantial reductions in susceptibility to nirsevimab/1G7 (K65Q/K68N and K203I) were seen in one sequence each (0.1%).

Table 180. Nirsevimab Neutralization of RSV Variants Containing Polymorphic Changes in the Nirsevimab Binding Site

RSV Subtype	Location of Polymorphism in F Protein	Polymorphic Changes in Binding Site[a]	Number of Sequences	Frequency of Polymorphism (%)	EC_{50} (ng/mL)[b]	Fold Reduction[c]
RSV A (=N1,525)	F2	N63T	7	0.5	8.8	1.6
		N63S	36	2.4	4.8[d]	1.5
		K65N	1	0.1	NA	NA
		K65R/C69G	1	0.1	NA	NA
		E66K	9	0.6	5.6	1.0
		K68M	1	0.1	4.3	0.8
	F1	N197T	1	0.1	7.4	1.3
		I199M	1	0.1	6.8	1.2
		D200N	2	0.1	5.3[d]	1.7
		L203F	1	0.1	3.7	0.7
		I206T	1	0.1	3.2[d]	1.0
		I206V	10	0.7	4.5[d]	1.5
		V207A	1	0.1	6.7	1.2
		K209R	1	0.1	4.2	0.8
		S211G	1	0.1	4.3[d]	1.4
	F1 and F2	E66K, L203V	1	0.1	3.2	0.6
RSV B (N=860)	F2	K65Q	11	1.3	23[d]	**3.8**
		K65Q, K68N	1	0.1	7,372	**1,239**
		K65T	2	0.2	23	**3.8**
		E66D	5	0.6	1.2[d]	0.4
		T67I	8	0.9	5.3[d]	1.8
		K68R	1	0.1	NA	NA
	F1	N197S	5	0.6	6.0	1.0
		N201S/Q209K	16	1.9	2.8 [d]	0.9
		Q202R	2[e]	0.2	21.6	**3.6**
		L203I	1	0.1	17,878	**3,005**
		I206L	1	0.1	2.9	0.5
		V207L	1	0.1	3.2	0.5
		Q209K	32	3.7	3.6[d]	1.2
		S211N	2	0.2	2.7	0.5

RSV Subtype	Location of Polymorphism in F Protein	Polymorphic Changes in Binding Site[a]	Number of Sequences	Frequency of Polymorphism (%)	EC_{50} (ng/mL)[b]	Fold Reduction[c]
		S211I	1	0.1	2.8	0.5
	F1 and F2	K65Q, S211N	6	0.7	107[d]	**35.7**

Source: page 17, study report ID8897-0011-amend-1; page 13, study report ID8897-0013
[a] One RSV A F protein sequence containing amino acid changes at positions from 63 to 66 (N63D/I64M/K65R/E66G) was excluded due to a potential sequence error
[b] Values from one or two (average value) independent experiments with 4 replicates. The E66K and N197S polymorphisms occur in the rRSV A2 and B9320 reference viruses, respectively
[c] Fold reductions calculated by dividing the EC_{50} values with the EC_{50} value for wild type RSV (median RSV-A EC_{50}=3.1 ng/mL and median RSV-B EC_{50}=3.0 ng/mL) or recombinant reference strains (rRSV-A2 EC_{50}=5.60 ng/mL and rRSV B9320 EC_{50}=5.95 ng/mL). EC_{50} values for all variants except isolate containing N201S/Q209K were from report ID8897-0011-amend-1
[d] EC_{50} value determined using clinical isolate containing indicated polymorphism
[e] One sequence contains a mixed Q/R at position 202
Bold values: ≥3-fold change compared with reference
Polymorphisms were evaluated in microneutralization assays as substitutions in recombinant RSV-A2 (for RSV A) or recombinant RSV-B9320 (for RSV B) unless otherwise noted. RSV-A polymorphisms were tested against 1G7, RSV-B polymorphisms were tested against nirsevimab or IG7.
Abbreviations: EC_{50}, 50% effective concentration; NA, not available; RSV, respiratory syncytial virus

Conclusions

Most of the polymorphisms seen in the nirsevimab binding site did not appear to impact susceptibility to nirsevimab. Substitutions at position K65 in RSV B generally caused moderate reductions in susceptibility, although K65Q in combination with K68N and the single substitution L203I conferred 1,239- and 3,005-fold reductions, respectively; however, these were each only seen in one of the 860 RSV B database sequences analyzed.

20.7.2. Cell Culture Selection of Resistance

Study reports: ID8897-0003, ID8897-0003-amend-1, ID8897-0011-amend-1.

To determine the potential for selection of RSV resistance to nirsevimab, and to identify amino acid residues associated with resistance, cell culture passage experiments were conducted of RSV A and RSV B in the presence of nirsevimab.

Methods

RSV A2 and RSV B9320 viruses were serially passaged three times in HEp-2 cells in the presence of 250 ng/mL of nirsevimab. Viruses were diluted into medium containing nirsevimab, and incubated for 1 hour at 37°C. In the first round of selection, virus/mAb mixture containing approximately $1x10^6$ pfu/mL of RSV A2 or $5x10^5$ pfu/mL of RSV B9320 was added to each well of 24-well plates, which were seeded 1 day prior with approximately $1x10^5$ HEp-2 cells per well. The first passage was allowed to proceed for up to 7 days while cells were observed microscopically for syncytia formation. Cells and supernatants were harvested when syncytia were observed.

Similar methodology was used for the second and third passages, with supernatants containing virus from the first or second passages initially being diluted 1:4,000 in medium containing 250 ng/mL of nirsevimab, then added to HEp-2 cells as for the first passage. Whether harvested supernatants contained virus was determined by ELISA of pellets obtained following centrifugation of the supernatants, using mAb 1331H-HRP conjugate for detection of F protein.

Reference ID: 5208810

Following the third passage, RNA was extracted from pellets containing cell-associated virus, and emergent substitutions in the F gene identified by population nucleotide sequence analysis.

Recombinant RSV harboring substitutions of interest was generated as described in Section 20.7.

Results

From the serial passage experiments, six RSV A2 viruses and nine RSV B9320 viruses were independently selected and isolated for characterization. All six RSV A2 viruses contained N208Y substitution in the F1 epitope region of RSV F, and a likely cell culture adaptive substitution in the F2 subunit, N67I, was seen in both the parental and cell culture passaged viruses (Table 181). Virus with the N67I/N208Y substitutions was 475-fold less susceptible to nirsevimab neutralization compared with the parental isolate. It is unclear if the emergence of N208Y in any of the six RSV A2 passaged cultures was the result of independent events or a single event (i.e., the N208Y-expressing subspecies may have been present in the RSV A2 stock used to initially infect the six separate cultures; nucleotide sequence analysis cannot inform as there is only possible sequence change).

For RSV B, single substitutions of N208D or N208S were observed, and double substitutions of K68N/N201S or K68N/N208S. These substitutions conferred reduced susceptibility to nirsevimab compared with the parental virus, of 5,532-fold for K68N/N201S, 14,623-fold for N208S, and >250,000-fold for N208D and K68N/N208S, with no detectable neutralization activity at the highest concentration tested (Table 181).

Palivizumab showed similar activity against the wild-type and variant RSV A and RSV B viruses, with <2-fold reductions in susceptibility of variants compared with the parental viruses.

Table 181. Neutralization of Nirsevimab Resistance-Associated Substitutions by Nirsevimab and Palivizumab

Subtype	Variants	Amino Acid Substitution	Location of Substitutions in F Subunit	Average EC_{50} of Nirsevimab nM (ng/mL)	Fold Reduction[a]	EC_{50} of Palivizumab nM (ng/mL)	Fold Reduction [a]
RSV-A	Parental RSV A2	N67I	F2	0.014 (2.1)	1	1.3 (194)	1
	RSVA2 A17-B10	N67I, N208Y	F2, F1	6.7 (998.3)	475	1.1 (167)	0.9
RSV-B	Parental RSV B9320	-	-	0.016 (2.4)	1	1.4 (211)	1
	RSV B9320 B14-B6	N208S	F1	237 (35,095)	14,623	0.7 (107)	0.5
	RSV B9320 B15-C2	N208D	F1	>4,000 (>600,000)	>250,000	2.4 (350)	1.7
	RSV B9320 B12-B3	K68N/N208S	F2, F1	>4,000 (>600,000	>250,000	1.2 (182)	0.9
	RSV B9320 B22-B4	K68N/N201S	F2, F1	89.7 (13276)	5,532	0.5 (70)	0.3

Source: page 14, study report ID8897-0003-amend-1
a Fold reduction was calculated from the variant EC_{50} value divided by the EC_{50} value for the parental strains
Abbreviations: EC_{50}, 50% effective concentration; RSV, respiratory syncytial virus

Individual and double substitutions identified in the cell culture selection studies were further assessed by microneutralization assay of recombinant viruses (Table 182). For RSV A, neither N67I nor N208Y individually conferred a reduction in susceptibility to nirsevimab, but N67I/N208Y together caused a 106-fold reduction in susceptibility compared with the parental virus.

K68N and N201S on their own conferred a decrease in RSV-B9320 susceptibility to nirsevimab of 4- and 65-fold, respectively, and when tested together reduced susceptibility by 13,439-fold (Table 182). Unlike RSV-A2, individual substitutions at N208 conferred large reductions in susceptibility to RSV B9320, with N208D and N208S conferring >90,000-fold and 24,619-fold reductions compared with the parental virus. For the combination of K68N and N208S, >90,000-fold reduction in susceptibility was observed, greater than the reductions seen with the individual substitutions.

Table 182. Nirsevimab Neutralization of Recombinant RSV A2 and RSV 9320 Variants With Substitutions Identified in Cell Culture Selection Studies

rRSV Subtype	Amino Acid Substitution	Location in F Subunit	Average EC_{50} (ng/mL)	Fold Reduction[b]
rRSV A2[a]	Parental wild type	-	1.7	1
	N67I	F2	2.6	1.5
	N208Y	F1	1.8	1.1
	N67I, N208Y	F2, F1	174.3	**102.5**
rRSV B9320	Parental wild type	-	2.2	1
	K68N	F2	8.3	**3.8**[c]
	N201S	F1	142	**64.5**[c]
	N208D	F1	>200,000	**>90,000**
	N208S	F1	54,161	**24,618.6**
	K68N, N201S	F2, F1	29,565	**13,438.6**
	K68N, N208S	F2, F1	>200,000	**>90,000**

Source: page 19, study report ID8897-0011-amend-1
[a] Average EC_{50} values were from two independent studies using nirsevimab and/or 1G7, the non-YTE version of nirsevimab
[b] Fold reduction was calculated from the variant EC_{50} value divided by the EC_{50} value for the parental strains.
[c] Further evaluation of RSV B K68N and N201S substitutions in surveillance studies showed a 29.9-fold and 126.7-fold reductions, respectively, compared with the reference (Section 18.1)
Bold values: ≥3-fold change compared with reference
Abbreviations: EC_{50}, 50% effective concentration; rRSV, recombinant RSV; RSV, respiratory syncytial virus

Conclusions

Resistance to nirsevimab was selected in cell culture passage for both RSV A and RSV B and was mediated through acquisition of one or two F protein substitutions. All amino acid substitutions identified at positions 68, 201 and 208 in the variants with reduced susceptibility to nirsevimab were localized in the nirsevimab binding site defined by the 1G7 Fab/RSV F co-crystal structure analysis (Section 20.2; (Zhu et al. 2017)). Individual substitutions at positions 68, 201 and 208 were assessed using recombinant RSV, and shown to confer reduced susceptibility when tested individually in RSV B9320, with greater reductions seen for the combinations of N67I/N208Y in RSV A2 and K68N with N201S or N208S in RSV B9320. For both RSV A and RSV B, the impacts on neutralization correlated with the changes in prefusion F binding activity, although a 5-fold reduction in binding had been seen for N208Y in RSV A (Section 20.3). Hence, in general, it appears that reduction in neutralization activity is mediated

through acquisition of substitutions which reduce or prevent binding to prefusion F. These amino acid residues are highly conserved, with >98% conservation at positions 68, 201 and 208 based on sequences from isolates collected from 1956-2014 (Section 20.2).

Additional published studies by (Zhu et al. 2018) evaluated the growth kinetics in HEp-2 cells of rRSV variants harboring resistance-associated substitutions. Similar growth kinetics were observed for RSV A2 variant with N67I/N208Y substitutions compared with the parental strain, and likewise for RSV B variants with N208S, N208D, K68N/N201S, or K68N/N208S substitutions, indicating that these variants have similar growth properties, at least in cell culture.

RSV B9320 harboring K65Q/S211N substitutions has also been evaluated in the cotton rat model of RSV infection (Zhu et al. 2017) , using similar methodology as described in Section 20.8. Weight-based doses of 1G7 or palivizumab ranging from 0.3 to 6 mg/kg were administered intramuscularly one day prior to intranasal challenge with the RSV B variant. Dose-dependent reductions in viral RNA in lungs harvested 4 days postinfection were seen for both 1G7 and palivizumab, although IG7 inhibiting viral replication with approximately 4.4-fold reduced activity (24.65 µg/mL) compared to RSV B9320 (5.6 µg/mL). Hence, it is likely that reduced activity seen in cell culture predicts a reduction in clinical activity, although it is not clear whether the magnitude of reduction would be the same.

20.7.3. Cross-Resistance

Study report: ID8897-0002.

To determine the potential for cross-resistance, a panel of viruses with reduced susceptibility to palivizumab was assessed against nirsevimab using a microneutralization assay.

Methods

Microneutralization assays were performed as described in Section 20.6. Variants with reduced susceptibility palivizumab were assessed, which included ones described in (Zhu et al. 2011).

Results

Palivizumab was not tested in the same experiment, but in cell culture it has >25,000-fold reduction in activity against variants containing K272E/M/Q/T or S275F/L substitutions, and 5,164-fold reduction for variants with K272N substitution (AstraZeneca 1998). 1G7 neutralized all variants with reduced susceptibility to palivizumab with EC_{50} values ranging from 3pM (0.44 ng/mL) to 21pM (3.2 ng/mL) (Table 183), similar or lower than the median EC_{50} value determined across RSV A and RSV B clinical isolates (Section 20.6). EC_{50} values of nirsevimab against polymorphic changes N197T, L203F, I206L and S211N, were similar to values reported previously (Section 20.7).

Table 183. 1G7 Neutralization of Palivizumab-Resistant Viruses and Recombinant Viruses

Substitution	Source	1G7 EC_{50} (ng/mL)	$TCID_{50}$
K272E	Palivizumab-resistant variant	1	841
K272N	Palivizumab-resistant variant	0.44	106
K272T	Palivizumab-resistant variant	3.2	1,679
K272Q	Palivizumab-resistant variant	0.8	53
N197T	rA2	5.8	708
L203F	rA2	3.7	63
S275F	rA2	3.1	1,413
S275L	rA2	2.2	53
I206L	rB9320	12.4	178
S211N	rB9320	1.1	596

Source: page 15, study report ID8897-0002
Abbreviations: EC_{50}, half-maximal effective concentration; r, recombinant; $TCID_{50}$, tissue culture half-maximal infectious dose

Conclusions

Nirsevimab was active against variants harboring palivizumab resistance-associated substitutions, with EC_{50} values similar to those observed across RSV A and RSV B clinical isolates. Hence, there appears to be limited potential for cross-resistance, at least in the case of palivizumab, which is also supportive evidence that the two mAbs target different sites on RSV F protein. Likewise, palivizumab did not show reduction in activity against cell culture selected variants harboring substitutions at positions 201 and 208, which had shown reduction in susceptibility to nirsevimab (Section 20.7.2).

20.8. Animal Models

20.8.1. Antiviral Activity in a Cotton Rat Model of RSV Infection

Study reports: ID8897-0006, ID8897-0007.

The ability of 1G7 to inhibit replication of RSV A2 and RSV B9320 in the lungs of cotton rats was assessed, with the objective of determining the serum concentrations of 1G7 which correlate with a 2 $\log_{10}$ and >3 $\log_{10}$ decrease in RSV lung titers.

Methods

Eight groups of 4 animals each were dosed intramuscularly with 1G7, ranging from 0.125 to 3 mg/kg, one day prior to intranasal challenge with $1x10^6$ pfu of RSV A2. For comparison, palivizumab doses ranging from 0.25 to 8 mg/kg were evaluated in parallel experiments, also using 8 groups of 4 animals each. One additional group was included as a control, with no mAb administered. Activity against RSV B9320 was assessed similarly, with the same 1G7 dosing groups, but only 3 groups testing different doses of palivizumab (0.25, 2.0, 8.0 mg/kg), and with two control groups with no mAb administered. Animals were sacrificed four days postinfection.

RSV titers in cotton rat lung homogenates were determined for each dose group. Lungs were harvested and homogenized and frozen prior to analyses. Thawed homogenates were diluted in minimal essential medium supplemented with 5% fetal bovine serum, and RSV titers determined by plaque assay on HEp-2 cells, using standard methodology (see Section 20.5.2).

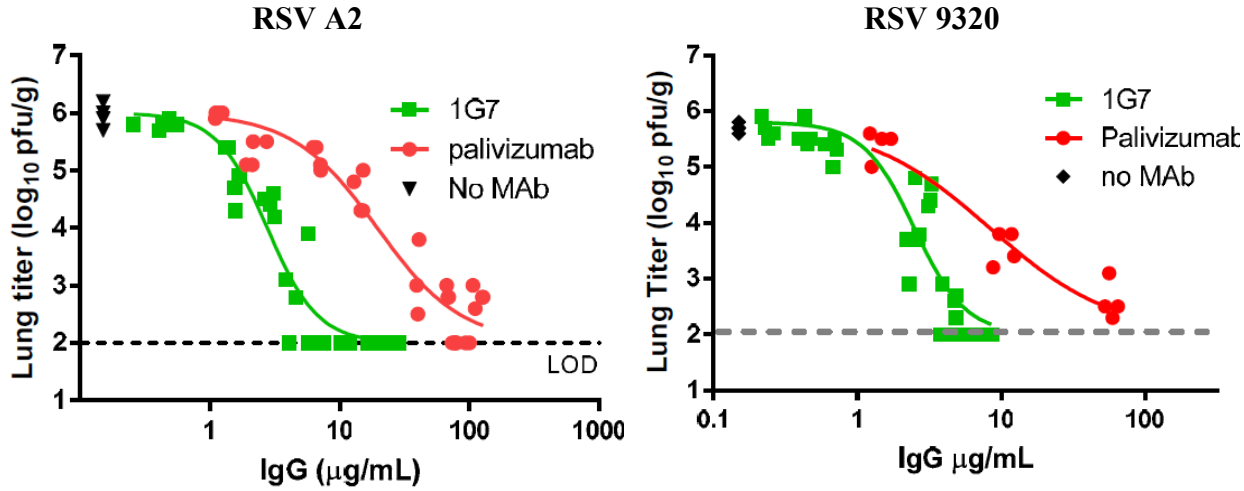
MAb concentrations were measured in serum by ELISA, using standard methodology. Human antibodies were captured by a goat anti-human antibody bound to microtiter plates. Bound human antibody was detected using a goat anti-human IgG peroxidase conjugate, and 3,3',5,5'-tetramethylbenzidine (TMB) as substrate. Absorbance was measured at 450 nm.

Results

For each dose assessed, the serum concentration of human IgG at the time of lung harvest was plotted against the corresponding viral titer measured in the lung homogenate and converted to $\log_{10}$ pfu per gram of lung tissue (Figure 79). A nonlinear regression analysis was conducted, in which the maximum was set to the maximal $\log_{10}$ concentration of untreated animals, and the minimum set to the limit of detection. Half-maximal effective concentration (EC_{50}) and 90% effective concentration (EC_{90}), resulting in 50% and 90% reductions in $\log_{10}$ pfu/gram, respectively, were calculated (Table 184).

When administered one day prior to infection, the 1G7 mAb inhibited RSV A2 with mean EC_{50} and EC_{90} values of 2.9 and 6.8 µg/mL, respectively, and inhibited RSV B9320 with mean EC_{50} and EC_{90} values of 1.8 and 5.8 µg/mL, respectively. In contrast, palivizumab inhibited RSV A2 with mean EC_{50} and EC_{90} values of 19 and 76 µg/mL, respectively, and inhibited RSV B9320 with mean EC_{50} and EC_{90} values of 6.9 and 51 µg/mL, respectively. Based on a comparison of mean EC_{50} serum concentrations, 1G7 was approximately 6-fold more potent than palivizumab in the cotton rat model of RSV A2 infection and 4-fold more potent than palivizumab in the cotton rat model of RSV B9320 infection.

Figure 79. Antiviral Activity of 1G7 in the Cotton Rat Model of RSV Infection



Source: page 13, study reports ID8897-0006 and ID8897-0007
Viral titers from the lungs of infected animals were determined 4 days after infection using a plaque assay and are represented as log plaque forming units (pfu) per gram of lung tissue. Serum concentrations of each mAb (IgG) were determined 4 days after dosing. The dashed line represents the limit of detection (100 pfu/gm) for the viral titer quantification. Data points represent measurements from individual animals. EC_{50} and EC_{90} values were calculated using a nonlinear regression analysis (GraphPad Prism). Data are from representative experiments.
Abbreviations: IgG, immunoglobulin G; RSV, respiratory syncytial virus

Table 184. Serum Concentrations Resulting in 2 log10 and >3 log10 Reductions in RSV Titers in the Lungs of Cotton Rats

	1G7		Palivizumab	
Experiment	EC_{50} (µg/mL)	EC_{90} (µg/mL)	EC_{50} (µg/mL)	EC_{90} (µg)/mL
RSV A2				
TR023	3.00	6.08	17.94	51.38
TR025	2.76	7.43	19.38	101.41
Mean	2.88	6.76	18.66	76.40
RSV B9320				
TR022	1.14	5.47	5.37	30.65
TR034	2.50	6.13	8.38	72.08
Mean	1.82	5.80	6.88	51.37

Source: page 13, study reports ID8897-0006 and ID8897-0007
Abbreviations: EC_{50}, 50% effective concentration; EC_{90}, 90% effective concentration; RSV, respiratory syncytial virus

Conclusions

Assessment of 1G7 in the cotton rat model of RSV infection showed a reduction in RSV lung titers following challenge, which were approximately 6-fold and 4-fold greater than seen with palivizumab for RSV A and RSV B, respectively. These differences are lower than seen in cell culture, where 1G7 was approximately 180-fold and 150-fold more potent than palivizumab against RSV A2 and RSV B9320, respectively (Section 20.6).

20.8.2. Assessment of Nirsevimab Impact on RSV Immunogenicity in Cotton Rat Model

Study report: ID8897-0032.

The cotton rat model of RSV infection was used to determine whether nirsevimab impacted the immune response to RSV infection.

Methodology

Cotton rats (n=12 animals per group) were injected intramuscularly at Day -1 with the test article 1G7 at 6 mg/kg, the nonrelevant control antibody R347 at 6 mg/kg or were untreated. Animals treated with mAbs were infected intranasally on Days 0 and 77 with 1 x 10^6 pfu RSV A2, and the untreated control group was infected similarly only on Day 0. One other control group of 9 animals was treated with PBS and was only infected on Day 77, and another control group of 4 animals was treated with 6 mg/kg 1G7 and infected on Day 77. Animals were sacrificed 4 days after viral challenge, with 6 animals at Day 4 and Day 81 for each of the untreated, 1G7 and R347 groups and 3 for the PBS group at Day 4 and 6 at Day 81. The 4 animals in the 1G7 control group were sacrificed on Day 81.

RSV titers in lung and nasal turbinate samples were determined by plaque assay of homogenized tissues as described in Section 20.5.2. Human IgG was quantitated using ELISA as described in Section 20.8.1.

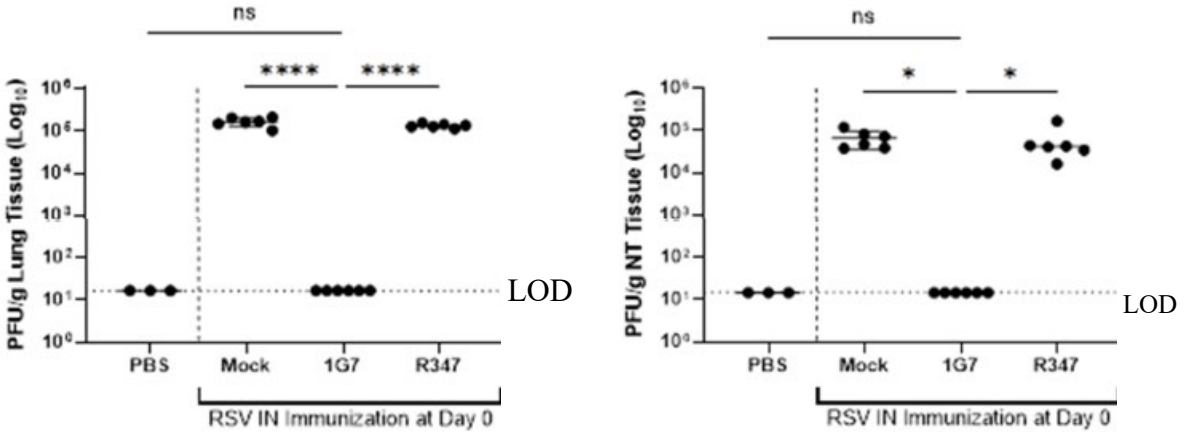
Neutralizing antibodies in serum samples were assessed using a cell-based fluorescent focus assay, using RSV expressing green fluorescent protein (GFP). Serum samples were heat inactivated at 56°C for 45 minutes and 3-fold serially diluted from a 1:8 starting dilution (final dilution 1:16) in 96-well plates. Palivizumab as a control was diluted similarly. Each dilution was mixed with RSV A2 GFP at 1,500 focus-forming units (FFU) per well and incubated for 2 hours at 37°C. HEp-2 cells at 5 x 10^4 cells per well were added, and plates incubated for 20-22 hours at 37°C. Cells were then fixed with formalin and GFP positive cells counted using an ImageXpress Micro XLS plate reader (Molecular Devices), and the $\log_2$ of the IC_{50} value for each serum sample determined. The limit of detection for this assay was 4 $\log_2$.

Results

Figure 80 shows the RSV titers in lung and nasal turbinate tissue at 4 days postinfection. The viral titers in both lung and nasal turbinate tissues of the animals in the 1G7 group were below the limit of detection (16.7 pfu/g of tissue and 14.6 pfu/g of tissue, respectively). For the untreated group, an average viral titer of 1.66 x 10^5 pfu/g of lung tissue was observed, similar to the mean viral titers measured in animals treated with the control mAb R347 (1.33 x 10^5 pfu/g lung tissue).

The remaining animals were left until Day 68 to allow for complete clearance of serum mAb, at which point they were bled, and human IgG titers measured in serum. 1G7 serum concentrations were not detectable in both 1G7-treated groups, and the control R347 mAb was decreased to an average of 0.2 mg/mL (data not shown).

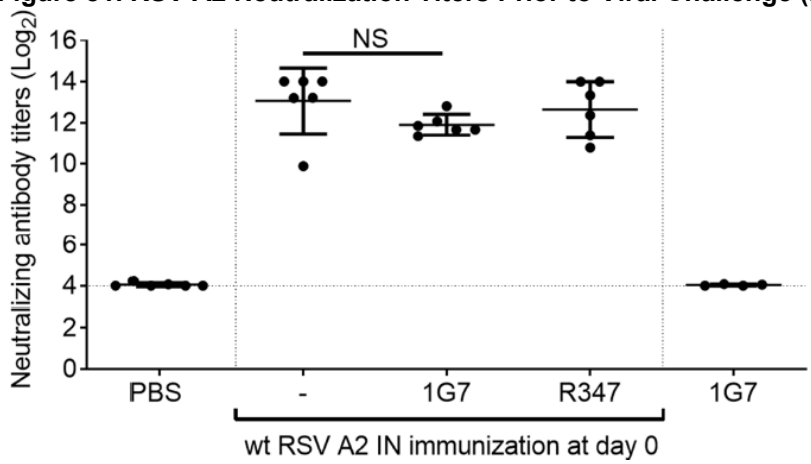
Figure 80. RSV Titers in the Lower and Upper Respiratory Tracts Four Days Post-RSV Challenge (Day 4)



Source: page 15, study report ID8897-0032
Viral titers were determined in lung and nasal turbinate homogenates by plaque assay four days postintranasal challenge with RSV. The mean of individual results +/- SD are shown. The horizontal dashed line represents the LOD (16.7 pfu/g of tissue and 14.6 pfu/g of tissue for lung and NT, respectively). Significance determined by one-way ANOVA. N=3 animals for PBS. N=6 animals for mock-treated, 1G7, and R347 groups, respectively. *p<0.05, ****p<0.0001
Abbreviations: IN, intranasal; LOD, limit of detection; ns, not significant; NT, nasal turbinates; RSV, respiratory syncytial virus

Animals were bled on Day 76 postchallenge and RSV neutralizing antibody titers determined (Figure 81). Neither group which was mock-infected on Day 0 showed detectable titers of anti-RSV neutralizing antibodies, whereas anti-RSV antibodies were detected for all groups infected on Day 0. Anti-RSV titers were not statistically significantly different between challenge groups, with mean antibody titers of 8.5 x 10^3, 3.8 x 10^3, and 12.8 x 10^3 for the mock-treated, 1G7-treated, and R347-treated groups, respectively.

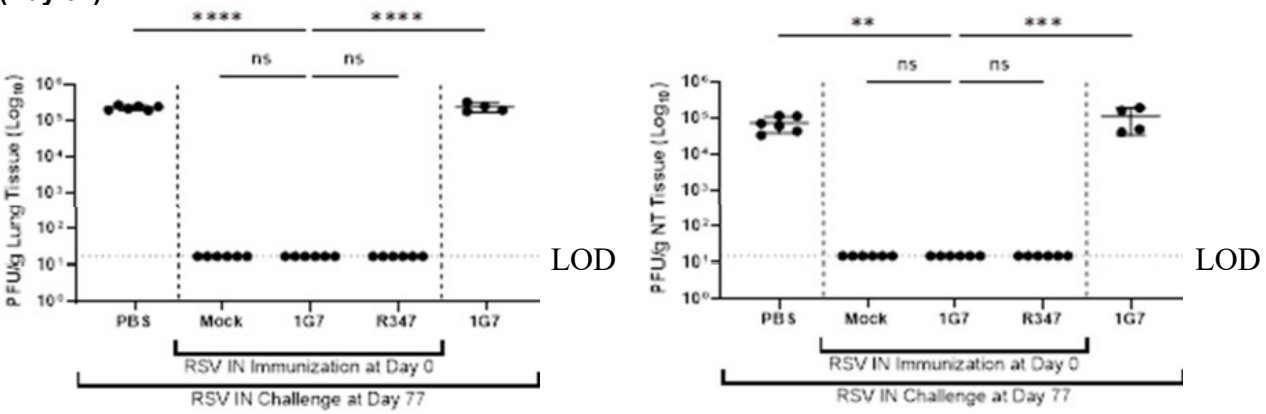
Figure 81. RSV A2 Neutralization Titers Prior to Viral Challenge (Day 76)



Source: page 17, study report ID8897-0032
RSV neutralization titers were determined in serum harvested from animals at Day 76, prior to the second RSV challenge. Titers are presented as the log2 dilution of serum that provides 50% reduction of RSV A2 virus in HEp-2 cells. The mean of individual results +/- SD are shown. Kruskal-Wallis test followed by Dunn's multiple comparison test was applied to compare untreated and 1G7-prophylaxed groups. The horizontal dashed line represents the limit of detection. N=6 animals for PBS (buffer) group that was not challenged on Day 0. N=6 animals for mock, 1G7 and R347 groups that were challenged IN on Day 0 with RSV. N=4 animals for 1G7 group that was not challenged on Day 0.
: - = mock-treated
Abbreviations: IN, intranasal; NS, nonsignificant; RSV, respiratory syncytial virus; wt, wildtype

Cotton rats were challenged with RSV A2 on Day 77 and RSV titers measured in lung and nasal turbinates on Day 81 (Figure 82). High viral titers were seen in groups treated with PBS or 1G7 and were mock infected on Day 0 (lung: 2.33 x 10^5 and 2.43 x 10^5 pfu/g of tissue, respectively; nasal turbinates: 7.53 x 10^4 and 1.15 x 10^5 pfu/g of tissue, respectively). Animals which were untreated or treated with 1G7 or R347 and challenged with RSV on Day 0 did not have measurable titers of RSV at Day 81 following the second challenge (limit of detection [LOD] for lung and nasal turbinate tissue =16.7 pfu/g and 14.6 pfu/g, respectively). Hence, these animals appeared to have developed an effective immune response to RSV which was protective against a second challenge.

Figure 82. RSV Titers in Lung and Nasal Turbinates Four Days Postintranasal Challenge With RSV (Day 81)



Source: page 18, study report ID8897-0032
Viral titers were determined in lung homogenates and nasal turbinate by plaque assay at Day 81, four days postintranasal challenge with wt RSV A2. The mean of individual results +/- SD are shown. The horizontal dashed line represents the LLD (16.7 pfu/g of tissue and 14.6 pfu/g of tissue for lung and NT, respectively). Significance determined by one-way ANOVA. N=6 animals for PBS (buffer) group that was not challenged on Day 0. N=6 animals for mock, 1G7 and R347 groups that were challenged IN on Day 0 with RSV. N=4 animals for 1G7 group that was not challenged on Day 0. **p<0.01, ***p<0.001, ****p<0.0001
Abbreviations: IN, intranasal; LOD, limit of detection; ns, not significant; NT, nasal turbinates; RSV, respiratory syncytial virus

Conclusions

In a cotton rat challenge study, animals treated prophylactically with 1G7 and challenged one day later with RSV, developed an immune response to RSV, as measured by the presence of serum neutralizing antibodies on Day 76, which was protective against subsequent challenge on Day 77. 1G7 is not modified with YTE substitutions in the Fc region, so would be expected to have been cleared by the time of the second challenge (although in rodents, YTE substitutions decrease antibody exposure; (Dall'Acqua et al. 2006)). Hence, in this model, 1G7 did not appear to significantly impact the development of an immune response to RSV, despite preventing the establishment of measurable titers of RSV in lung and nasal turbinates following the initial challenge. It is not clear whether these results can be extrapolated to humans, however.

Antibody-dependent enhancement (ADE) of infection, in terms of enhancement of RSV titers, was not observed in this study, or other experiments with the cotton rat model (Section 20.8.1), at sub-neutralizing doses down to 0.125 mg/kg for the RSV A2 challenge study and 0.03125 mg/kg for RSV B9320. While ADE of RSV infection has been demonstrated for mAbs in immune cell culture, no correlation between cell culture ADE and disease severity in infants has been demonstrated to date (van Erp et al. 2019).

Reference ID: 5208810

21. Other Drug Development Considerations

Not applicable.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

Four sites were inspected in support of this application. Based on the results of these inspections, the trials (Trials 03, 04, and 05) appear to have been conducted adequately, and the data generated by the clinical investigator sites appear acceptable in support of the indication.

Office of Science Investigations Inspection Reports

Site #2002937 for Trials 03, 04, and 05, Dr. Shabir Madhi, Soweto, South Africa

Inspection Dates: February 20-March 3, 2023

- For Protocol 03, 92 subjects were screened, 78 subjects were randomized, and 77 subjects had completed the study.
- For Protocol 04, 191 subjects were screened, 171 subjects were randomized, and 151 subjects had completed the study. Per the Applicant's data line listings, eight subjects were withdrawn by their parent/guardian for unspecified reasons, and ten subjects were lost to follow-up.
- For Protocol 05, 45 subjects were screened, 39 subjects were randomized and completed the study. Per the Applicant's data line listings, four subjects were withdrawn by their parent/guardian for unspecified reasons, one subject was lost to follow-up, and one subject relocated.

An audit of the study records was conducted for 23 of the 78 randomized subjects for Trial 03, 7 of the 171 randomized subjects for Trial 04, and 6 of the 41 randomized subjects for Trial 05. Source records were reviewed for missed cases of MA RSV LRTI as well as comparing the dates of respiratory sample collection, central laboratory results, visit settings, exam findings, and clinical signs in the source documents at the site against the Applicant's data line listings for 23 of the 78 randomized subjects in study Trial 03 and 7 of the 171 randomized subjects in Trial 04.

The findings included the following:

- No discrepancies or missed cases of MA RSV LRTI were noted.
- For Trial 03, there were unreported adverse events of cough, nasal congestion and runny nose for Subject - (b) (6) (randomized to nirsevimab on (b) (6)) documented in the adverse event (AE) log.
- For Trial 05, there were three unreported adverse events for two subjects. Subject - (b) (6) (randomized to nirsevimab on (b) (6) was documented on the AE log and progress notes as having a mild heat rash on (b) (6), and mild nasal congestion

on (b) (6). Per the AE log and progress notes, Subject - (b) (6) (randomized to palivizumab on (b) (6)) was documented as having an upper respiratory tract infection (URTI) that was then deleted and upgraded to a moderate lower respiratory tract infection (LRTI), which ended on (b) (6). No start date was recorded in the source documents for this adverse event.

Site #2004169 for Trial 05, Joseph Domachowske, SUNY Upstate Medical University, Syracuse, NY

Inspection Dates: January 9-12, 2023

At this site for Protocol 05, Dr. Domachowske screened and randomized 20 subjects. Out of 20 randomized subjects, 14 subjects had completed one season of the study. Per the Applicant's data line listings, three subjects withdrew consent, two subjects were lost to follow-up, and one subject was removed from the study due to a family move. Records were reviewed for all 20 screened subjects at this site. Records reviewed included, but were not limited to, protocol versions, eligibility, informed consent, adverse events reporting, protocol adherence, protocol deviations, IRB approvals/acknowledgments, sponsor/IRB correspondence, monitoring reports, investigational product accountability records, and financial disclosures. All source records were in paper format. The primary endpoint of Trial 05 was safety. There was no evidence of underreporting of adverse events or of unreported protocol deviations.

Site #2004872 for Trial 04, Dr. Xavier Saenz Llorens, Tocumen, Panama

Inspection Dates: February 13-17, 2023

At this site for Protocol 04, 228 subjects were screened, 181 subjects were randomized, and 171 subjects had completed the study at the time of inspection as the study was still in the follow-up period. Per the source records, two subjects withdrew from the study after moving, two subjects withdrew consent, and six subjects were lost to follow-up. An audit of the study records was conducted for 135 subjects for adverse events reporting, 51 subjects for reporting of protocol deviations, eligibility, concomitant medications, and informed consent, and 32 subjects for efficacy endpoint data verification.

One protocol violation was reported at this site. Subject - (b) (6) was enrolled despite meeting exclusion criterion #5 in the protocol. The subject was taking a prescription medication (isoniazid) within a week of screening. No discrepancies or missed cases of MA LRTI were noted. There was no evidence of underreporting of adverse events or of unreported protocol deviations.

23. Labeling: Key Changes and Considerations

This Prescribing Information (PI) review includes a high-level summary of the rationale for major changes to the finalized PI as compared to the (FOR NDAs/BLAs: Applicant's draft PI / FOR EFFICACY SUPPLEMENTS: currently approved PI and the Applicant's draft PI) (Table 185). The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is

consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

Prescribing Information Labeling Review

Applicant's proposed labeling submitted on September 26, 2022, was compared with final agreed upon labeling. This review summarizes the major label changes and provides a cross reference to other sections of the Integrated Review for additional details and rationale for the labeling changes. Edits to highlights and table of contents were made to capture changes to the full prescribing information.

(b) (4)

[redacted]

3 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

Reference ID: 5208810

(b) (4)

[redacted]

23.1. Approved Labeling Types

Upon approval of this application, the following labeling documents will be FDA-approved:

- Beyfortus (nirsevimab) prescribing information
- Beyfortus (nirsevimab) patient package information

24. Postmarketing Requirements and Commitments

The following postmarketing requirements (PMRs) and commitments PMCs) have been negotiated with the Applicant and are agreed upon.

Table 186 shows the draft postmarketing requirements and corresponding timetables

Table 186. Postmarketing Requirements

PMR Number	PMR Description	Timetable
1	Provide reports on the prevalence of current and emerging RSV variants, including the frequency of known nirsevimab resistance-associated substitutions, on an annual (AZ-sponsored surveillance studies) or 6-monthly (clinical trials, public sequence databases) basis with the periodic safety report. These study reports should include genotypic data from public sequence database (i.e., GISAID, NCBI GenBank), and both genotypic and phenotypic data from ongoing clinical studies and surveillance activities, including all new variants and substitutions showing ≥ 5-fold reductions in susceptibility: • OUTSMART-RSV and INFORM-RSV studies • Clinical trials of nirsevimab	Final Protocol Submission: N/A Study completion: N/A Interim Report Submissions: • Annual reports of AstraZeneca sponsored surveillance studies • 6-monthly reports of clinical trials, and public sequence databases Final Report Submission: January 2030
2*	Based on surveillance studies and a pooled analysis of RSV A and RSV B isolates from nirsevimab-treated subjects (inclusive of all subjects meeting primary, secondary, or exploratory case definitions), assess phenotypically the individual and concurrent substitutions shown below. **RSV A** <u>Individual substitutions</u> S62G, K65Q, K65R, E110G, L111I, L119I, K123Q, D200N, V247L, W341R, K419E, N515H **RSV B** <u>Individual substitutions</u> R42K, K68R, S190N, N200Y, L204S, N208I, K272R, S389P <u>Concurrent substitutions</u> K68N+I206M+Q209R I206M+Q209R+K272R F15L+A103V+L172Q+S173L F15L+A103V+L172Q+S173L+K191R+I206M+Q209R F15L+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P F15L+R42K+A103V+L172Q+S173L+S190N+K191R+I206M+Q209R+S211N+S389P L204S+I206M+Q209R+S211N	Final Protocol Submission: N/A Study Completion: N/A Interim Report Submissions: • Annual reports of AstraZeneca-sponsored surveillance studies • 6-monthly reports of clinical trials, public sequence databases Final Report Submission: 12/2025

Source: Virology Reviewer
*FDA has also requested to be notified within 2 months of receipt of new phenotypic data for individual or concurrent substitutions showing ≥5-fold reduction in susceptibility, and as soon as possible for substitutions showing ≥100-fold reduction in susceptibility.
Abbreviations: AZ, AstraZeneca; N/A, not applicable; PMR, postmarketing requirements; RSV, respiratory syncytial virus

Table 187 shows the draft postmarketing commitments and corresponding timetables:

Table 187. Postmarketing Commitments

PMC Number	PMC Description	Timetable
1	Conduct a long-term follow-up study (Study D5290N00001: HARMONIE study extension for the UK cohort) to evaluate antibody dependent	Final Protocol Submission: 10/2023 Study Completion for HARMONIE extension: 04/2025

PMC Number	PMC Description	Timetable
	enhancement of RSV disease after nirsevimab administration to neonates and infants prior to or during their first RSV season. The assessment for antibody dependent enhancement of RSV disease should include RSV lower respiratory tract infection (LRTI) hospitalization events. The follow-up period should continue through Day 511 post-dosing.	Final Study Report for HARMONIE extension: 10/2025
2	Conduct an observational, U.S.-based long-term follow-up study of infants eligible to receive nirsevimab in their first year of life to assess the impact of RSV disease through Day 511 post dosing. This study should include assessment of MA RSV LRTI and RSV hospitalization. The study may be conducted using existing databases for the 2023 and 2024 RSV seasons.	Final Protocol Submission: 03/2025 Study Completion: 03/2028 Interim Study Report Submission: 08/2026 Final Study Report Submission: 03/2029
3	Submit the interim and the final study reports and datasets for the ongoing HARMONIE trial, "A Phase IIIb Randomized Open-label Study of Nirsevimab (Versus no Intervention) in Preventing Hospitalizations Due to Respiratory Syncytial Virus in Infants."	Final Protocol Submission: N/A Study Completion: 03/2024 Interim Report Submission: 10/2023 Final Report Submission: 07/2024
4	Submit the final study report and datasets for Trial D5290C00004, a phase 3 randomized, double-blind, placebo-controlled trial to evaluate the safety and efficacy of nirsevimab for the prevention of medically attended RSV respiratory tract infection (MA RSV LRTI) in preterm and term infants.	Final Protocol Submission: N/A Study Completion: 03/2023 Final Report Submission: 10/2023
5	Submit the final study report and datasets for Trial D5290C00005, a double-blind, active-controlled trial to evaluate the safety, efficacy, and pharmacokinetics of nirsevimab for the prevention of medically attended RSV respiratory tract infection (MA RSV LRTI) in high-risk infants and children.	Final Protocol Submission: N/A Study Completion: 03/2023 Final Report Submission: 10/2023
6	Submit the final study report and datasets for Trial D5290C00008, "A Phase 2, Open-label, Uncontrolled, Single-dose Study to Evaluate the Safety and Tolerability, Pharmacokinetics, and Occurrence of Antidrug Antibody for Nirsevimab in Immunocompromised Children ≤ 24 Months of Age."	Final Protocol Submission: N/A Study Completion: 04/2023 Final Report Submission: 10/2023

PMC Number	PMC Description	Timetable
7	Submit the final study report and datasets for the CHIMES trial (D5290C00006; NCT05110261), "A Phase 3, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Safety and Efficacy of Nirsevimab, a Monoclonal Antibody With Extended Half-life Against Respiratory Syncytial Virus, in Healthy Preterm and Term Infants in China."	Final Protocol Submission: N/A Study Completion: 12/2025 Final Report Submission: 08/2026
8	Provide data from real world shipping studies covering all transportation configurations, temperatures, modes, and routes of commercial transportation to evaluate product quality of the final drug product in the commercial container closure system pre- and post-shipment.	Final Protocol Submission: 12/2023 Study Completion: 06/2024 Final Report Submission: 12/2024

Source: Correspondence with Applicant, received July 7, 2023
Abbreviations: M, month; N/A, not applicable; PMC, postmarketing commitment; RSV LRTI, respiratory syncytial virus lower respiratory tract infection; U.K, United Kingdom; U.S., United States; Y, year

25. Financial Disclosure

Table 188. Covered Clinical Studies

Was a list of clinical investigators provided:	Yes ☒☐	No ☐ (Request list from Applicant)
Total number of investigators identified: 2,620		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): None		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 2		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Payment of >%25,000 to 2 investigators. One enrolled one subject. The other enrolled one subject, who was a screen failure. Therefore, neither payments affected the study results. Significant payments of other sorts: None Proprietary interest in the product tested held by investigator: None Significant equity interest held by investigator: None Sponsor of covered study: None		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 2,422		
Is an attachment provided with the reason:	Yes	No ☐ (Request explanation from Applicant)

Abbreviation: FDA, Food and Drug Administration

Reference ID: 5208810

26. References

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Guidance for Industry

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Other

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27. Review Team

Table 189. Reviewers of Integrated Assessment

Role	Name(s)
Clinical Reviewer	Melisse Baylor, MD
Clinical Team Leader Cross-Disciplinary Team Leader	Mary Singer, MD, PhD
Clinical Virology Reviewers	Michael Thomson, PhD Eric Donaldson, PhD
Clinical Virology Team Leader	Jules O'Rear, PhD
Pharmacology/Toxicology Reviewer	Ilona Bebenek, PhD, DABT
Pharmacology/Toxicology Team Leader	Laine Peyton Myers, PhD, DABT
Division director (Pharm/Tox)	Hanan Ghantous, PhD, DABT
OCP Reviewer(s)	Yang Zhao, PhD Justin Earp, PhD Mohsen Rajabiabhari, PhD
OCP Team Leader(s)	Kunyi Wu, PharmD Hao Zhu, PhD Yow-Ming Wang, PhD
Division Director (OCP)	Kellie Reynolds, PharmD
Biometrics Reviewer	Anna Kettermann, Dipl. Math, MA
Biometrics Secondary Reviewer	Thamban Valappil, PhD
Supervisory Mathematical Statistician (OB)	Scott Komo, DrPH
Regulatory Project Manager	Saebyeol Jang, PhD, RAC-US
Associate Director for Labeling	Stacey Min, PharmD
Deputy Director (Safety)	Poonam Mishra, MD, MPH
Deputy Division Director (clinical)	Wendy Carter, DO
Division Director (clinical)	Debra Birnkrant, MD
Associate Director for Therapeutic Review	Yodit Belew, MD
Office Director	John Farley, MD, MPH

Abbreviations: OCP, Office of Clinical Pharmacology; OB, Office of Biostatistics

Table 190. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	
RBPM	Andrew Shiber, PharmD
Review Chief	Rachel Novak, PhD
Reviewer	Deborah Schmiel, PhD
Labeling Assessor	Scott Dallas, RPh
OPMA Facility Reviewer	Wendy Tan, PhD
OPMA Facility Team Leader	Zhong Li, PhD
OPMA Micro Reviewer	Wendy Tan, PhD
OPMA Micro Team Leader	Maxwell Van Tassell, PhD
CMC ATL	Anshu Rastogi, PhD
OPDP	LaToya (Shenee) Toombs, PharmD Sam Skariah, PharmD
DMPP	Susan Redwood, MPH, BSN, RN Jessica Chung, PharmD, MS Barbara Fuller, PharmD (TL)
OSI	Suyoung (Tina) Chang, MD Philip Kronstein, MD
OSE/DMEPA	Melina Fanari, PharmD Madhuri Patel, PharmD (TL)
OSE/OPE IO	Neha Gada, PharmD, BCPS
OSE/OPE/DPV	Kimberley Swank, PharmD Rachna Kapoor, PharmD, MBA Christopher Jones, PharmD, MPH, MS
Other	
Medical Editor	Katelyn Wakefield Aisha Khan Quinn Laffin
Clinical Data Scientist	Anh-Thu Lam, PhD Deangelo McKinley, PhD (TL)
AC Designated Federal Officer	She-Chia Jankowski, PharmD Michael Gu, PharmD (Chief of DACCM/ACMB)

Abbreviations: AC, Advisory Committee; ACMB, Advisory Committee Management Branch; ATL, Application Technical Lead; DACCM, Division of Advisory Committee and Consultant; Management DMEPA, Division of Medication Error Prevention and Analysis; DMPP, Division of Medical Policy Program; DPV, Division of Pharmacovigilance; IO, Immediate Office; OPDP, Office of Prescription Drug Promotion; OPE, Office of Pharmacovigilance and Epidemiology; OPMA, Office of Pharmaceutical Manufacturing Assessment; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations; RBPM, Regulatory Business Process Manager; TL, Team Leader.

27.1. Reviewer Signatures

See next page.

Reference ID: 5208810

Table 191. Signatures of Reviewers

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Reviewer	Melisse Baylor Office of Infectious Diseases DAV	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 1, 2, 3, 4, 6.3, 7, 10, 17, 22, 24, 25	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Melisse S. Baylor -S Digitally signed by Melisse S. Baylor -S Date: 2023.07.11 15:27:43 -04'00'					
Clinical Cross-Disciplinary Team Lead	Mary Singer Office of Infectious Diseases DAV	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections:	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Mary Singer Digitally signed by Mary Singer Date: 2023.07.11 12:31:53 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Virology Reviewer	Michael Thomson Office of Infectious Diseases DAV	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 3.1.1.3, 5.1, 6.3.3, 18, 20	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Michael Thomson -S Digitally signed by Michael Thomson -S Date: 2023.06.29 13:30:32 -04'00'					
Clinical Virology Reviewer	Eric Donaldson Office of Infectious Diseases DAV	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 18.5.3, 18.6	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Eric F. Donaldson -S Digitally signed by Eric F. Donaldson -S Date: 2023.07.11 10:17:06 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Virology Team Leader	Jules O'Rear Office of Infectious Diseases DAV	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 3.1.1.3, 5.1, 6.3.3, 18, 20	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Julian J. O'rear -S Digitally signed by Julian J. O'rear -S Date: 2023.07.11 13:13:39 -04'00'					
Pharmacology/Toxicology Reviewer	Ilona Bebenek Office of Infectious Diseases DPTID	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 7.1, 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Ilona Bebenek -S Digitally signed by Ilona Bebenek -S Date: 2023.07.11 13:37:56 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Pharmacology/Toxicology Supervisor	Laine Peyton Myers Office of Infectious Diseases DPTID	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 7.1, 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Laine P. Myers -S Digitally signed by Laine P. Myers -S Date: 2023.07.12 09:02:58 -04'00'					
Pharmacology/Toxicology Division Director	Hanan Ghantous Office of Infectious Diseases DPTID	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 7.1, 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Hanan N. Ghantous -S Digitally signed by Hanan N. Ghantous -S Date: 2023.07.12 09:17:23 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Pharmacology Primary Reviewer	Yang Zhao Office of Clinical Pharmacology DIDP	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5, 6, 8, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Yang Zhao -S Digitally signed by Yang Zhao -S Date: 2023.07.11 17:28:05 -04'00'					
Clinical Pharmacology Team Leader	Kunyi Wu Office of Clinical Pharmacology DIDP	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 6.3.2, 8.1, 8.2, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Kunyi Wu -S Digitally signed by Kunyi Wu -S Date: 2023.07.12 08:50:02 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Pharmacology/Pharmacometrics Primary Reviewer	Justin Earp Office of Clinical Pharmacology Division of Pharmacometrics	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5, 6, 8, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Justin C. Earp -S Digitally signed by Justin C. Earp -S Date: 2023.07.13 13:08:52 -04'00'					
Clinical Pharmacology/Pharmacometrics Secondary Reviewer	Hao Zhu Office of Clinical Pharmacology Division of Pharmacometrics	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5, 6, 8, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☐ Yes ☐ No
Signature/date/time stamp: Hao Zhu -S Digitally signed by Hao Zhu -S Date: 2023.07.11 18:42:26 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Pharmacology Primary Reviewer	Mohsen Rajabi Abhari Office of Clinical Pharmacology Therapeutic Biologics Program	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Mohsen Rajabi-abhari -S Digitally signed by Mohsen Rajabi-abhari -S Date: 2023.07.11 18:31:36 -04'00'					
Clinical Pharmacology Secondary Reviewer	Yow-Ming Wang Office of Clinical Pharmacology Therapeutic Biologics Program	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Yow Ming C. Wang -S Digitally signed by Yow Ming C. Wang -S Date: 2023.07.11 19:17:15 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Pharmacology Division Director	Kellie Reynolds Office of Clinical Pharmacology DIDP	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 6.3.2, 8.1, 8.2, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Kellie S. Reynolds -S Digitally signed by Kellie S. Reynolds -S Date: 2023.07.11 16:55:02 -04'00'					
Biometrics Primary Reviewer	Anna Kettermann Office of Biostatistics DBIV	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 6.2, 6.3.1, 16	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Anna Kettermann -S Digitally signed by Anna Kettermann -S Date: 2023.07.11 17:11:53 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Biometrics Secondary Reviewer	Thamban Valappil Office of Biostatistics DBIV	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 6.2, 6.3.1, 16	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Thamban I. Valappil -S Digitally signed by Thamban I. Valappil -S Date: 2023.07.11 19:01:27 -04'00'					
Biometrics Tertiary Reviewer	Scott Komo Office of Biostatistics DBIV	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 6.2, 6.3.1, 16	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Scott S. Komo -S Digitally signed by Scott S. Komo -S Date: 2023.07.11 20:12:23 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Regulatory Project Management Regulatory Project Manager	Saebyeol Jang Other DROID	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 12	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Saebyeol Jang -S Digitally signed by Saebyeol Jang -S Date: 2023.07.11 09:53:16 -04'00'					
Clinical Associate Director for Labeling	Stacey Min Office of Infectious Diseases DAV	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Stacey Min -S Digitally signed by Stacey Min -S Date: 2023.07.11 10:21:12 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Other Cross Discipline Safety Advisor	Neha Gada Office of Surveillance and Epidemiology OPE IO	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Section: 7.7.3	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Neha Gada -S Digitally signed by Neha Gada -S Date: 2023.07.11 16:24:30 -04'00'					
Other Division Director	Christopher Jones Office of Surveillance and Epidemiology OPE/DPV II	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Section: 7.7.3	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Steven C. Jones -S Digitally signed by Steven C. Jones -S Date: 2023.07.11 16:53:32 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Deputy Director (Safety)	Poonam Mishra Office of Infectious Diseases DAV	☒ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 24	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Poonam Mishra -S Digitally signed by Poonam Mishra -S Date: 2023.07.13 14:32:13 -04'00'					
Clinical Deputy Director	Wendy Carter Office of Infectious Diseases DAV	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections:	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒Yes ☐ No
Signature/date/time stamp: Wendy W. Carter -S Digitally signed by Wendy W. Carter -S Date: 2023.07.13 14:53:24 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Division Director	Debra Birnkrant Office of Infectious Diseases DAV	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections:	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒Yes ☐ No
Signature/date/time stamp: Debra B. Birnkrant -S Digitally signed by Debra B. Birnkrant -S Date: 2023.07.13 15:45:13 -04'00'					

Reference ID: 5208810

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Associate Director for Therapeutic Review	Yodit Belew Office of Infectious Diseases DAV	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections:	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Yodit Belew -S Digitally signed by Yodit Belew -S Date: 2023.07.13 14:30:45 -04'00'					

Abbreviations: DAV, Division of Antivirals; DBIV, Division of Biometrics IV; DIDP, Division of Infectious Disease Pharmacology; DPTID, Division of Pharm/Tox for Infectious Diseases; DPV II, Division of Pharmacovigilance II; DROID, Division of Regulatory Operations for Infectious Diseases; IO, Immediate Office; OPE, Office of Pharmacovigilance and Epidemiology

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This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MARY E SINGER
07/14/2023 09:14:00 AM

YODIT BELEW
07/14/2023 09:14:56 AM

JOHN J FARLEY
07/14/2023 09:57:27 AM

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW

BLA#: 761328 **DATE REVIEWED: 12/10/2022 to 3/23/2023**

Reviewers: Eric F. Donaldson, Ph.D.

SDN	Date Submitted	Date Received	Date Assigned
000e 0002	09/26/2022	09/26/2022	09/26/2022

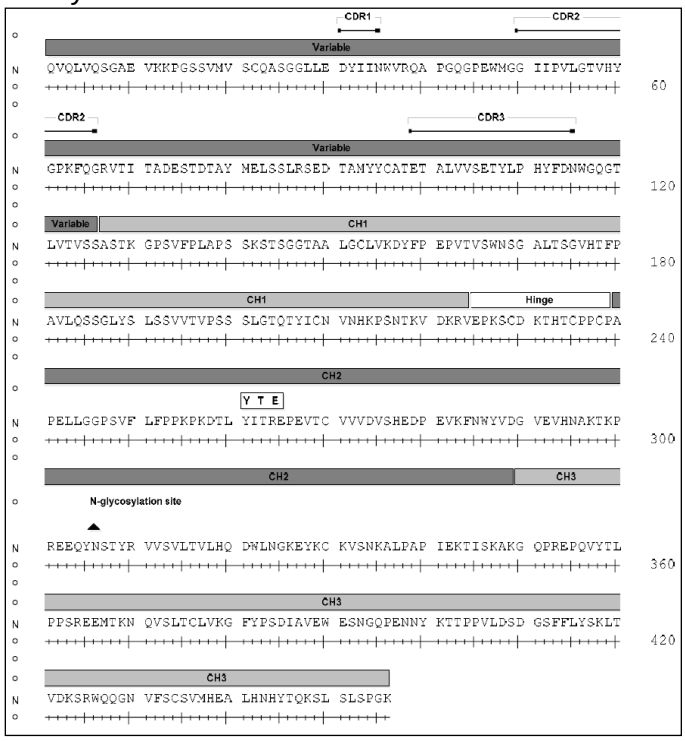
Applicant: AstraZeneca AB
Sodertalje, Stockholm County, Sweden, SE-151 85

US Agent: AstraZeneca Pharmaceuticals LP
1800 Concord Pike
Wilmington, DE 19803
Leslie Sands
Regulatory Affairs Director
Email: leslie.sands@astrazeneca.com
Phone: 202-815-0006
Fax: 301-398-4018

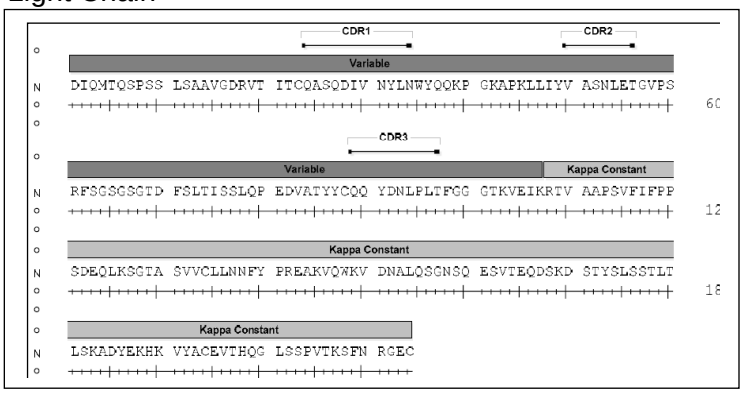
Product Names: MEDI8897, nirsevimab (recombinant human IgG1κ monoclonal antibody)

Structure:

Heavy Chain



Light Chain



Molecular Weight: ~149 kDa

Drug category: Antiviral (Anti-RSV)

Indication: Prevention of (b) (4) lower respiratory tract disease caused by respiratory syncytial virus (RSV) infection.

Dosage Form/Route of Administration: 100 mg/mL (b) (4) intramuscular injection

Supporting Doc.: IND118524

Abbreviations: AA, amino acid; F, fusion protein; LRTI, lower respiratory tract illness; MA, medically attended; mAb, monoclonal antibody; NGS, next generation nucleotide sequencing; NIR, nirsevimab; PAL, palivizumab; PBO, placebo; RSV, respiratory syncytial virus; RT-PCR, reverse transcription-polymerase chain reaction;

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
BLA#: 761328 DATE REVIEWED: 12/10/2022 to 3/23/2023

Executive Summary

This submission is part of the Biologics License Application for nirsevimab, which is a respiratory syncytial virus (RSV) F protein inhibitor monoclonal antibody (mAb) indicated for the prevention of RSV lower respiratory tract disease in: neonates and infants entering or during their first RSV season and children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season. Nirsevimab is a recombinant human IgG1κ mAb that binds with high affinity to a highly conserved, discontinuous neutralizing epitope (AA 62 to 69 and AA 196 to 212) within antigenic site Ø (AA 62 to 96 and AA 195 to 227) on the pre-fusion confirmation of the RSV F protein. The primary Clinical Virology review of this application was performed by Dr. Michael Thomson, Ph.D. (see review BLA761328.000). This review was specifically performed for the assessment of next generation nucleotide sequencing (NGS) data submitted in support of the BLA.

A spot check independent review of the NGS data from a total of 43 subjects from Study 3 (D5290C00003; NCT02878330), MELODY (D5290C00004; NCT03979313), and MEDLEY (D5290C00005; NCT03959488) who met the primary case definition of medically attended (MA) RSV lower respiratory tract infection (LRTI), with or without hospitalization was performed by mapping fastq files from each subject to an RSV A or RSV F-gene reference sequence and comparing the substitutions and frequencies. The NGS sequencing results generated by DAV were largely in agreement with the results reported by the Applicant. No information associated with resistance was obtained from the independent review of NGS data in large part because of the prevention trial design precluding a treatment-emergent resistance analyses. This application is approvable from the Clinical Virology perspective based on the review of the primary Clinical Virology reviewer (see review BLA761328.000).

BACKGROUND

Respiratory syncytial virus (RSV), a member of the Pneumoviridae family (Rima et al., 2017), is a non-segmented, negative-sense, enveloped RNA virus and is classified into two subtypes, A and B, based on sequence homology of the RSV surface glycoprotein G (Wertz and Moudy, 2004). RSV entry into host cells and cell-to-cell spread is dependent on the fusion (F) protein, which mediates fusion of the viral and host cell membranes (Walsh et al., 1984, Walsh et al., 1989). The F protein is a highly conserved protein across RSV subtypes (Wertz and Moudy, 2004) and is produced as an inactive full-length precursor (AA 1-574) that is cleaved by host cell proteases into the biologically active, disulfide-linked F1 and F2 subunits, known as the extracellular region (F2: AA 24-109 and F1: AA 137-524) of the mature RSV F protein. The F protein is expressed on virions in a prefusion conformation. Juxtaposing of the virus and host cell membranes leads to the F protein-mediated fusion event and the accompanying irreversible refolding of F protein into a post-fusion conformation (Chang and Dutch, 2012). Six antigenic sites (Ø and I–V) have been identified in pre-fusion and/or post-fusion F proteins (Gilman et al., 2016) with target epitopes for prophylactic neutralizing mAbs (Broadbent et al., 2015).

Nirsevimab is a recombinant human IgG1κ mAb that binds with high affinity to a highly conserved, discontinuous neutralizing epitope (AA: 62 to 69 and AA: 196 to 212) within antigenic site Ø (AA: 62 to 96 and AA: 195 to 227) on the pre-fusion confirmation of the RSV F protein. Nirsevimab has been engineered with a triple amino acid substitution (M252Y/S254T/T256E [YTE]) in the Fc region to prolong serum half-life of the mAb. For a comprehensive review of this application and all pertinent background information, please see the primary Clinical Virology review of this application, which was performed by Dr. Michael Thomson, Ph.D. (see review BLA761328.000).

Communications Pertaining to the NGS Data Submission

The following comment was communicated to the Applicant in response to a Mock NGS data submission that was received in IND118524 SDN 116 and reviewed under BBI118424.116:

Clinical Virology comment: In general, the mock next generation sequencing data, including FASTQ files, a frequency table, and a key summary table, are in an acceptable format for review. However, we have the following recommendations that will help expedite the review of NGS data when submitted to the BLA:

a. We note that the nomenclature for the FASTQ files is incorrect. Please consult section ***6.1.2***

Labeling fastq sequence files in the Guidance for Industry Technical Specifications Document entitled, Submitting Next Generation Sequencing Data to the Division of Antiviral Products.

i. Please provide a directory for each Clinical Study that contains subdirectories for each RSV genotype. The FASTQ files for subjects infected with each genotype should be included in the appropriate subdirectory

ii. Please name each FASTQ file with the unique subject identifier and time point

b. Please provide frequency tables for each RSV genotype independently.

Applicant's Response (included in the BLA): AstraZeneca thanks the Agency for their careful review of the NGS data and corresponding clinical virology CRT packages.

Response to comment 1a.

i. A subdirectory has been created for each RSV subtype (RSV A and RSV B) and FASTQ files reside in the appropriate subdirectory based upon RSV G subtype analysis.

ii. FASTQ file names are provided by our partner laboratory ([redacted] (b) (4)) performing the NGS assay in accordance with their SOPs for this validated assay. Given quality concerns with manual renaming of the FASTQ files by AstraZeneca without an established reconciliation process in place at this time, we cannot rename FASTQ files with their subject ID and timepoint. Rather, we have provided information for linking the FASTQ file name with unique subject identifier and time point in the key file, also provided on the portable hard drive as part of this submission.

Response to comment 1b.
Individual frequency tables have been provided for each RSV subtype (RSV A and RSV B).

Clinical Virology response: The nomenclature for the NGS files was not optimal in allowing for the substantial review of the NGS data and would not be acceptable for review of large treatment-emergent resistance assessments under normal circumstances. However, given that the sequence data were primarily of a surveillance nature and were comprised of limited temporal samples, a spot check independent review of the NGS data from a total of 43 subjects from 3 clinical trials was performed.

Comment (communicated in letter to sponsor): Please be advised that the nomenclature for the NGS fastq files submitted in BLA761328 was not acceptable for regulatory review, despite our communication to you on August 16, 2022 requesting that these files be renamed in accordance with section ***6.1.2 Labeling fastq sequence files*** in the Guidance for Industry Technical Specifications Document entitled, Submitting Next Generation Sequencing Data to the Division of Antiviral Products. We remind you that data in a BLA or NDA must be submitted in a reviewable format and strongly encourage you to communicate nomenclature requirements to your partner laboratory ([redacted] (b) (4)) performing the NGS assay.

Review of Next Generation Sequencing Data

An independent assessment of the NGS data submitted by the Applicant was performed for a total of 43 subjects from Study 3 (D5290C00003; NCT02878330), MELODY (D5290C00004; NCT03979313), and MEDLEY (D5290C00005; NCT03959488) who met the primary case definition of medically attended (MA) RSV lower respiratory tract infection (LRTI), with or without hospitalization. Several subjects were selected from each time period (through Day 150 or Day 151-360, etc.) in an attempt to have at least one subject from each treatment arm, RSV subtype, and hospitalization status where possible, for each time period.

Overview of the Applicant's NGS Methodology

The Applicant reported that the potential emergence of neutralization escape variants that may impact efficacy and effectiveness of nirsevimab was monitored throughout the development program with clinical (ID8897-cVAP-001; cVAP) and surveillance (ID8897-svap-002; sVAP) virology analysis plans to track the prevalence and emergence of F protein sequence variations among RSV isolates and evaluate the impact of these

changes on cell culture susceptibility to neutralization. The sVAP (ID8897-svap-002) describes samples analyzed, sample collection (nasal samples) and workflow, performance characteristics of assays and methodologies, and analysis and reporting plans to support resistance monitoring of surveillance RSV isolates. The data for the independent review of NGS samples were derived via the cVAP (ID8897-cVAP-001).

The cVAP (ID8897-cVAP-001) for all pivotal studies contributing to the clinical efficacy of nirsevimab was previously described in Report ID8897-cVAP-001. Respiratory samples were obtained from all subjects found to have an LRTI and all subjects who required hospitalization for a respiratory infection (even without diagnosis of an LRTI). The resistance analysis population included any subject in any treatment group and any reporting period with a respiratory sample that tested positive for RSV by central laboratory RT-PCR with an evaluable NGS sequence.

All RT-PCR confirmed RSV isolates were subtyped and evaluated by genotypic and phenotypic resistance analysis methodologies as previously described (see Report ID8897-cVAP-001). Genotypic analyses were performed using a validated NGS method to detect major (≥25% frequency within each sample) and minor (≥4% and ≥5% LLOQ frequency within each sample of RSV A and B, respectively) viral variants. For completeness, data up to the limit of detection (≥2% frequency) are additionally provided. The data were divided into major (substitutions that were observed in ≥25% of the viral sequences from each sample) and minor variants (substitutions that were observed in ≥4% for RSV A and ≥5% RSV B). Subjects were counted once for each category within a reporting period, with the exception of subjects who had different RSV subtype infections within a reporting period. For subjects with multiple RSV positive samples within a reporting period, the first specimen from each illness episode is analyzed in summary data. Listings are provided for subjects with more than one respiratory specimen.

The NGS analyses were performed by [redacted] (b) (4) using Illumina MiSeq technology generating 250 bp paired-end reads. FASTQ files were analyzed in CLC Genomics Workbench to determine frequency of minor variants. RSV F nucleotide reads were mapped to Netherlands RSV A/13-5275 or Netherlands RSV B/13-1273 reference sequences, followed by variant calling and amino acid annotation. Results were exported from CLC Genomics Workbench as tab-delimited text files.

Variant frequency tables were generated to depict amino acid changes identified in the full-length RSV F protein (AA 1 to 574), including the nirsevimab (AA 62 to 69 and AA 196 to 212) and palivizumab (AA 262 to 275) binding sites, that differ from 2013 Netherlands reference strains (Netherlands RSV A/13-5275 or Netherlands RSV B/13-1273). RSV F protein sequence variations detected among clinical isolates were categorized as polymorphic if they were detected among surveillance RSV isolates since 2015 and nonpolymorphic if they had not been detected. Prevalence of all RSV F protein sequence variations were reported relative to consensus sequences of recent circulating RSV A and RSV B strains since February 2015.

Overview of DAV NGS Analysis Methodology

The CLC Genomics Workbench (V22.0, Qiagen Ca. USA) was used to perform the independent assessment of the NGS data for this BLA. The CLC Genomics Workbench tool contains specific tools that allow the reviewer to batch rename files to meet nomenclature rules for the analysis pipeline, trim reads, map reads to a reference sequence, determine low-frequency variant calls, and export variant call files (VCF) in Excel.

The CLC Genomics Workbench. Briefly, the workflow in CLC Genomics workbench was as follows: trimmed reads were mapped to the RSV A (2013 Netherlands RSV A/13-005275; KX858757.1) or RSV B (Netherlands RSV B/13-001273; KX858756.1) F gene reference sequence as appropriate and variants in the RSV F gene were called at the amino acid level using the low frequency variant caller. The parameters used in CLC Genomics were the following:

1. Mapping using CLC read mapping aligner
    a. Match score: 1
    b. Mismatch cost: 2
    c. Insertion cost: 3
    d. Deletion cost: 3
    e. Length fraction of read required for mapping: 0.5

   f. Similarity fraction: 0.8
   g. Local alignment
2. InDels and Structural Variants
   a. p-value threshold of unaligned end breakpoints: 0.0001
   b. maximum number of mismatches: 3
   c. minimum number of reads to filter variants: 2
3. Local realignment
   a. multi-pass realignment: 2
   b. maximum guidance-variant length (defined by InDels and Structural Variants): 100
4. Low frequency variant detection
   a. Minimum frequency: 1%
   b. Minimum coverage was set to 100
   c. Maximum coverage was set to 1,500,000
   d. Frequency was set to 1%
   e. Significance: 1%

Each step of the NGS analysis process is briefly described below.

A. Processing fastq files. Data were submitted to the FDA on a portable hard drive, which included fastq files for each subject's samples that were sequenced using paired end chemistry and the Illumina MiSeq sequencing platform. The nucleotide sequences were uploaded via the CLC Genomics interface and renamed to conform with DAV nomenclature preferences.

B. Preparing sequence reads and reference sequences prior to mapping. The fastq files were imported into CLC Genomics and reads were mapped to the RSV A (2013 Netherlands RSV A/13-005275; KX858757.1) or RSV B (Netherlands RSV B/13-001273; KX858756.1) F gene reference sequence as appropriate. The F gene was annotated as a coding sequence to establish the correct amino acid number for the gene. The individual reads from each fastq file were subjected to trimming using the default parameters for CLC Genomics Workbench (when used).

C. Mapping reads to the appropriate reference sequence. The reads from each fastq file were aligned to the appropriate reference sequence to generate a mapping for each sample. The mapping contained the target of interest (F gene sequences) and was used to identify variants that differed from the reference sequence. In general, the mappings were assessed to determine the depth of coverage at each nucleotide position and to evaluate read directionality (ratio of forward to reverse reads) to identify regions of bias.

D. Generating frequency tables of amino acid substitutions. From the read mappings, variants were identified and variant files were generated using the Low Frequency Variant Detector in CLC Genomics workbench. The variant call files were exported to Excel and converted into amino acid frequency tables. Here is a description of frequency table generation:

   a. **Low Frequency Variant Detection (LFVD)** – according to the CLC Genomics Workbench Manual: *"a statistical test is performed at each site to determine if the nucleotides observed in the reads at that site could be due simply to sequencing errors, or if they are significantly better explained by there being one (or more) alleles than the reference present in the sample at some unknown frequency. If the latter is the case, a variant corresponding to the significant allele will be called, with estimated frequency".*
   b. **Frequency tables** – Tables were generated using from the CLC genomics VCF exported in Excel. Information for each position of each gene and each subject for which variation from the reference occurs was exported. The frequency table contains the following columns: unique subject identifier (USUBJID), treatment regimen (ARM), visit (VISIT), the amino acid position within the gene of interest (AAPOS), total coverage at the nucleotide position (TCOV), the amino acid found in the reference sequence (AAREF), the amino acid substitution (AASUB), the coverage at the nucleotide level for the variant (VCOV), and the frequency by which the variant was detected (AAFREQ). Frequency tables are generated by:
      i. The variant tables were combined by subtype (RSV A or RSV B)

ii. The variant tables were filtered to remove synonymous substitutions
iii. Any amino acid substitution ≥1% was presented in the table
iv. The variant tables generated by DAV were directly compared to the frequency tables submitted by the Applicant

Independent Review of NGS Data

This review focused on NGS data derived from subject nasal samples that originated from three studies: STUDY 3 (D5290C00003; NCT02878330), MELODY (D5290C00004; NCT03979313), and MEDLEY (D5290C00005; NCT03959488). In STUDY 3, in the nirsevimab (NIR) arm 11/969 subjects (1.1%) had an RSV A infection and 14/969 (1.4%) had an RSV B infection compared to 22/484 (4.5%) and 22/484 (4.5%), respectively, for the placebo (PBO) group (Table 1). In MELODY, in the NIR arm 23/2009 subjects (1.1%) had an RSV A infection and 10/2009 (0.5%) had an RSV B infection compared to 25/1003 (2.5%) and 29/1003 (2.9%), respectively, for the PBO group (Table 1). In MEDLEY, in the NIR arm 4/161 subjects (0.6%) had an RSV A infection and 0/616 (0.0%) had an RSV B infection compared to 1/309 (0.3%) and 2/309 (0.6%), respectively, for the palivizumab (PAL) group (Table 1).

Table 1. RSV A and RSV B infections in the three clinical trials (compiled by DAV).

Study	# Subjects with RSV A		# Subjects with RSV B	
STUDY 3	Placebo	Nirsevimab	Placebo	Nirsevimab
	22/484 (4.5%)	11/969 (1.1%)	22/484 (4.5%)	14/969 (1.4%)
MELODY	Placebo	Nirsevimab	Placebo	Nirsevimab
	25/1003 (2.5%)	23/2009 (1.1%)	29/1003 (2.9%)	10/2009 (0.5%)
MEDLEY	Palivizumab	Nirsevimab	Palivizumab	Nirsevimab
	1/309 (0.3%)	4/616 (0.6%)	2/309 (0.6%)	0/616 (0.0%)

An independent assessment of the NGS data submitted by the sponsor was performed for a total of 43 subjects from STUDY 3 (D5290C00003; NCT02878330), MELODY (D5290C00004; NCT03979313), and MEDLEY (D5290C00005; NCT03959488) who met the primary case definition of medically attended (MA) RSV lower respiratory tract infection (LRTI), with or without hospitalization (Table 2). Several subjects were selected from each time period (through Day 150 or Day 151-360, etc.) in an attempt to have at least one subject from each treatment arm, RSV subtype, and hospitalization status where possible, for each time period.

Table 2. Subjects selected for independent assessment of NGS data (compiled by DAV). Dosing in STUDY 3 was 50 mg NIR; dosing for MELODY and MEDLEY: 50 mg NIR for subjects <5 kg, 100 mg NIR for >= 5 kg. Dosing of PAL in MEDLEY was 5 x monthly doses of 15 mg/kg.

Clinical Trial	Subtype	SUBJID	ARM	Event
Study 3 (D5290C00003)	RSV A	(b) (6)	PBO	Protocol Defined RSV LRTI Outpatient
			PBO	Protocol Defined RSV LRTI Hospitalization
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Hospitalization
			NIR	Protocol Defined RSV LRTI Outpatient
			PBO	Protocol Defined RSV LRTI Outpatient
			PBO	Protocol Defined RSV LRTI Hospitalization
	RSV B		PBO	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			PBO	Protocol Defined RSV LRTI Hospitalization
			PBO	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Hospitalization
			NIR	Protocol Defined RSV LRTI Hospitalization
			NIR	Protocol Defined RSV LRTI Outpatient

MELODY (D5290C00004)	RSV A	(b) (6)	PBO	Protocol Defined RSV LRTI Hospitalization
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Hospitalization
			PBO	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Hospitalization
			NIR	Protocol Defined RSV LRTI Outpatient
			PBO	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			PBO	Protocol Defined RSV LRTI Outpatient
	RSV B		PBO	Protocol Defined RSV LRTI Hospitalization
			NIR	Protocol Defined RSV LRTI Hospitalization
			PBO	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			PBO	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Outpatient
MEDLEY (D5290C00005)	RSV A	(b) (6)	NIR	Protocol Defined RSV LRTI Outpatient
			PAL	Protocol Defined RSV LRTI Outpatient
			NIR	Protocol Defined RSV LRTI Hospitalization
			NIR/NIR	Protocol Defined RSV LRTI Hospitalization
			PAL/NIR	Protocol Defined RSV LRTI Hospitalization
			PAL/NIR	Protocol Defined RSV LRTI Outpatient
	RSV B		NIR/NIR	Protocol Defined RSV LRTI Hospitalization
			PAL	Protocol Defined RSV LRTI Outpatient
			PAL/NIR	Protocol Defined RSV LRTI Hospitalization

Highlighted subjects were duplications as follows: for subject (b) (6) two sets of paired end files were examined; for subject (b) (6) RSV A and RSV B infections were both detected, but no fastq files were provided. NIR, nirsevimab; PBO, placebo; PAL, palivizumab.

The NGS sequencing results generated by DAV were largely in agreement with the results reported by the Applicant (see Appendix 1 for details). For the clinical trial known as STUDY 3 (D5290C00003; NCT02878330), there were 14 subjects included in the spot check population, including seven subjects with RSV A (3 nirsevimab and 4 placebo) and seven with RSV B (4 nirsevimab and 3 placebo) and the following substitutions were identified by both the Applicant and DAV at similar frequencies and coverages:

- **STUDY 3 (D5290C00003; NCT02878330; n=14) agreements:**
  - **RSV A (n=7):** A23T (n=1), R113K (n=1), Y117H (n=1), N120S (n=1), K123E (n=1), and S213R (n=1)
  - **RSV B (n=7):** F15L (n=7*), Y33F (n=1), A103V (n=7*), L172Q (n=7*), S173L (n=7*), K191R (n=1), I206M (n=1), Q209R (n=1), Y457H (n=1*), and I527M (n=1).
    - * No fastq files were provided for subject (b) (6) but the Applicant reported this

substitution in the sample derived from the subject.

- **STUDY 3 (D5290C00003; NCT02878330) disagreements:** The substitution Y306H was detected by DAV in both nirsevimab and placebo subjects but not reported by the Applicant at a frequency range of 7-10% in all six of the subjects who had RSV B and had sequence data available. Of note, no fastq files were provided for subject (b) (6)

For the clinical trial MELODY (D5290C00004; NCT03979313) there were 22 subjects included in the spot check population, including 10 subjects with RSV A (6 nirsevimab and 4 placebo) and 12 subjects with RSV B (9 nirsevimab and 3 placebo) and the following substitutions were identified by both the Applicant and DAV at similar frequencies and coverages:

- **MELODY (D5290C00004; NCT03979313; n=22) agreements:**
  - **RSV A (n=10):** T12I (n=3), T13A (n=1), A23S (n=3), T122A (n=5), K123Q (n=2), E387D (n=1), I384V (n=2**), S443T (n=1), N515H (n=2**), A540S (n=2**), K551R (n=1), and D562N (n=1).
    - ** The Applicant detected this substitution at a frequency of 2 to 12% with much higher overall coverage at the position whereas DAV detected the substitution at much greater frequencies of 40 to 100% but at much lower total coverage. Of note, I384V is harbored by the reference isolate for phenotyping.
    - Substitutions highlighted in yellow have been recommended for phenotypic analysis (see BLA761328.000).
  - **RSV B (n=11):** F12I (n=2), F15L (n=11), K87R (n=1), A103V (n=11), N120S (n=1), L172Q (n=9), S173L (n=9), S190N (n=4), K191R (n=9), L204S (n=1), I206M (n=9), Q209R (n=9), S211N (n=4), S330T (n=2), S389P (n=5), and T522A (n=1).
- **MELODY (D5290C00004; NCT03979313) disagreements:**
  - E2G (n=1) was detected by DAV but not the Applicant at 99% frequency in subject (b) (6) who was infected with RSV B.
  - The substitution Y306H was detected by DAV but not reported by the Applicant at a frequency range of 6-11% in all 11 of the subjects who had RSV B and had sequence data available.

For the clinical trial MEDLEY (D5290C00005; NCT03959488) there were nine subjects included in the spot check population, including six subjects with RSV A (3 nirsevimab, 2 palivizumab/nirsevimab and 1 palivizumab) and 3 subjects with RSV B (1 nirsevimab, 1 palivizumab/nirsevimab and 1 palivizumab) and the following substitutions were identified by both the Applicant and DAV at similar frequencies and coverages:

- **MEDLEY (D5290C00005; NCT03959488; n=9 subjects) agreements:**
  - **RSV A (n=6):** T12I (n=1), A103T (n=1), E110G (n=1), M115T (n=1), T122A (n=2), N126Y (n=1), K272T (n=1), K272M (n=1), and S275L (n=1).
  - **RSV B (n=3):** L4P (n=1), F12L (n=1), F12I (n=1), F15L (n=3), R42K (n=1), A103V (n=3), L172Q (n=3), S173L (n=3), S190N (n=2), K191R (n=3), I206M (n=3), Q209R (n=3), S211N (n=2), K272T (n=1), S389P (n=2), and I525V (n=1).
- **MEDLEY (D5290C00005; NCT03959488) disagreements:** The substitution Y306H was detected by DAV but not reported by the Applicant at a frequency range of 9-10% in all three of the subjects who had RSV B.

Overall, the results obtained in the independent analysis were consistent with the results reported by the Applicant with the following exceptions:

- E2G (n=1) was detected by DAV but not the Applicant at 99% frequency in subject (b) (6) who was infected with RSV B. This substitution was detected in one subject who received NIR; however, the substitution is outside of the NIR epitope and has not been associated with resistance. This was likely

in the primer region and was trimmed by the Applicant during NGS analysis,

- The substitution Y306H was detected by DAV but not reported by the Applicant at a frequency range of 6-11% in all 20 subjects who had RSV B and had sequence data available, including subjects who received PBO (n=5), NIR (n=13) or PAL (n=2). Of note, the positioning of this substitution within the sequence reads that contained this substitution was analyzed to determine if it was associated with anomalies characterized in the known error profile for Illumina MiSeq platform. This substitution resulted from a TAT to CAT codon mutation, occurred in various portions of the read at a high-quality score, and does not appear to be associated with a homopolymer sequence. The substitution cannot be ruled out based on sequence error, and it is unknown why it is detected by DAV but not the Applicant. However, given that it occurred in all treatment groups in subjects with RSV B and the amino acid position does not fall within the NIR epitope, it is unlikely to be associated with NIR treatment or resistance.
- In clinical trial MELODY (D5290C00004; NCT03979313) there was a frequency and coverage discrepancy between DAV and the Applicant pertaining to substitutions I384V (subject (b) (6) from the NIR treatment group), N515H (subjects (b) (6) from the NIR treatment group and (b) (6) from the PBO treatment group), and A540S (subjects (b) (6) from the NIR treatment group and (b) (6) from the PBO treatment group). In general, this discrepancy appears to be related to differences in coverage as the Applicant reported coverages of 10-fold greater depth at these positions compared to the data generated by DAV. The exact reason for the differences in coverage are not clear; however, given that these substitutions are outside of the NIR epitope at positions that are not associated with resistance, occurred in subjects from the NIR and PBO treatment groups, and were detected by both DAV and the Applicant (albeit at different frequencies) it is unlikely that these substitutions are associated with NIR treatment or resistance.

Conclusion: The NGS sequencing results generated by DAV were largely in agreement with the results reported by the Applicant. No information associated with resistance was obtained from the independent review of NGS data in large part because the sequence information provided was not supportive of a treatment-emergent resistance analyses. This application is approvable from the Clinical Virology perspective based on the review of the primary Clinical Virology reviewer (see review BLA761328.000).

CONCLUSIONS

- This submission is part of the Biologics License Application for nirsevimab, which is a RSV F protein inhibitor mAb indicated for the prevention of RSV lower respiratory tract disease in: neonates and infants entering or during their first RSV season and children up to 24 months of age who remain vulnerable to severe RSV disease through their second RSV season.
- Nirsevimab is a recombinant human IgG1κ mAb that binds with high affinity to a highly conserved, discontinuous neutralizing epitope (AA 62 to 69 and AA 196 to 212) within antigenic site Ø (AA 62 to 96 and AA 195 to 227) on the pre-fusion confirmation of the RSV F protein.
- The primary Clinical Virology review of this application was performed by Dr. Michael Thomson, Ph.D. (see review BLA761328.000).
- This review was specifically performed for the assessment of NGS data submitted in support of the BLA.
- The nomenclature for the NGS files was not optimal in allowing for the substantial review of the NGS data and would not be acceptable for review of large treatment-emergent resistance assessments under normal circumstances. However, given that the sequence data were primarily of a surveillance nature and were comprised of limited temporal samples, a spot check independent review of the NGS data from 43 subjects from 3 clinical trials was performed.
- The NGS sequencing results generated by DAV were largely in agreement with the results reported by

the Applicant. No information associated with resistance was obtained from the independent review of NGS data in large part because the sequence information provided was not supportive of a treatment-emergent resistance analyses.

- This application is approvable from the Clinical Virology perspective based on the review of the primary Clinical Virology reviewer (see review BLA761328.000).

RECOMMENDATIONS

1. Please be advised that the nomenclature for the NGS fastq files submitted in BLA761328 was not acceptable for regulatory review, despite our communication to you on August 16, 2022 requesting that these files be renamed in accordance with section ***6.1.2 Labeling fastq sequence files*** in the Guidance for Industry Technical Specifications Document entitled, Submitting Next Generation Sequencing Data to the Division of Antiviral Products. We remind you that data in a BLA or NDA must be submitted in a reviewable format and strongly encourage you to communicate nomenclature requirements to your partner laboratory [redacted] (b) (4) performing the NGS assay.

SIGNATURES

Eric Donaldson, Ph.D.
Clinical Virology Reviewer

CONCURRENCES

Date:

Clin Virology TL/J O'Rear

cc:
HFD-530/IND 118524
HFD-530/Division File
HFD-530/RPM/Jang

Appendix 1

NGS Analysis Data Generated by DAV

Table A1. DAV analysis of Study 3 spot check population (RSV A).

SUB	SUBJID	Visit	AZ	CLC
A23T	[redacted] (b) (6)	V5-D91	1.00	1.00
R113K		V6-D151	1.00	1.00
Y117H		V6-D151	1.00	1.00
N120S		V5-D91	1.00	1.00
K123E		V1-SCR	1.00	1.00
S213R		V1-SCR	1.00	1.00

SUB, substitution detected; SUBJID, subject ID; AX, detected by AstraZeneca; CLC, identified by DAV using CLC Genomics Workbench; V, visit number; D, Study Day; SCR, screening.

Table A2. DAV analysis of Study 3 spot check population (RSV B).

SUB	SUBJID	Visit	AZ	CLC
F15L	(b) (6)	V1-Scr	1.00	1.00
		V6-D151	1.00	1.00
		V6-D151	1.00	1.00
		V5-D91	1.00	1.00
		V1-Scr	1.00	1.00
		V1-Scr	1.00	nsd
		V5-D91	1.00	1.00
Y33F		V6-D151	0.99	0.99
A103V		V1-Scr	0.99	0.99
		V6-D151	0.99	0.99
		V6-D151	0.99	0.99
		V5-D91	0.99	0.99
		V1-Scr	0.99	0.99
		V1-Scr	0.99	nsd
		V5-D91	0.99	0.99
L172Q		V1-Scr	1.00	1.00
		V6-D151	1.00	1.00
		V6-D151	1.00	1.00
		V5-D91	1.00	1.00
		V1-Scr	1.00	1.00
		V1-Scr	1.00	nsd
		V5-D91	1.00	1.00
S173L		V1-Scr	0.99	0.99
		V6-D151	0.99	0.99
		V6-D151	0.99	0.99
		V5-D91	0.99	0.99
		V1-Scr	0.99	0.99
		V1-Scr	0.99	nsd
		V5-D91	0.99	0.99
K191R		V5-D91	1.00	1.00
I206M		V5-D91	1.00	1.00
Q209R		V5-D91	1.00	1.00
Y306H		V1-Scr	nd	0.09
		V6-D151	nd	0.08
		V6-D151	nd	0.10
		V5-D91	nd	0.07
		V1-Scr	nd	0.10
		V5-D91	nd	0.09
Y457H		V5 DAY 91	0.12	nsd
I527M		V5-D91	1.00	1.00

SUB, substitution detected; SUBJID, subject ID; AX, detected by AstraZeneca; CLC, identified by DAV using CLC Genomics Workbench; V, visit number; D, Study Day; SCR, screening. Yellow indicates substitutions detected by DAV but not AZ.

Table A3. DAV analysis of MELODY spot check population (RSV A).

SUB	SUBJID	AZ	CLC
T12I	(b) (6)	1.00	0.99
		1.00	0.99
		1.00	0.99
T13A		1.00	1.00
		1.00	1.00
A23S		0.99	0.99
		0.98	0.98
		0.99	0.99
T122A		1.00	1.00
		1.00	1.00
		0.99	0.99
		0.96	0.96
		1.00	1.00
K123Q		1.00	1.00
		0.99	0.99
E378D		1.00	1.00
I384T		1.00	1.00
		0.98	0.97
I384V		0.02	0.03
		0.03	1.00*
S443T		1.00	1.00
N515H		0.05	0.44*
		0.04	0.40*
A540S		0.12	0.99*
		0.09	0.99*
K551R		0.94	0.94
D562N		0.86	0.99

SUB, substitution detected; SUBJID, subject ID; AX, detected by AstraZeneca; CLC, identified by DAV using CLC Genomics Workbench; V, visit number; D, Study Day; SCR, screening. Orange indicates substitutions with frequency discrepancies.

Table A4. DAV analysis of MELODY spot check population (RSV B)

SUB	SUBJID	AZ	CLC
E2G	(b) (6)	nd	0.99
F12I		0.99	0.99
		1.00	1.00
F15L		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00

K87R	(b) (6)	0.17	0.17
A103V		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
N120S		1.00	1.00
L172Q		1.00	1.00
		1.00	1.00
		0.99	0.99
		0.99	0.99
		1.00	1.00
		0.99	0.99
		1.00	1.00
		1.00	1.00
		0.99	0.99
S173L		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	0.99
		0.99	1.00
		0.99	0.99
		0.98	0.98
S190N		1.00	1.00
		1.00	1.00
		0.99	0.99
		1.00	1.00
K191R		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	1.00
		1.00	0.99
L204S		0.99	0.99
I206M		1.00	1.00
		0.99	0.99
		0.99	0.99
		1.00	1.00
		1.00	1.00
		1.00	1.00
		0.99	0.99
		1.00	1.00
		0.99	0.99

Q209R	(b) (6)	.00	1.00
		.00	1.00
		.00	1.00
		.00	1.00
		.00	1.00
		.00	1.00
		.00	0.99
		.00	1.00
		.99	0.99
S211N		.00	1.00
		.00	1.00
		.99	0.99
		.00	1.00
Y306H		nd	0.09
		nd	0.07
		nd	0.09
		nd	0.09
		nd	0.09
		nd	0.11
		nd	0.08
		nd	0.08
		nd	0.08
		nd	0.06
		nd	0.09
S330T		.00	1.00
		.00	1.00
S389P		.00	1.00
		.00	1.00
		.00	1.00
		.99	0.99
		.00	1.00
T522A		.00	1.00

SUB, substitution detected; SUBJID, subject ID; AX, detected by AstraZeneca; CLC, identified by DAV using CLC Genomics Workbench; V, visit number; D, Study Day; SCR, screening. Yellow indicates substitutions detected by DAV but not AZ.

Table A5. DAV analysis of MEDLEY spot check population (RSV A)

SUB	SUBJID	AZ	CLC
T12I	(b) (6)	.99	0.99
A103T		.00	1.00
E110G		.00	1.00
M115T		.99	0.99
T122A		.00	1.00
		.00	1.00
N126Y		.99	0.99
K272T		.20	0.20
K272M		.07	0.07
S275L		.28	0.32

SUB, substitution detected; SUBJID, subject ID; AX, detected by AstraZeneca; CLC, identified by DAV using CLC Genomics Workbench; V, visit number; D, Study Day; SCR, screening.

Table A6. DAV analysis of MEDLEY spot check population (RSV B)

SUB	SUBJID	AZ	CLC
L4P	(b) (6)	1.00	0.99
F12L		1.00	1.00
F12I		1.00	1.00
F15L		1.00	1.00
		1.00	1.00
		1.00	1.00
R42K		1.00	1.00
A103V		0.99	0.99
		0.99	0.99
		0.99	0.99
L172Q		1.00	1.00
		0.99	0.99
		1.00	1.00
S173L		0.99	0.99
		0.99	0.99
		0.99	0.99
S190N		1.00	0.99
		1.00	1.00
K191R		1.00	1.00
		1.00	1.00
		1.00	1.00
I206M		1.00	1.00
		0.99	0.99
		1.00	1.00
Q209R		1.00	1.00
		1.00	0.99
		1.00	1.00
S211N		1.00	1.00
		1.00	1.00
K272T		0.04	0.04
Y306H		nd	0.09
		nd	0.10
		nd	0.10
S389P		0.99	0.99
		1.00	0.99
I525V		0.99	0.99

SUB, substitution detected; SUBJID, subject ID; AX, detected by AstraZeneca; CLC, identified by DAV using CLC Genomics Workbench; V, visit number; D, Study Day; SCR, screening. Yellow indicates substitutions detected by DAV but not AZ.

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