CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

021246Orig1s045 and 021087Orig1s062

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 021087 SE-062/021246 SE-045 **SDN:** 875/369 **DATE REVIEWED:** 11/01/12
Virology Reviewer: Damon J. Deming, Ph.D.

Reviewer: Damon J. Deming, Ph.D.
Date Submitted: 06/21/2012 **Date Assigned:** 06/22/2012
Date Received: 06/21/2012

Applicants Name and Address:

Hoffman-LaRoche Inc.
340 Kingsland Street
Nutley, NJ 07110-1199
973-235-6141
973-562-3700 (FAX)

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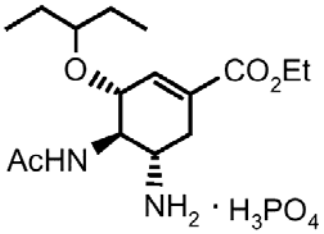
SDN 888/379 (eCTD 0019/0042)	September 14, 2012

Related Supporting documents: IND 53,093; NDA 21087; NDA 21246

Product Names: Oseltamivir phosphate, Tamiflu®

Chemical Names: (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester, phosphate

Structure:



OSELTAMIVIR PHOSPHATE

Molecular formula: $C_{16}H_{28}N_2O_4$ (free base)
Molecular weight: 312.4 for the free base, 410.4 for the phosphate salt
Drug category: Antiviral
Indication: Treatment of uncomplicated influenza A and B virus infection in infants with a post conceptional age of at least (b) (4) weeks.

Dosage Form/Route of administration: 3 mg/kg BID/Oral

Supporting documents: IND 053093, IND 071826

Abbreviations: CASG, Collaborative Antiviral Study Group; cDNA, complementary deoxyribonucleic acid; HA, hemagglutinin; HAI, hemagglutination inhibition; MDCK, Madin-Darby Canine Kidney Cells; MUNANA, methylumbelliferyl N-acetylneuraminic acid; NA, neuraminidase; NAI, neuraminidase inhibition; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction

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EXECUTIVE SUMMARY

Antiviral drugs provide therapeutic and prophylactic treatment options for seasonal and pandemic influenza virus infections. There are two approved classes of anti-influenza virus drugs, the M2 ion inhibitors, or adamantanes, and the neuraminidase inhibitors (NAIs). The adamantanes include amantadine (NDAs 016020, 016023, 017118, and 018101) and rimantadine (NDAs 019649 and 019650), approved for the treatment of uncomplicated influenza A virus infection in patients ≥ 1 year of age in October 1966 and September 1993, respectively. However, the emergence of adamantane-resistant influenza strains has rendered the adamantanes ineffective against currently circulating strains (Deyde et al., 2007; Dharan et al., 2009; CDC FluView, Week 42, 2012).

The approved NAIs include oseltamivir phosphate (NDAs 021087 and 021246) and zanamivir (NDA 021036). Oseltamivir phosphate was approved for treatment of uncomplicated influenza A and B virus infections in patients ≥ 1 year of age on October 27, 1999, for prophylaxis of adults and adolescents ≥ 13 years of age on November 17, 2000, and for prophylaxis of children ≥ 1 year of age on December 21, 2005. Zanamivir was approved for treatment of uncomplicated influenza A and B virus infection in patients ≥ 12 years of age on July 26, 1999, for treatment of children ≥ 7 years of age on April 26, 2000, and for prophylaxis of adults and children ≥ 5 years of age on March 29, 2006. Neither of the neuraminidase inhibitors has been approved for the treatment or prophylaxis of influenza virus infection in pediatric populations < 1 year of age, an age group that is highly vulnerable to influenza virus-related morbidity and mortality. Hoffman-LaRoche, Inc. has submitted the results of two bridging-studies as part of a supplemental application to fulfill part of this unmet medical need:

1) Study CASG 114, entitled "A pharmacokinetic/pharmacodynamic and safety evaluation of oseltamivir (TAMIFLU®) for the treatment of children less than 24 months of age with confirmed influenza infection," was conducted at 16 U.S. sites under the NIH sponsored IND 071826.

2) Study WP22849, entitled "An open label, prospective, pharmacokinetic/ pharmacodynamic, and safety evaluation of oseltamivir (TAMIFLU®) in the Treatment of Infants 0 to < 12 months of age with confirmed influenza infection," was not conducted under IND at sites in the European Union.

There were no key virology issues that arose during the review of this supplemental application. The pivotal studies were not designed to demonstrate antiviral activity or efficacy in subjects < 1 year old; both were small, open-labeled, uncontrolled studies that evaluated a narrow dose range and enrolled subjects at times exceeding the anticipated therapeutic window of oseltamivir. The observed resistance rates determined in the FDA analysis were within the expected ranges for the influenza virus types identified among study subjects and given the types of assays used in the resistance analyses.

1 Recommendations

1.1 Recommendation and Conclusion on Approvability

The amendments to the TAMIFLU® labeling proposed by the applicant to include safety information related to the use of oseltamivir phosphate for the treatment of influenza virus infection in children less than 1 year old is acceptable from a Clinical Virology perspective.

1.2 Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management

There are no Phase 4 commitments for this supplemental application.

2 Summary of OND Virology Assessments

2.1 Nonclinical Virology

No new nonclinical virology data describing the mechanism of action or antiviral activity of oseltamivir carboxylate were included in this application. However, the relevant nonclinical studies have been reviewed previously under NDA 021087/021246 and IND 053093. In summary, oseltamivir phosphate is an ethyl ester prodrug requiring ester hydrolysis for conversion to the active form, oseltamivir carboxylate. Oseltamivir carboxylate is an inhibitor of influenza virus neuraminidase affecting release of viral particles. The antiviral activity and neuraminidase inhibitory activity of oseltamivir carboxylate against laboratory strains and clinical isolates of influenza virus has been determined in cell culture infectivity and biochemical neuraminidase activity assays.

The concentrations of oseltamivir carboxylate required for inhibition of influenza virus in cell culture were highly variable depending on the assay method used and the virus tested. The 50% and 90% effective concentrations (EC_{50} and EC_{90} values) were in the range of 0.0008 µM to >35 µM and 0.004 µM to >100 µM, respectively. The median IC_{50} values of oseltamivir against influenza A/H1N1, influenza A/H3N2, and influenza B clinical isolates were 2.5 nM (range 0.93-4.16 nM, N=74), 0.96 nM (range 0.13-7.95 nM, N=774), and 60 nM (20-285 nM, N=256), respectively, in a neuraminidase inhibition assay that relied upon quantification of a fluorescently labeled MUNANA substrate. The relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, inhibition of *in vivo* influenza virus replication, and inhibition or reduction of infection-related symptoms in humans has not been established.

2.2 Clinical Virology

Clinical data supporting the proposed indication were derived from two pharmacokinetic bridging studies:

1) A pharmacokinetic/pharmacodynamic and safety evaluation of oseltamivir (TAMIFLU®) for the treatment of children less than 24 months of age with confirmed influenza infection (CASG 114), and

2) An open label, prospective, pharmacokinetic/pharmacodynamic, and safety evaluation of oseltamivir (TAMIFLU®) in the Treatment of Infants 0 to < 12 months of age with confirmed influenza infection (WP22849)

The incidence of emergent resistant viruses was at least 8% (3/37) among subjects infected with the 2009 pandemic strain of influenza A/H1N1 in CASG 114, at least 22% (7/32) among subjects infected with the 2009 pandemic strain of influenza A/H1N1 in WP22849, and at least 10% (1/10) among subjects infected with influenza A/H3N2 in WP22849. No correlations

between oseltamivir pharmacokinetics and pharmacodynamics, including antiviral activity, were observed.

3 Administrative

3.1 Reviewer's Signature

_____
Damon J. Deming, Ph.D.
Microbiologist, HFD-530

3.2 Concurrence

_____ Date_____
HFD-530/J. O'Rear /TL Micro

cc:

HFD-530/NDA
HFD-530/Division File
HFD-530/RPM/Thompson

OND Microbiology Review

1 Introduction and Background

Influenza is an acute respiratory disease caused by infection with influenza virus that is characterized by the sudden onset of fever, coryza, and cough. In healthy adults and children the illness is often self-limiting and causes only limited morbidity. However, influenza in infants can develop into croup, bronchiolitis, or pneumonia, conditions that are associated with significant morbidity. Indeed, the highest hospitalization and mortality rates typically occur in children aged < 6 months (Dawood et al., 2010). Antiviral drugs and annual vaccinations are the only means of prophylaxis for seasonal influenza virus infections; however, children aged < 6 months are too young to be vaccinated (CDC, 2012). Therefore, antiviral drugs represent the sole therapeutic and prophylactic option for this highly susceptible population.

There are two approved classes of anti-influenza virus drugs, the M2 ion inhibitors, or adamantanes, and the neuraminidase inhibitors (NAIs). The adamantanes include amantadine (NDAs 016020, 016023, 017118, and 018101) and rimantadine (NDAs 019649 and 019650), approved for the treatment of uncomplicated influenza A virus infection in patients ≥ 1 year of age in October 1966 and September 1993, respectively. However, the emergence of adamantane-resistant influenza strains has rendered the adamantanes ineffective against currently circulating strains (Deyde et al., 2007; Dharan et al., 2009; CDC FluView, Week 42, 2012). From the 1999-2000 up to the 2007-2008 influenza seasons, less than 1% of the isolates tested worldwide demonstrated reduced susceptibility to NAIs when evaluated using virus amplified in cell culture and evaluated with a neuraminidase assay (CDC, 2008; Dharan et al., 2009). The number of oseltamivir resistant H1N1 isolates rose to approximately 19% in the 2007-2008 season and to > 99% in the 2008-2009 season (CDC FluView, Week 39, 2009). The novel 2009 pandemic A(H1N1) virus, which retains susceptibility to oseltamivir carboxylate, has since become the dominant circulating A(H1N1) strain (CDC, 2011; CDC FluView, Week 42, 2012).

The approved NAIs include oseltamivir phosphate (NDAs 021087 and 021246) and zanamivir (NDA 021036). Oseltamivir phosphate was approved for treatment of uncomplicated influenza A and B virus infections in patients ≥ 1 year of age on October 27, 1999, for prophylaxis of adults and adolescents ≥ 13 years of age on November 17, 2000, and for prophylaxis of children ≥ 1 year of age on December 21, 2005. Zanamivir was approved for treatment of uncomplicated influenza A and B virus infection in patients ≥ 12 years of age on July 26, 1999, for treatment of children ≥ 7 years of age on April 26, 2000, and for prophylaxis of adults and children ≥ 5 years of age on March 29, 2006. Neither of the neuraminidase inhibitors has been approved for the treatment or prophylaxis of influenza virus infection in pediatric populations < 1 year of age. Hoffman-LaRoche, Inc. has submitted the results of two bridging-studies as part of a supplemental application to fulfill this unmet medical need:

Hoffman-LaRoche, Inc. has submitted the results of 2 studies to support a supplemental application to expand the treatment indication of oseltamivir phosphate to full-term (post conceptional age of at least (b) (4) weeks) infants:

- *Study CASG 114 (WP20749)*: "A pharmacokinetic/pharmacodynamic and safety evaluation of oseltamivir (TAMIFLU®) for the treatment of children less than 24 months of age with confirmed influenza infection." The study was conducted under the NIH

sponsored IND 071826 and enrolled a total of 87 subjects from 16 U.S. centers over the course of 4 influenza seasons (2006-2007 to 2009-2010).

- *Study WP22849*: "An open label, prospective, pharmacokinetic/pharmacodynamic, and safety evaluation of oseltamivir (TAMIFLU®) in the Treatment of Infants 0 to < 12 months of age with confirmed influenza infection." Protocol WP22849 was not conducted under IND at sites within the European Union.

Notably, neither of these studies was designed to demonstrate antiviral activity or efficacy.

1.1 Important Milestones in Product Development

NDA 21087 received FDA approval on October 27, 1999 for Tamiflu® capsule treatment of uncomplicated acute illness due to influenza virus infections in adults who have been symptomatic for no more than two days. NDA 21246 received FDA approval on December 14, 2000 for Tamiflu® oral suspension for the treatment of uncomplicated acute illness due to influenza virus infections in patients older than one year of age who have been symptomatic for no more than two days.

The following key-supplements and label-changes have also received approval:

- December 21, 2005 NDA 21-087/S-030 and NDA 21-246/S-017 for prophylaxis of influenza for patients between 1-12 years of age
- February 7, 2011 NDA 21-087/S-057 and NDA 21-246/S-040; new safety information relating to higher than previously reported rates of treatment-associated resistance in children infected with influenza A viruses was added to the label

1.2 Methodology

1.2.1 Methods and Materials for CASG 114

Screening and sample collection. Volunteers with symptoms consistent with influenza virus infection were screened at local sites using virus-specific diagnostic culture or rapid antigen tests. Subjects must have developed symptoms within 96 hours of enrollment, which is longer than the approved 48 hour oseltamivir treatment window. Nasopharyngeal specimens were obtained from study subjects on Days 1, 3, 5 (± 1 day), and 10 (± 2 days) with Copan swabs and placed in viral transport media. Hemagglutinin (HA) and neuraminidase (NA) type (e.g., H3N2, B, etc.) were determined for isolates that replicated on cell culture.

Virus culture. Swabs were shipped in viral transport medium to the CASG Central Unit Laboratory within 32 hours of being obtained, where they were aliquoted and frozen at -80°C. Specimens were then processed further for virus culture in the laboratory of Dr. Marilyn

Menegus, Rochester, NY. Viral specimens were thawed in batches and used to inoculate culture tubes containing monolayers of Madin-Darby canine kidney (MDCK) cells, incubated at 33°C, and the cultures were examined daily for cytopathology. Virus positive cultures were subsequently titrated on MDCK cells.

RT-PCR methods for influenza A and B virus quantification. Viral loads in clinical specimens were determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) in the laboratory of Dr. Fred Lakeman, Birmingham, AL. Nucleic acids were isolated from 190 µL of viral specimens using the MagNA Pure® LC 1.0 or the MagNA Pure® LC 2.0 kits. A 5 µL sample of RNA was then reverse transcribed with SuperScript® III reverse transcriptase and gene specific primers to generate cDNA. Primer and probe nucleic acid sequences were not provided.

NA and HA nucleotide sequencing. The nucleotide sequences of the NA or HA open reading frames of cell culture amplified viruses amplified by AmpliTaq Gold® PCR and the resulting DNA fragments were purified by MSB® HTS PCRapace kit. Full length sequencing was performed for the NA and HA genes of cell culture amplified virus using the Big Dye® Terminator 3.1 Cycle Sequencing kit and sequenced on an ABI 3031 XL automated sequencer. Four reference virus strains were used in the genotype comparisons: A/Brisbane/59/2007 for seasonal H1N1, A/California/07/2009 for 2009 pandemic H1N1, A/Brisbane/10/2007 for H3N2, and B/Brisbane/60/2008 for influenza B. The inferred amino acid sequence from the NA or HA nucleotide sequences were aligned with the corresponding reference sequences using Align X® in Vector NTI® and amino acid differences between each sequence and the reference were determined. Influenza virus type and subtype were confirmed by phylogenetic analysis using BLAST®.

Known amino acid polymorphisms were defined as sequence variants that appear within the public NCBI influenza database. All human NA and HA protein sequences for influenza A/H1N1, A/H3N2, and B strains were retrieved from the NCBI Influenza Virus Resource (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html).

Neuraminidase inhibition phenotyping. Antiviral susceptibility testing was performed by the central laboratory (ViroClinics) on the first and last culturable isolates. The susceptibilities of cell culture amplified viruses were assessed using a methylumbelliferyl N-acetylneuraminic acid (MUNANA)-based biochemical inhibitory assay, and the results presented as the IC_{50} value, the concentration of oseltamivir carboxylate sufficient to reduce NA activity by 50%.

(b) (5)

1.2.2 Methods and Materials for WP22849

Sample collection. Influenza virus infection was confirmed at screening by locally analyzed nasal and throat swabs. Nasopharyngeal specimens were obtained from study subjects on Days 1 (baseline), 3 or 4, 6, 11, 18 (± 2 days), and 30 (± 2 days) with swabs, placed in UTM-RT transport medium, and shipped to the central lab for confirmatory testing and virological analyses. The Day 18 and 30 samples were only collected if clinically indicated.

Influenza A and B virus detection, subtype determination, and quantification. Confirmatory testing and quantification of influenza A and B virus by quantitative RT-PCR (TaqMan® EZ) culture assay using MDCK cells were conducted by the central laboratory (b) (4)

The matrix-specific primers and probes used to detect influenza A and B viruses by quantitative RT-PCR were:

Primers and Probe for the Detection of Influenza A Viruses
Forward: 5'—aagaccaatcctgtcacctctga
Reverse: 5'—caaagcgtctacgctgcagtcc
Probe: 5'—tttgtgttcacgctcaccgtgcc

Primers and Probe for the Detection of Influenza B Viruses
Forward: 5'—gagacacaattgcctacctgctt
Reverse: 5'—ttctttcccaccgaaccaac
Probe: 5'—agaagatggagaaggcaaagcagaactagc

The hemagglutinin-specific primers and probes used for identifying influenza A virus subtypes in influenza A positive samples are shown below. Note that neuraminidase-specific primers and probe, in addition to hemagglutinin-specific primers and probe, were used for verifying the presence of 2009 pandemic H1N1 virus in clinical samples.

Primers and Probe for the Detection of Seasonal Influenza A/H1 viruses
Forward: 5'—gaatagccccactacaattgggtaa
Reverse: 5'—gtaattcgcattctgggtttcc
Probe: 5'—aagatccatccggcaacgctgca

Primers and Probe for the Detection of Influenza A/H3 viruses
Forward: 5'—gggaaaagctcaataatgagatcag
Reverse: 5'—ttgggaatgcttccatttgg
Probe: 5'—tgcacccattggcaaatgcaattc

Primers and Probe for the Detection of 2009 Pandemic Influenza A/H1 viruses
For (H1): 5'—ggaaagaaatgctggatctggta
Rev (H1): 5'—atgggaggctggtgtttatagc

Probe H1: 5'—tgcaatacaacttgtcagacacccaaggg
For (N1): 5'—acatgtgtgtgcagggataactg
Rev (N1): 5'—tccgaaaatcccactgcatat
Probe N1: 5'—atcgaccgtgggtgtctttcaacca

Detection of NA H275Y by allele-specific RT-PCR. Baseline influenza A positive samples were tested by allele-specific RT-PCR for the presence of NA H275Y-expressing variants. The primers and probe used for the detection of seasonal and pandemic influenza A/H1N1 viruses were:

Primers and Probe for the Detection of H275Y in Seasonal H1N1*

Forward:	5'—aaaagggaaaggttactaaatcaatagagt
Reverse:	5'—cagtgtctgggtaacaggaacatt
Probe (W/T):	5'—caCcCattTttCatTa
Probe (275Y):	5'—caCcCaatTttTatTa

Primers and Probe for the Detection of H275Y in Pandemic H1N1*

Forward:	5'—cagtcgaaatgaatgcccctaa
Reverse:	5'—tgcacacatgtgatttcatag
Probe (W/T):	5'—ttaTCActAtgAggaatg
Probe (275Y):	5'—ttaTTActAtgAggaatg

* Capital letters represent locked nucleic acids with modified ribose rings containing an extra bridge connecting the 2' oxygen and 4' carbon that can enhance hybridization properties, increasing the binding strength of a primer for its target, thereby increasing the sensitivity of PCR assays (Ballantyne et al., 2008).

NA and HA nucleotide sequence analysis. Full length sequencing of NA and HA was attempted on all RT-PCR–positive clinical samples. Clinical isolates that failed the direct nucleotide sequence analysis were amplified on cell culture and the analysis repeated on the cultured virus.

Neuraminidase inhibition phenotyping. All culture-positive MDCK cell supernatants were tested for NA sensitivity to oseltamivir using the NA-Star® assay (ABI). The inhibitory potency of oseltamivir was expressed as an IC_{50} value (concentration of inhibitor necessary to reduce NA activity by 50% relative to a reaction containing no inhibitor) and compared with the inhibitory potency of a reference strain virus. Reference strains used in this study were A/PR/8/34 and B/Lee/40.

1.3 Prior FDA Virology reviews.

Supporting nonclinical and clinical virology studies for oseltamivir phosphate were previously reviewed under NDA 021087/021246. Please see the Clinical Virology reviews by Narayana Battula, Ph.D. for detailed descriptions of these analyses.

1.4 Major Virology issues that arose during product development

There were no major Clinical Virology issues that arose during the review of this supplemental application.

1.5 State of antimicrobials used for the indication sought

No drugs are currently approved for treatment of uncomplicated influenza A or B virus infection in children less than 1 year old.

2 Nonclinical Virology

No new nonclinical studies to characterize the mechanism of action, antiviral activity, or combination antiviral activity of oseltamivir carboxylate were included in this supplemental application. Please see the Clinical Virology review conducted by N. Battula, Ph.D. of NDA 21087 for detailed analyses of the relevant studies submitted as part of the original NDA application.

3 Relevant Findings from Other Disciplines.

3.1 Pharmacokinetics

No new pharmacokinetic data relevant to Clinical Virology were included in the application. For a detailed pharmacokinetic analysis, see the review by Huimin Zheng, Pharm.D.

6.2 Pharmacodynamics

No pharmacodynamic data relevant to Clinical Virology were included in the application. For detailed pharmacodynamic analyses, see the reviews by Huimin Zheng, Pharm.D. and Jee Eun Lee, Ph.D.

6.3 Clinical

The safety data indicate that the tested dosages of oseltamivir phosphate were reasonably well tolerated by children less than 1 year old. For a detailed clinical safety analysis, see the review by Tafadzwa Vargas-Kasambira, M.D., M.P.H.

4 Clinical Virology

4.1 CASG 114

The primary objective of CASG 114 was to define oseltamivir carboxylate pharmacokinetics following administration of an oral dose of oseltamivir phosphate in children aged 0.5 to 34 months with influenza virus infection confirmed by diagnostic cell-culture or RT-PCR–based assays. Subjects were enrolled into one of five age cohorts.

Cohort I: 271 to 364 days; 30 mg BID
Cohort II: 181 to 270 days; 3 mg/kg BID
Cohort III: 91 to 180 days; 3 mg/kg BID
Cohort IV: 31 to 90 days; 3 mg/kg BID
Cohort V: 13-30 days; 3 mg/kg BID

The dosages were selected to match plasma oseltamivir exposure levels comparable to those of adults and children > 1 year old treated with the approved regimen. During the study, doses were adjusted by predetermined rules to achieve the targeted exposure levels; doses in Cohorts I and II were increased to 3.5 mg/kg (Cohorts I-B and II-B, respectively) after an interim analysis indicated that exposure levels were below the lower limit of the targeted range in some subjects.

The disposition of subjects in CASG 114 is summarized in Figure 1, including the numbers and ages (in days) of subjects enrolled into each cohort, the doses administered to subjects in each cohort (all were administered BID), as well as the number, type, and subtype of influenza viruses isolated from subjects within each cohort.

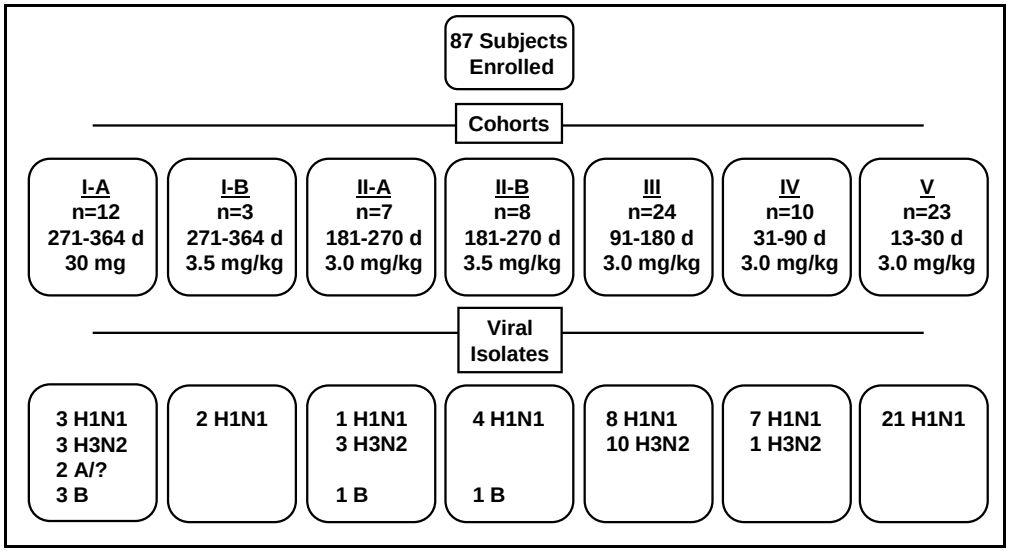


Figure 1. CASG 114 subjects and viruses per cohort

The time of collection, virus type/subtype, and host-subject age (by cohort) of each isolate is shown in Figure 2. Notably, the cohorts were not balanced with respect to numbers, influenza virus type and subtype, or timing of enrollment (i.e., flu-season). These imbalances complicate virological comparisons between the treatment groups.

Specimens from 17 of 87 subjects were culture-negative and therefore excluded from subsequent virological analyses. In total, viruses from 70 subjects were successfully amplified on culture and characterized, including 46 influenza H1N1 viruses, 17 H3N2 viruses, 2 influenza A viruses of unknown subtype, and 5 influenza B viruses.

The sponsor estimated time to clearance by determining the amount of time between baseline, which was the first day of oseltamivir phosphate administration, and the first day that virus became undetected by either RT-PCR or MDCK culture infectivity assay. The estimated times to clearance by RT-PCR and cell-culture, along with summary statistics, are presented in Figure 3.

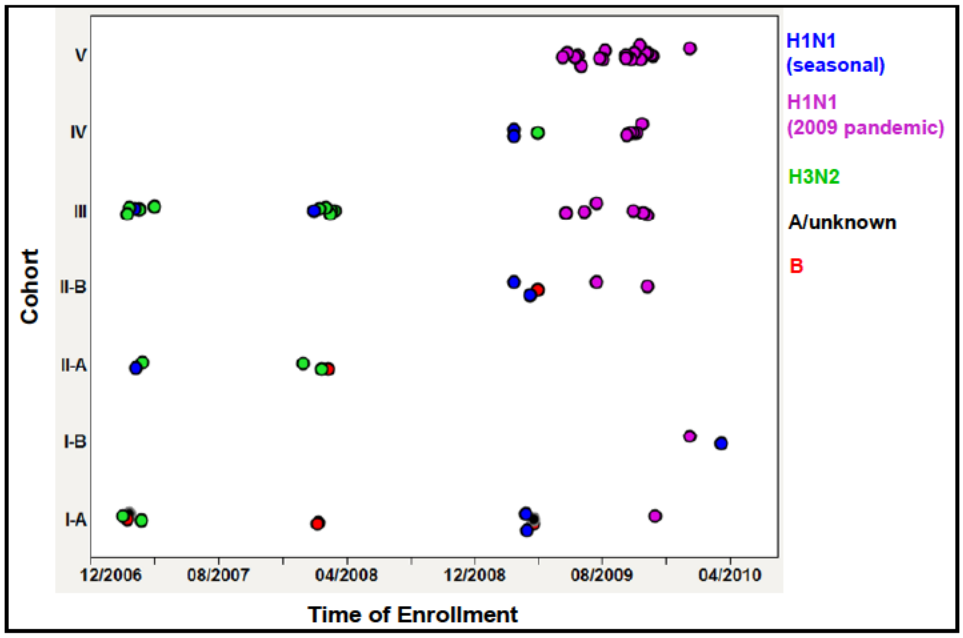
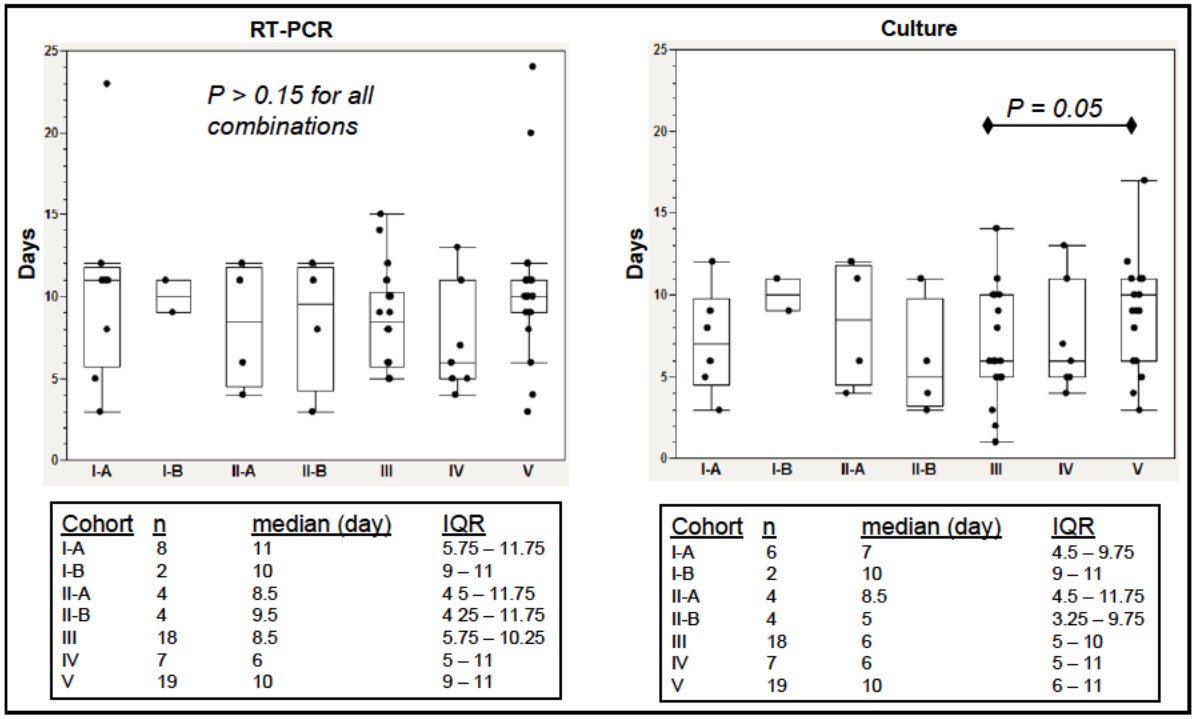


Figure 2. CASG 114 influenza type and subtype per cohort at enrollment



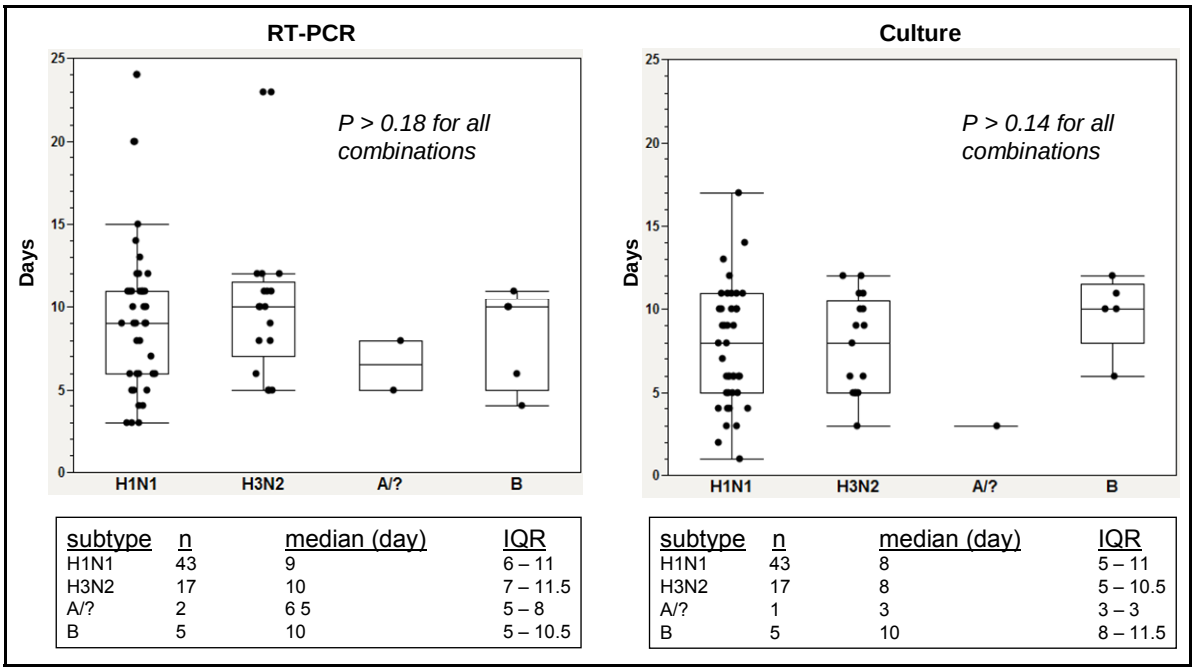
Cohort	n	median (day)	IQR
I-A	8	11	5.75 – 11.75
I-B	2	10	9 – 11
II-A	4	8.5	4 5 – 11.75
II-B	4	9.5	4 25 – 11.75
III	18	8.5	5.75 – 10.25
IV	7	6	5 – 11
V	19	10	9 – 11

Cohort	n	median (day)	IQR
I-A	6	7	4.5 – 9.75
I-B	2	10	9 – 11
II-A	4	8.5	4.5 – 11.75
II-B	4	5	3.25 – 9.75
III	18	6	5 – 10
IV	7	6	5 – 11
V	19	10	6 – 11

Figure 3. Influenza A time to undetected by cohort and assay type

Data for seasonal H1N1, 2009 pandemic H1N1, H3N2, and influenza A virus isolates of unknown subtype were pooled for this analysis due to the small sample size. The median number of days before viral load diminished to undetected levels by RT-PCR ranged from 6 to 11 days, with no significant difference between cohorts (P > 0.15 for all comparisons). The median times to undetected virus by cell-culture assay were similar, ranging from 5 to 10 days.

However, there was a significant difference between subjects in Cohort III (6 to 8 month olds treated with 3.0 mg/kg BID) and Cohort V (0-2 month olds treated with 3.0 mg/kg BID); the median (interquartile range; IQR) time from baseline to undetected virus by cell-culture for subjects in Cohorts III and V were 6 (5-10) and 10 (6-11) days, respectively (P = 0.05). The clinical significance of this difference is unknown. Subjects in Cohort V were younger than those in Cohort III, and it is possible that the delayed reduction in viral infectivity on cell culture reflects a slower induction of anti-influenza antibodies in the younger children with less developed immune systems. It is also possible that the difference is related to virological imbalance between the cohorts; 8 H1N1 and 10 H3N2 viruses were isolated from subjects in Cohort III while 19 H1N1 and no H3N2 isolates were collected from subjects in Cohort V. A similar analysis comparing the estimated clearance rates by virus type and subtype did not demonstrate statistically significant differences by either RT-PCR (P > 0.18 for all comparisons) or cell-culture assay (P > 0.14 for all comparisons) (Figure 4). The individual RNA load and cell culture infectivity assay data for each subject are illustrated in Appendix A.



subtype	n	median (day)	IQR
H1N1	43	9	6 – 11
H3N2	17	10	7 – 11.5
A/?	2	6 5	5 – 8
B	5	10	5 – 10.5

subtype	n	median (day)	IQR
H1N1	43	8	5 – 11
H3N2	17	8	5 – 10.5
A/?	1	3	3 – 3
B	5	10	8 – 11.5

Figure 4. Influenza Virus Time to Undetected by Subtype

Resistance to NAIs is associated with substitutions in the viral NA and/or HA glycoproteins. Resistance conferring NA substitutions can occur at amino acid positions within or outside of the catalytic domain and are often subtype specific. Genotypic resistance is typically associated with expression of H275Y in H1N1 viruses, E119V, R292K, or N294S in H3N2 viruses, and D197E/N in B viruses (Nguyen et al., 2012). However, reduced susceptibility has also been associated with the expression of several other NA substitutions identified in nonclinical, surveillance, and clinical studies including E119G/V, D151G/N/E, Y155H, D199G/E/N, I223K/M/R/V, S247G/N, G249K/R + I427T, or N295S in H1N1 viruses; E119D/I, D151E/D/G/N/V, I222L, R224K, E276D, N294S, or R371K in H3N2 viruses; and E105K, E117A/D/G/V, R150K, D197Y, I221I/V/T, H273Y, R292K, N294S, R374K, or G407S in subtype B viruses (Ferraris and Lina, 2008; Fujisaki et al., 2012; Hurt et al., 2012; Monto et al., 2006; Nguyen et al., 2012; Sheu et al., 2008).

HA substitutions associated with reduced susceptibility to NAIs typically involve amino acid changes that reduce the affinity of HA for its sialic acid-bearing receptors, thereby decreasing the dependence of the virus on NA function (Gubareva et al., 1998; McKimm-Breschkin et al., 1996). Most HA NAI-resistance-associated substitutions have been identified in isolates from cell culture studies or from viruses found in immunocompromised patients, and the clinical impact of HA substitutions selected by NAIs is unclear. HA substitutions that have been associated with reduced susceptibility to NAIs include G155E (Mckimm-Breschkin et al., 2012) and A196T (Baz et al., 2007) in H1N1; K189G (Smee et al., 2001), V226I (Ison et al., 2006), S262N (Kiso et al., 2004), A28T + R124M (Tai et al., 2008), and R142G + Y195F + I239R (Ison et al., 2006) in H3N2; and H99Q (Cheam et al., 2004), N150S (Staschke et al., 1995), N155S, T208I (Gubareva et al., 1998), S285A (Ison et al., 2006), and V90A + L242Q (Barnett et al., 1999) in B viruses.

Resistance analyses included the genotypic and phenotypic characterization of cell culture amplified isolates from 68 subjects, including 45 subjects infected with H1N1 virus (37 with 2009 pandemic H1N1 and 8 with seasonal H1N1), 18 with H3N2 virus, and 5 with influenza B virus. The phenotypic analysis consisted of oseltamivir carboxylate susceptibility determinations by MUNANA–based neuraminidase inhibition (NAI) assays, while the genotypic analysis consisted of population nucleotide sequence analysis of the influenza neuraminidase (NA) and hemagglutinin (HA) genes.

Summary tables of the genotypic resistance analysis for the NA and HA proteins of seasonal influenza H1N1, 2009 pandemic H1N1, and H3N2 isolates are presented in Tables 1, 2, and 3, respectively. Each table contains data for any isolate with an emergent substitution (indicated by a highlighted cell) or that contained a known resistance-associated substitution. Summary tables containing the genotypic data from all isolates are presented in Appendix B. Missing genotypic data are indicated by black cells. The genotypic summary includes any amino acid deviations from the reference strain's sequence. The results of the phenotypic analysis are presented under the "NAI Susceptibility" heading, include the results of the MUNANA-based NAI assay for the isolate and a wild-type NA reference, and are presented as the 50% inhibition concentration (IC_{50} value) in nM units.

There were 8 cases with seasonal H1N1 infection. Six of the eight viruses isolated from subjects expressed NA H275Y, a known resistance-conferring substitution, at baseline (Table 1). Virus from Subject 228 and Subject 6 experienced on-treatment substitutions within NA and HA, respectively. Subject 228's baseline isolate contained a mixed population of variants expressing NA S59S/G at baseline (Day 1) that shifted to S59 at Day 3. Subject 6's baseline isolate expressed HA N203D at Day 1 and N203 at Day 5. Both of the amino acid substitutions experienced by isolates in these subjects resulted in a higher degree of concordance with the reference strain and are unlikely to represent a treatment-related selection, particularly not in the presence of NA H275Y, which is expected to reduce the antiviral activity of oseltamivir carboxylate. Indeed, all 6 of the isolates had IC_{50} values ≥ 191.5 nM, which was > 100-fold higher than those of the reference isolates.

In contrast to the seasonal H1N1 isolates, none of the cell culture amplified isolates from the 39 subjects who were infected with pandemic H1N1 viruses had detectable levels of variants expressing known oseltamivir resistance-associated substitutions at baseline (Appendix B). However, 7 subjects harbored viruses that experienced amino acid changes during or after

treatment, including the emergence of NA H275Y-expressing variants in Subjects 627, 321, and 363 (Table 2). Interestingly, the emergence of the H275Y variant in Subject 627 by Day 7 was only associated with a 4.3-fold shift, whereas emergence of H275Y variants in Subject 321 on Day 3 and Subject 363 on Day 10 resulted in 54.0-fold and 117.1-fold shifts, respectively, and it is possible that the identification of the resistant isolate would not have been identified if the resistance analysis had been limited to the NAI phenotypic assay.

Table 1. Summary of Resistance for Seasonal Influenza A H1N1 Isolates

Specimen			NA Substitutions								HA Substitutions									NAI Susceptibility	
Cohort	SubID	Day	L24	N28	G41	S42	S59	H126	H275	D354	S158	E186	N200	G202	N203	A206	H209	D289	N393	IC_{50}	IC_{50}_REF
I-A	441	1							Y	G	N	G		A	D	T				191.5	1.59
I-A	441	5							Y	G	N	G		A	D	T				556.6	1.36
II-B	7	1	F	S					Y	G				V	D	T	R			570	2.57
II-B	7	4	F	S					Y	G				V	D	T	R			592.9	1.36
II-B	503	1		S					Y	G				I	D	T	R		T	671.9	1.7
II-B	503	5		S					Y	G				I	D	T	R		T	656.8	1.7
III	228	1					S/G		Y	G					D					389.62	1.35
III	228	3							Y	G										852.14	1.03
IV	6	1				N			Y	G			S	S	D	T				647	1.7
IV	6	5				N			Y	G			S	S		T				215.3	1.59
IV	502	1		S	E			Q	Y	G				V	D	T	R	G		380.2	1.7
IV	502	5		S	E			Q	Y	G				V	D	T	R	G		640.9	1.7

Table 2. Summary of Resistance for Pandemic Influenza A H1N1 Isolates

Specimen			NA Substitutions									HA Substitutions													NAI Susceptibility	
Cohort	SubID	Day	I8	F74	V106	S145	N248	I258	H275	S340	S442	P66	K71	P100	I133	P141	P176	S220	D_G239	I284	P314	I338	E391	D453	IC_{50}	IC_{50}_REF
III	627	1			I		D							S				T	D			V	K		0.89	3.04
III	627	7			I		D		Y					S				T	D			V	K		3.83	1.92
III	641	1			I	S/F	D							S				T	D			V	K		1.7	1.82
III	641	3			I		D							S				T	D			V	K		0.78	2.1
IV	237	1			I		D					S	R	S				T	D	T		V			1.02	1.9
IV	237	3			I		D							S				T	D	T		V			0.52	1.81
V	28	1			I		D							S				T	D			V			0.82	1.5
V	28	6			I		D	M						S				T	D			V			0.35	1.57
V	42	1			I		D							S		L	T	T	D			V		N	0.64	1.9
V	42	4			I		D							S				T	D			V			0.4	1.78
V	321	1			I		D							S				T	E		S	V			0.55	2.1
V	321	3			I		D		H/Y	S/F				S				T	E		S	V			29.68	2.1
V	363	1			I		D							S	M			T	D			V			1.75	1.82
V	363	10			I		D		Y					S	M			T	D			V			204.9	1.78

Six of the eight remaining genotypic changes represented an amino acid change back to the consensus sequence, and it is unlikely that these quasispecies shifts were the result of oseltamivir selection (Table 2). Two of the eight substitutions, NA I258M and S340S/F in Subjects 28 and 321, respectively, represent a deviation from the reference strain's sequence. The I258M variant, which had an IC_{50} value of 0.35 nM, was no less susceptible to oseltamivir than the reference strain, which was 1.57 nM, or the baseline clinical isolate, which had an IC_{50} value of 0.82 nM. Evaluating the impact of the NA S340S/F substitution on drug susceptibility is difficult because of the co-emergence of H275H/Y.

None of the cell culture amplified viruses isolated from the 18 H3N2 infected subjects had detectable levels of resistant variants at baseline (Appendix B). Isolates from 8 subjects that did experience genotypic shifts between baseline and during- or post-treatment are summarized in Table 3. Emergent substitutions that deviated from the reference strain's amino acid sequence included NA I443V (Subject 4, Day 4 isolate), HA K308Q, P309S, and A352S (Subject 5, Day 4

isolate), NA D147D/N (Subject 161, Day 3 isolate), NA T434P (Subject 23 Day 3 and 5 isolates), NA N465I/N (Subject 301, Day 3 isolate), NA D151D/N (Subject 522, Day 3 and 5 isolates), and HA I377R (Subject 522, Day 5 isolate). However, none of the amino acid changes involved known oseltamivir resistance-conferring substitutions, occurred in isolates from more than 1 subject, or were associated with shifts in susceptibility > 1.4-fold higher than those of the reference strain, which appeared to be within the range of the assay's variability. The contribution of HA to phenotypic susceptibility was not evaluated using a cell culture infectivity assay.

Table 3. Summary of Resistance for Influenza A H3N2 Isolates

Specimen			NA Substitutions													HA Substitutions																NAI Susceptibility	
Cohort	SubID	Day	I26	V143	D147	D151	R210	I215	I254	K296	T312	R400	T434	I443	N465	T16	D117	S126	L173	K189	V202	N206	N294	K308	P309	A352	G353	I377	E465	K524	IC_{50}	IC_{50}_REF
I-A	4	1			N			V			I					A				Q	G	D						R	D		0.48	1.35
I-A	4	4			N			V			I			V																	0.66	1.35
II-A	5	1			N			V			I					A				Q	G	D						R			0.41	1.03
II-A	5	4			N			V			I					A				Q	G	D		Q	S	S		R			0.35	0.75
II-A	161	1						V			I					A			S	Q	G	D						R			0.33	1.46
II-A	161	3			N/D			V			I					A			S	Q	G	D						R			1.45	1.46
II-A	161	10									I																				0.52	0.75
III	23	1		L							I					A	N				G	D								T	1.5	1.26
III	23	3									I		P																		1.85	1.26
III	23	5									I		P																		1.99	1.26
III	24	1		A							I					A					G	D									1.22	0.7
III	24	4									I					A					G	D									1.76	1.26
III	224	1	I/T		N/D		K/R				I					A					G	D									0.49	1.31
III	224	5									I					A					G	D									0.69	1.31
III	301	1							V	R	I	K				A		A	S		G	D	K				R				0.09	0.43
III	301	3							V	R	I	K			N/I																0.27	0.43
III	522	1			N			V			I					A				Q	G	D									2.09	0.65
III	522	3			N	N/D		V			I																				0.8	0.65
III	522	5			N			V			I					A				Q	G	D						R			0.72	0.65

None of the cell culture amplified viruses from the influenza B virus infected subjects had detectable levels of resistant variants at baseline, nor were any emergent substitutions in NA or HA detected during or after treatment (Appendix B). However, the phenotypic susceptibility of influenza B viruses to NAIs is < 10-fold that of influenza A viruses, and the lower level of antiviral activity may reduce the probability of selecting resistant variants.

In summary, the incidence of emergent resistant viruses was at least 8% (3/37) among subjects infected with the 2009 pandemic strain of influenza A/H1N1 in CASG 114, including Subject 627 of Cohort III, who was 219 days old at the time of enrollment, and Subjects 321 and 363 of Cohort V, who were 34 and 20 days old at the time of enrollment, respectively.

4.2 WP22849

The primary objective of WP22849 was to define oseltamivir pharmacokinetics in children aged 0 to 12 months with influenza virus infections confirmed by diagnostic rapid antigen test or by RT-PCR–assay. The trial enrolled 65 subjects from 11 centers in Europe, including Spain, Italy, Germany, France, Belgium, and Poland, from the 2010/2011 to the 2011/2012 influenza seasons.

Infants were enrolled into three cohorts according to their postnatal age:

Cohort I: infants 91 to 364 days, 3 mg/kg BID

Cohort II: infants 31 to 90 days, 2.5 mg/kg BID
Cohort III: 14 to 30 days, 2 mg/kg BID

Patients with influenza symptoms for up to 96 hours prior to first dose were allowed into the study in order to allow for the inclusion of infants whose parents/guardians may not have initially recognized the illness. However, this delay of treatment may have limited drug efficacy.

The disposition of subjects in WP22849 is summarized in Figure 5, including the numbers and ages (in months) of subjects enrolled into each cohort, the doses administered to subjects in each cohort (all were administered BID), as well as the number, type, and subtype of influenza viruses isolated from subjects within each cohort.

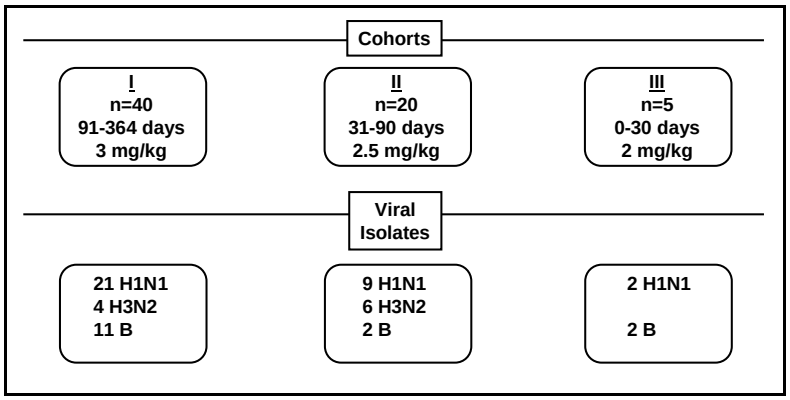


Figure 5. WP22849 Subject and Virus Disposition

The time of collection, virus type/subtype, and cohorts of each isolate are shown in Figure 6. Notably, the cohorts were not balanced with respect to numbers, influenza virus type and subtype, or timing of enrollment (i.e., flu-season). These imbalances complicate virological comparisons between the cohorts.

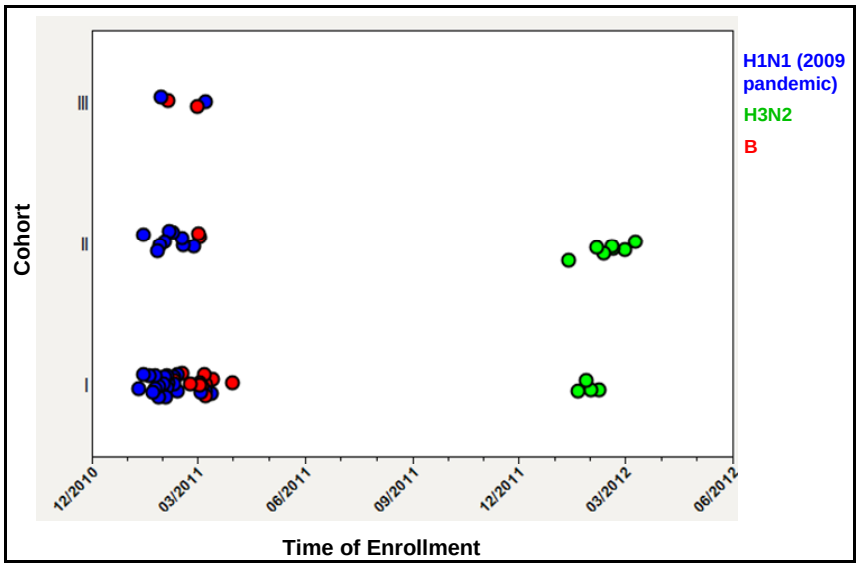
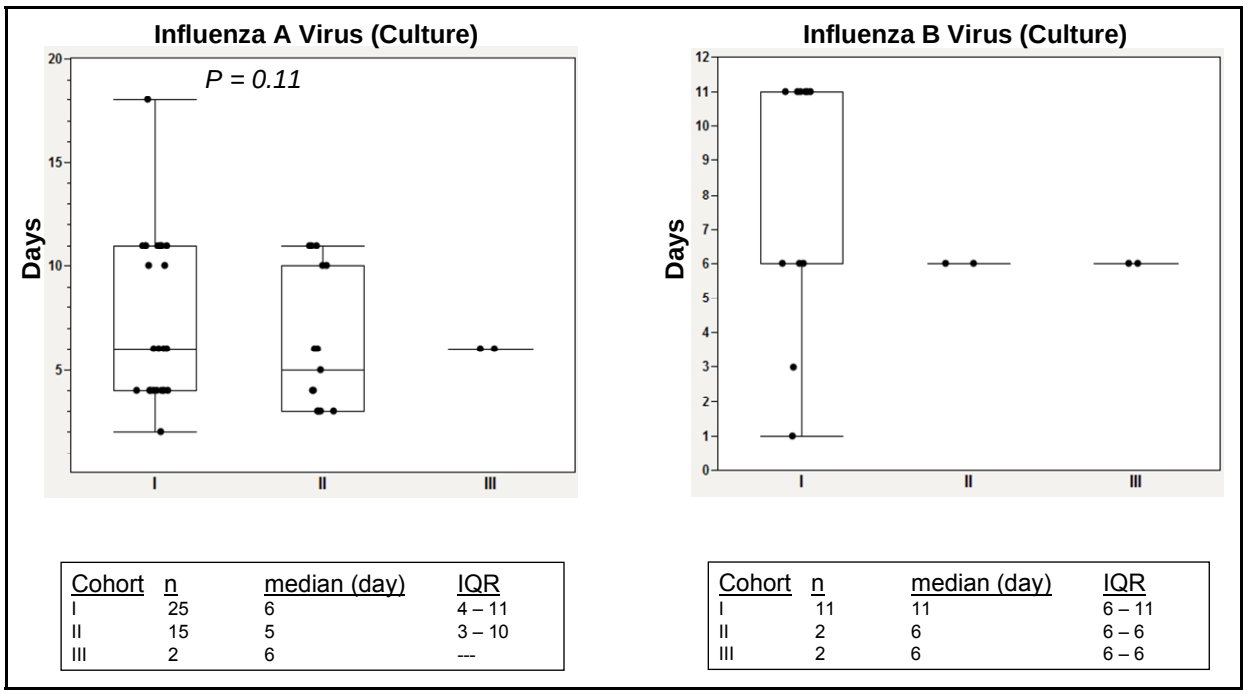


Figure 6. WP22849 influenza Type and Subtype per Cohort at Enrollment

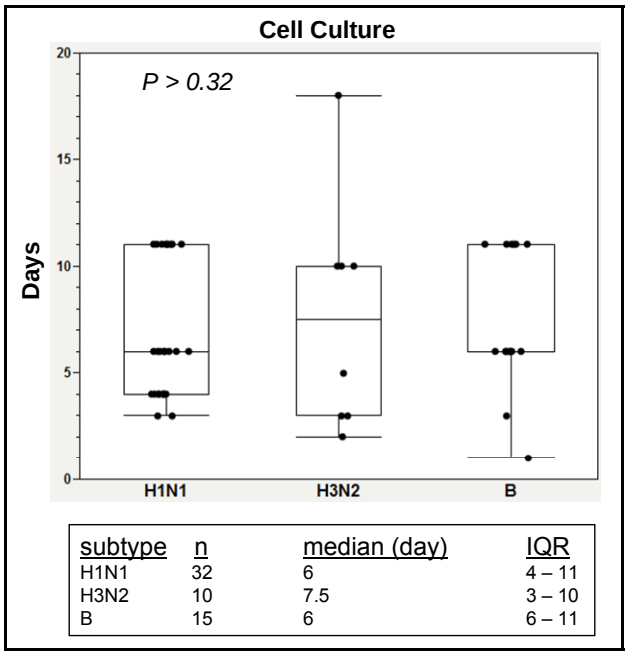
Influenza isolates from 57 subjects were successfully amplified on culture and characterized, including 32 influenza H1N1 viruses (pandemic 2009), 10 H3N2 viruses, and 15 influenza B viruses. The time to viral clearance was estimated by determining the amount of time that passed between baseline, which was the first day of oseltamivir phosphate administration, and the first day that virus became undetected by MDCK culture infectivity assay. The estimated times to clearance by cell-culture, along with summary statistics, are presented in Figure 7. Data for the 2009 pandemic H1N1 and H3N2 isolates were pooled for this analysis (Figure 7, left) due to small sample number. The median number of days before virus dropped to undetected levels by cell culture assay ranged from 5 to 6 days, with no significant difference between Cohorts I and II (P > 0.11; Cohort III was too small for statistical analysis). The median times to undetected influenza B virus by cell culture assay were longer, ranging from 6 to 11 days, but the Cohorts had too few subjects to allow for meaningful comparisons (Figure 7, right). A similar analysis comparing the estimated clearance rates by virus type and subtype did not demonstrate statistically significant differences (P > 0.32 for all comparisons) (Figure 8). The individual RNA load and cell culture infectivity assay data for each subject are illustrated in Appendix C.



Cohort	n	median (day)	IQR
I	25	6	4 – 11
II	15	5	3 – 10
III	2	6	---

Cohort	n	median (day)	IQR
I	11	11	6 – 11
II	2	6	6 – 6
III	2	6	6 – 6

Figure 7. Influenza A (left) and B (right) Viruses versus Time to Undetected



subtype	n	median (day)	IQR
H1N1	32	6	4 – 11
H3N2	10	7.5	3 – 10
B	15	6	6 – 11

Figure 8. Time to Undetected by Subtype

Resistance analyses included the phenotypic and genotypic characterization of isolates from 57 subjects, including 32 subjects infected with H1N1 virus, 10 with H3N2 virus, and 15 with influenza B virus. The phenotypic analysis consisted of oseltamivir susceptibility determinations by NA-Star® assay (ABI), while the genotypic analysis consisted of population nucleotide sequence analysis of the influenza neuraminidase (NA) and hemagglutinin (HA) genes. The genotypic analysis was initially attempted on RNA collected directly from clinical samples; if the analysis failed, then the virus was amplified on cell culture and the analysis repeated.

Summary tables of the genotypic resistance analysis for the NA and HA proteins of influenza H1N1, H3N2, and influenza B virus isolates are presented in Tables 4, 5, and 6, respectively. Each table contains data for any isolate with an emergent substitution (indicated by a highlighted cell) or that contained a known resistance-associated substitution. Missing genotypic data are indicated by black cells. Summary tables for all isolates are provided in Appendix D. The genotypic summary includes any amino acid deviations from the reference strain's sequence. The results of the phenotypic analysis are presented under the "NAI Susceptibility" heading, include the results of the NAI assay for the isolate and a wild-type NA reference, and are presented as the 50% inhibition concentration (IC_{50} value) in nM units.

There were 32 subjects with H1N1 virus infection, and none of their baseline isolates expressed NA H275Y or other known oseltamivir resistance-associated substitutions (Table 4). However, substitutions associated with resistance emerged among the viruses of 21.9% (7/32) of the subjects. Specifically, variants expressing NA H275Y emerged in isolates from 6/7 subjects and a variant expressing NA N295S emerged 1/6 subjects. Among the subjects whose virus developed H275Y, 3/6 were detected as mixed populations, with susceptibility shifts ranging from 4.79-fold to 119-fold from baseline. The three subjects with dominant H275Y expressing variants experienced susceptibility shifts from 74-fold to 128-fold from baseline. Virus from Subject 3001, from whom the N295S-expressing variant was isolated on Day 6, experienced a 55-fold shift from baseline.

Table 4. Summary of resistance and NA/HA substitutions for H1N1 isolates

Specimen				NA Substitutions										HA Substitutions													NAI Susceptibility	
Cohort	SubID	Sample	Day	V13	M15	N44	V106	V241	N248	G249	H275	N295	N369	P100	D114	S160	G172	S202	A214	S220	D_G239	Q_R240	M274	D291	I338	E391	S468	IC_{50}	IC_{50}_REF
I	1701	Culture	1			S	I	I	D				K	S		G		T	T	T	D	Q			V	K	N	0.29	0.39
I	1701	Swab	4			S	I	I	D				K	S		G		T	T	T	D	Q			V	K	N	0.28	0.39
I	1701	Swab	6			S	I	I	D		Y		K	S		G		T	T	T	D	Q			V	K	N	79.69	0.39
I	3001	Culture	1				I	I	D				K	S	N			T		T	D	Q			V	K	N	0.49	0.8
I	3001	Culture	3				I	I	D				K															0.29	0.51
I	3001	Culture	6				I	I	D			S	K	S	N			T		T	D	Q			V	K	N	26.8	0.8
I	3004	Culture	1			S	I	I	D				K	S		G		T	T	T	D	Q			V	K	N	0.38	0.75
I	3004	Culture	4			S	I	I	D				K	S		G		T	T	T	D	Q			V	K	N	0.41	0.75
I	3004	Culture	6			S	I	I	D		H/Y		K	S		G		T	T	T	D	Q			V	K	N	3.01	0.75
II	2005	Culture	1				I	I	D	E			K	S	N			T		T	D	Q			V	K	N	0.31	0.8
II	2005	Swab	4				I	I	D	E			K	S	N			T		T	D	Q			V	K	N	0.91	1.01
II	2005	Culture	6				I	I	D	E	H/Y		K	S	N			T		T	D	Q			V	K	N	36.76	0.8
II	3801	Swab	1				I	I	D				K															0.29	0.75
II	3801	Culture	3				I	I	D				K															0.57	1.01
II	3801	Culture	6				I	I	D		H/Y		K															1.35	0.75
II	4203	Culture	1				I	I	D				K	S	N		E	T		T	D	Q		N	V	K	N	0.63	0.8
II	4203	Culture	4				I	I	D				K	S	N		E	T		T	D	Q		N	V	K	N	0.3	0.51
II	4203	Swab	6				I	I	D		Y		K	S	N		E	T		T	D	Q		N	V	K	N	80.53	0.8
III	4301	Culture	1	I	I		I	I	D				K	S	N			T		T	D	Q	V		V	K	N	0.49	0.68
III	4301	Culture	4	I	I		I	I	D		Y		K															36.44	0.68

None of the isolates from the 10 H3N2 infected subjects had detectable levels of resistant variants at baseline. Isolates from 3 subjects exhibited genotypic shifts between baseline and during- or post-treatment (Table 5). Emergent substitutions that deviated from the reference strain's amino acid sequence included NA R292K, a known oseltamivir resistance-associated substitution, in Subject 4225 on Day 3, and HA I538T in Subject 4227 on Day 6. Subject 1206 bore virus expressing NA A82T at baseline and a mixed variant population of A82A/T on Day 3. The NAI assay was not conducted on the R292K-expressing or HA I538T-expressing isolates.

Table 5. Summary of Resistance for Seasonal Influenza A H3N2 Isolates

Specimen				NA Substitution									HA Substitutions																		NAI Susceptibility	
Cohort	SubID	Source	Day	L81	A82	D93	R210	R292	S367	K369	N402	I464	T46	Q49	S61	T64	I67	L75	K78	G94	K160	N161	L173	A214	T228	S230	V239	N294	N328	D503	I538	IC_{50}	IC_{50}_REF
I	4225	Swab	1	P		E	K		N	T	D	L	A					I	E		N	S	S	S	A	I	I		S	N		0.35	0.5
I	4225	Culture	3	P		E	K	K	N	T	D	L	A					I	E		N	S	S	S	A	I	I		S	N			
I	4225	Culture	6	P		E	K		N	T	D	L	A					I	E		N	S	S	S	A	I	I		S	N		0.31	0.5
I	4227	Culture	1	P		G			N	T	D	L		R	N	I	M		E	S	N			S	A	I	I	K	S			0.59	0.5
I	4227	Culture	3	P		G			N	T	D	L		R	N	I	M		E	S	N			S	A	I	I	K	S			0.38	0.5
I	4227	Culture	6	P		G			N	T	D	L		R	N	I	M		E	S	N			S	A	I	I	K	S		T		
II	1206	Culture	1	P	T				N	T	D	L							E		N	S		S	A	I	I		S	N		0.28	0.5
II	1206	Culture	3	P	A/T				N	T	D	L							E		N	S		S	A	I	I		S	N		0.29	0.5

Cell-culture amplified influenza B isolates from 4/15 subjects experienced genotypic shifts from baseline (Table 6), although the Day 6 isolate for Subject 2602 likely represented a sampling error as its NA and HA amino acid sequences match that of isolates from Subject 3608 (Appendix D). The Day 6 isolate from Subject 4214 expressed NA M50T and A209A/T substitutions that are not known to be associated with resistance to oseltamivir and did not occur in isolates from more than 1 subject. The Day 4 isolate from Subject 2601 expressed NA A245D, a substitution that has not been previously linked to oseltamivir resistance but was associated with an IC_{50} value of 15.35 nM, which represented a 2.9-fold shift from baseline susceptibility in this study.

Table 6. Summary of Resistance for Seasonal Influenza B Isolates

Specimen			NA Substitution																													HA Substitution							NAI Susceptibility	
Cohort	SubID	Day	T8	S27	T49	M50	P51	Q61	L73	L132	N199	V204	A209	K220	L238	I240	A245	S246	F266	N329	N340	E358	M375	A389	A395	S397	M464	C474	P480	V481	S487	N490	V30	E63	L73	I161	A217	T275	I470	IC_{50}	IC_{50}_REF
I	2602	1			A																D							Y							V				4 29	2.64
I	2602	4			A																D							Y							V				4 32	2.69
I	2602	6	M					H	F			I		N	I					D		A	K	T		R			I		H	I							3 67	2.51
I	3606	1		L			S		F		D							L		D								L		P				P	V				1 82	2.64
I	3606	3		L			S		F		D							L		D								L		P				P	V				2 01	3.34
I	3606	6		L			S		F		D							L										L		P				P	V				2 37	2.64
I	4214	1														V				D					V									P	V			L	3 28	2.62
I	4214	4														V				D					V									P	V			L	5 06	2.69
I	4214	6				T							A/T			V				D					V															3 65	2.62
I	4216	1																			D						I						K		V				3.3	3.34
I	4216	4																															K		V		I		3 01	2.69
I	4216	6																			D						I						K		V		I		3.43	2.64
II	2601	1																			D			T											V	E			5.3	2.64
II	2601	4															D				D			T											V	E			15.35	2.64

In summary, at least 22% (7/32) of subjects infected with the 2009 pandemic strain of influenza A/H1N1 and at least 10% (1/10) among subjects infected with influenza A/H3N2 developed resistant virus. The subjects who developed oseltamivir-resistant H1N1 viruses included: Subjects 1701, 3001, and 3004 of Cohort I, who were 97, 202, and 189 days old at the time of enrollment respectively; Subjects 2005, 3801, and 4203 of Cohort II, who were 70, 56, and 57 days old at the time of enrollment, respectively; and Subject 4301 of Cohort III, who was 29 days old at the time of enrollment. The specific age of Subject 4225, who harbored the emergent oseltamivir resistant H3N2 virus, was not provided, but—based on cohort assignment—should have been between 91 and 364 days at the time of enrollment. The emergence of an influenza B virus expressing NA A245D may have been associated with oseltamivir resistance.

5 Conclusions

There were no key virology issues that arose during the review of this supplemental application. The pivotal studies were not designed to demonstrate antiviral activity or efficacy in subjects < 1 year old; both were small, open-labeled, uncontrolled studies that evaluated a narrow dose range and enrolled subjects at times exceeding the anticipated therapeutic window of oseltamivir.

The incidence of emergent resistant viruses was at least 8% (3/37) among subjects infected with the 2009 pandemic strain of influenza A/H1N1 in CASG 114, at least 22% (7/32) among subjects infected with the 2009 pandemic strain of influenza A/H1N1 in WP22849, and at least 10% (1/10) among subjects infected with influenza A/H3N2 in WP22849. These resistance rates are consistent with those observed in other trials (TAMFLU label).

6 Package Insert

No changes to Section 12.4 of the label were included with this supplemental application. However, all references to “Clinical Pharmacology (12.4)” should be changed to “Microbiology (12.4)” for consistency with other antiviral package inserts.

7 Recommendations

There are no recommendations to be communicated to the sponsor at this time.

8 References

Barnett JM, Cadman A, Burrell FM, Madar SH, Lewis AP, Tisdale M, Bethell R. In vitro Selection and Characterization of influenza B/Beijing/1/87 isolates with altered susceptibility to zanamivir. Virology. 1999 Dec 20;265(2):286-295.

Baz M, Abed Y, Boivin G. Characterization of drug-resistant recombinant influenza A/H1N1 viruses selected in vitro with peramivir and zanamivir. Antiviral Res. 2007 May;74(2):159-162

CDC. FluView: A Weekly Influenza Surveillance Report Prepared by the Influenza Division. 2008-2009 Influenza Season Week 39 ending October 3, 2009.

CDC. FluView: A Weekly Influenza Surveillance Report Prepared by the Influenza Division. 2012-2013 Influenza Season Week 42 ending October 20, 2012.

CDC. Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP) – United States, 2012-13 Influenza Season. MMWR. Aug 17, 2012; 61(32):613-618.

CDC. Update: Influenza Activity – United States, 2010-11 Season, and Composition of the 2011-12 Influenza Vaccine. MMWR. June 3, 2011; 60(21):705-712.

CDC. 2007-2008 Influenza (Flu) Season. Flu Season Summary (September 30, 2007 – May 17, 2008).

Cheam AL. Barr IG. Hampson AW. Mosse J. Hurt AC. In vitro generation and characterisation of an influenza B variant with reduced sensitivity to neuraminidase inhibitors. Antiviral Res. 2004 Sep;63:177-181.

Dawood FS, Fiore A, Kamimoto L, et al. Burden of Seasonal Influenza Hospitalizaiton in Children, United States, 2003 to 2008. *J Pediatr*. 2010; 157(5):808-814.

Deyde VM, Xu X, Bright RA, Shaw M, Smith CB, Zhang Y, Shu Y, Gubareva LV, Cox NJ, Klimov AI. Surveillance of Resistance to Adamantanes among Influenza A(H3N2) and A(H1N1) Viruses Isolated Worldwide. *J Infect Dis*. 2007; 196:249-257.

Dharan NJ, Gubareva LV, Meyer JJ, et al. Infections with Oseltamivir-Resistant Influenza A(H1N1) Virus in the United States. *JAMA*. 2009; 301(10):1034-1041.

Ferraris O, Lina B. Mutations of neuraminidase implicated in neuraminidase inhibitors resistance. J Clin Virol. 2008 Jan;41(1):13-19.

Fujisaki S, Takashita E, Yokoyama M, Taniwaki T, Xu H, Kishida N, Sato H, Tashiro M, Imai M, Odagiri T. A single E105K mutation far from the active site of influenza B virus neuraminidase contributes to reduced susceptibility to multiple neuraminidase-inhibitor drugs. Biochem Biophys Res Commun. 2012 Nov 3. [Epub]

Gubareva LV, Mastrosovich M N, Brenner M K, Bethell R C, and R G Webster. Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. J Infect Dis. 1998 Nov;178(5):1557-62.

Hurt AC, Chotpitayasunondh T, Cox NJ, Daniels R, et al., Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. Lancet Infect Dis. 2012 Mar;12(3):240-8.

Ison M, Gubareva LV, Atmar RL, Treanor J, Hayden FG. Recovery of Drug-Resistant Influenza Virus from Immunocompromised Patients: A Case Series JID. 2006 Mar 15;193(6):760-764.

Kiso M, Mitamura K, Sakai-Tagawa Y, Shiraishi K, Kawakami C, Kimura K, Hayden FG, Sugaya N, and Kawaoka Y. Resistant influenza A viruses in children treated with oseltamivir. Lancet. 2004 Aug28-Sep3;364(9436):759-765

McKimm-Breschkin JL, Blick TJ, Sahasrabudhe A, Tiong T, Marshall D, Hart GJ, Bethell RC, Penn CR. Generation and characterization of variants of NWS/G70C influenza virus after in vitro passage in 4-amino-Neu5Ac2en and 4-guanidino-Neu5Ac2en. Antimicrob Agents Chemother. 1996 Jan;40(1):40-6.

McKimm-Breschkin JL, Rootes C, Mohr PG, Barrett S, Streltsov VA. In vitro passaging of a pandemic H1N1/09 virus selects for viruses with neuraminidase mutations conferring high-level resistance to oseltamivir and peramivir, but not to zanamivir. J Antimicrob Chemother. 2012 Aug;67(8):1874-83.

Monto AS, Macken C, McKimm-Breschkin JL, Hampson AW, Hay A, Klimov A, Tashiro M, Webster RG, Aymard M, Hayden F, Zambon M, Influenza viruses resistant to the neuraminidase inhibitors detected during the first three years of their use. Antimicrob Agents Chemother. 2006;50:2395-2402.

Nguyen HT, Trujillo AA, Sheu TG, Levine M, Mishin VP, Shaw M, Ades EW, Klimov AI, Fry AM, Gubareva LV. Analysis of influenza viruses from patients clinically suspected of infection with an oseltamivir resistant virus during the 2009 pandemic in the United States. Antiviral Res. 2012 Mar;93(3):381-6.

Sheu TG, Deyde VM, Okomo-Adhiambo M, Garten RJ, Xu X, Bright RA, Butler EN, Wallis TR, Klimov AI, Gubareva LV. Surveillance for neuraminidase inhibitor resistance among human influenza A and B viruses circulating worldwide from 2004 to 2008. Antimicrob Agents Chemother. 2008 Sep;52(9):3284-92.

Smee DF, Sidwell RW, Morrison AC, Baily KW, Baum EZ, Ly L, Wagaman PC. Characterization of an influenza A (H3N2) virus resistant to cyclopentane neuraminidase inhibitor RWJ-270201. Antiviral Res. 2001 Dec;52:251-259

Staschke KA, Colacino JM, Baxter AJ, Air GM, Bansal A, Hornback WJ, Munroe JE, Laver WG. Molecular basis for the resistance of influenza viruses to 4-guanidino- Neu5Ac2en. Virology. 1995 Dec 20;214:642-646.

Tia CY, Escarpe PA, Sidwell RW, Williams MA, Lew W, Wu H, Kim CU, Mendel DB. Characterization of human influenza virus variants selected in vitro in the presence of the neuraminidase inhibitor GS 4071. Antimicrob. Agents Chemother. 1998 Dec;42:323-441.

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/s/

DAMON J DEMING
11/20/2012

JULIAN J O REAR
11/20/2012

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 21087 S-62/ 21246 S-45 **Applicant:** Hoffman-LaRoche Inc. **Stamp Date:** 06/21/2012

Drug Name: Oseltamivir **NDA Type:** Priority

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		Only new clinical information included.
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?			Not applicable
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			Not applicable
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?			Not applicable
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	X		
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?			Not applicable. No changes to the Microbiology section proposed.

File name: 5_Microbiology Filing Checklist for a NDA or Supplement 010908

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
11	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	X		
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? YES

Not to be communicated to the sponsor:
We will be updating the label to include additional resistance-associated substitutions that have been recently identified and published.

Damon J. Deming, Ph.D.	August 06, 2012
Reviewing Microbiologist	Date
Julian J. O'Rear, Ph.D.	August 06, 2012
Microbiology Team Leader	Date

File name: 5_Microbiology Filing Checklist for a NDA or Supplement 010908

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/s/

DAMON J DEMING
08/06/2012

JULIAN J O REAR
08/06/2012