CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 206426 **SDN:** 030 **DATE REVIEWED:** 11/4/14

Clinical Virology Reviewer: Takashi E. Komatsu, Ph.D., RAC

NDA #: (b) (4) **Supporting Document Numbers:** 030

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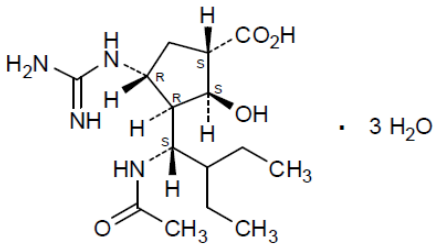
Reviewer's Name: Takashi E. Komatsu, Ph.D., RAC

Related/Supporting Documents: IND 69038, IND (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4)

Product Name(s):

Proprietary: RAPIVAB
Non-Proprietary/USAN: Peramivir
Code Name/Number: RWJ-270201 (b) (4) BCX-1812

Chemical Names: (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-ethylbutyl]-4-(aminoiminomethyl)amino]-2-hydroxy-cyclopentanecarboxylic acid-trihydrate
Structure:



PERAMIVIR

Molecular formula: $C_{15}H_{28}N_4O_4 \cdot 3H_2O$
Molecular weight: 382.45
Drug category: Antiviral
Indication(s): Treatment of acute uncomplicated influenza in patients 18 years and older
Dosage Form(s): 200 mg (10 mg/mL) in 0.9% sodium chloride solution
Route(s) of Administration: IV
Recommended Dosage: single 600 mg dose
Dispensed: Rx _X_ **OTC** ___ **(Discipline relevant)**

Abbreviations: AE, adverse event; AUC, area under the serum concentration; BID, twice daily; C_{max}, maximum serum concentration; CL, systemic clearance; EC, effective concentration; HA, hemagglutinin;

Reference ID: 3658925

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IC, inhibitory concentration; IP, intraperitoneal; IV, intravenous; mAb, monoclonal antibody; MCP-1, monocyte/macrophage chemoattractant protein; MDCK, Madin-Darby canine kidney; MIP, macrophage inhibitory protein; MOI, multiplicity of infection; NA, neuraminidase; NAI, neuraminidase inhibitor; NP, nuclear protein; OSE, oseltamivir; QD, once daily; RAT, rapid antigen test; RBV, ribavirin; RT-PCR, reverse transcription-polymerase chain reaction; $TCID_{50}$, 50% tissue culture infectious dose; TID, three times daily; T_{max}, time of maximum serum concentration; ZVR, zanamivir;

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 206426 **SDN:** 030 **DATE REVIEWED:** 11/4/14

Clinical Virology Reviewer: Takashi E. Komatsu, Ph.D., RAC

BACKGROUND and SUMMARY

This submission contains the sponsor's revised label for peramivir.

Microbiology Package Insert

The words with strikethroughs are the text the sponsor was requested to delete and words highlighted in blue are the sponsor's recommended insertions. The words highlighted in red are what the sponsor has requested to be deleted and this reviewer agrees with the sponsor's proposal. The words in green are what the sponsor has requested to be deleted but should be kept. The sponsor's rationale is presented below.

12.4 Microbiology

Mechanism of Action

Peramivir is an inhibitor of influenza virus neuraminidase, an enzyme that releases viral particles from the plasma membrane of infected cells. The median neuraminidase inhibitory activity (IC_{50} values) of peramivir in biochemical assays against influenza A/H1N1 virus, influenza A/H3N2 virus, and influenza B virus clinical isolates were 0.16 nM (n=44; range 0.01- 1.77 nM), 0.13 nM (n=32; range 0.05-11 nM), and 0.99 nM (n=39; range 0.04-54.2 nM), respectively, in a neuraminidase assay with a fluorescently labeled MUNANA substrate.

Antiviral Activity

The antiviral activity of peramivir against laboratory strains and clinical isolates of influenza virus was determined in cell culture. The concentrations of peramivir required for inhibition of influenza virus in cell culture varied depending on the assay method used and the virus tested. The median 50% effective concentrations (EC_{50} values) of peramivir in cell culture assays were 2.6 nM (n=13; range (b)(4)0.09-21 nM), 0.08 nM (n=17; range 0.01-1.9 nM) and 4.8 nM (n=11; range 0.06-120 nM) for influenza A/H1N1 virus, A/H3N2 virus, and B virus strains, respectively.

The relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, and the inhibition of influenza virus replication in humans has not been established.

Resistance

Cell culture: Influenza A and B virus isolates with reduced susceptibility to peramivir were recovered by serial passage of virus in cell culture in the presence of increasing concentrations of peramivir. Reduced susceptibility of influenza virus to inhibition by peramivir may be conferred by amino acid substitutions in the viral neuraminidase or hemagglutinin proteins (Table 3).

Table 3: Amino Acid Substitutions Selected by Peramivir in Cell Culture Studies

	Type/Subtype		
Protein	**A/H1N1**	**A/H3N2**	**B**
HA	D129S, R208K	N63K, G78D, N145D, K189E	T139N, G141E, R162M, D195N, T197N, Y319H
NA	N58D, I211T, H275Y		H275Y

In vivo: Influenza A and B virus isolates with amino acid substitutions associated with reduced susceptibility to peramivir were observed in clinical isolates collected during clinical trials with peramivir (Table 4). Amino acid substitutions have also been observed in viral isolates sampled during community

Reference ID: 3658925

surveillance studies which may be associated with reduced susceptibility to peramivir (Table 4). The clinical impact of this reduced susceptibility is unknown.

Table 4: Neuraminidase Amino Acid Substitutions Associated with Reduced Susceptibility to Peramivir in Clinical Virus Isolates

Protein		Type / Subtype		
		Influenza A/H1N1 (N1 numbering)	Influenza A/H3N2	Influenza B (B numbering in brackets)
NA	Clinical Trial	H275Y	R292K, N294S	-
	Community Surveillance Studies	(b) (4) I223R/V, S246N, H275Y (b) (4)	E119V, Q136K (b) (4) D151A/E/G/N/V	(b) (4) P141S (b) (4) D198E/N/Y (D197E/N/Y), I222T/V (I221T/V), (b) (4) R371K (R374K)

Circulating seasonal influenza strains expressing neuraminidase resistance-associated substitutions have been observed in individuals who have not received RAPIVAB. Prescribers should consider available information from the CDC on influenza virus drug susceptibility patterns and treatment effects when deciding whether to use RAPIVAB.

Cross Resistance

Cross-resistance between peramivir, oseltamivir and zanamivir was observed in neuraminidase biochemical assays and cell culture assays. The amino acid substitutions that resulted in reduced susceptibility to peramivir and either oseltamivir or zanamivir are summarized in Table 5. The clinical impact of this reduced susceptibility is unknown.

Table 5: Summary of Amino Acid Substitutions with Cross-Resistance between Peramivir and Oseltamivir or Zanamivir in Laboratory Experiments

		Type/ Subtype		
	Protein	A/H1N1 (N1 Numbering)	A/H3N2	B
Oseltamivir	HA	(b) (4)	N63K, N145D	-
	NA	E119V, D151G/N, Y155H, D198G, I223/R/V, S246N, H275Y, N294S	E119I/V, R292K, N294S	P139S, G140R, D197E/N/Y, I221T/V, H275Y, R371K
Zanamivir	HA	(b) (4)	N63K, N145D	-
	NA	(b) (4) Q136K, (b) (4) Y155H, D198G, (b) (4) S246N, N294S	E119G/V, T148I, D151A/G/N/V, R292K, N294S	E116A/D/G, (b) (4) P139S, (b) (4) R149K, D198E/N, R292K, R371K

No single amino acid substitution has been identified that could confer cross-resistance between the neuraminidase inhibitor class (peramivir, oseltamivir, zanamivir) and the M2 ion channel inhibitor class (amantadine, rimantadine). However, a virus may carry a neuraminidase inhibitor resistance-associated substitution in neuraminidase and an M2 ion channel inhibitor resistance-associated substitution in M2 and may therefore be resistant to both classes of inhibitors. The clinical relevance of phenotypic cross-resistance evaluations has not been established.

Immune Response
No influenza vaccine/peramivir interaction study has been conducted.

Reviewer's Note: The sponsor has provided a reasonable proposal. The sponsor divided Table 4 to separate substitutions that were observed only in community surveillance studies. (b) (4)

The sponsor's requested deletions for Table 5 that this reviewer agrees with: (b) (4)

The Division agrees with these changes. However, there are still a few substitutions that the sponsor needs to include:

- NA S246N (H1N1): Influenza A virus (H1N1) with the NA S247N substitution has been detected in community specimens ranging from ~1% to ~30% in the US, Europe, Asian and Australia (Hurt et al., 2011). The IC_{50} values of peramivir against WT, S246N, H275Y, and S246N + H275Y strains were 0.09 nM, 0.18 nM, 19.72 nM, and 60.91 nM, respectively (Hurt et al., 2011). The IC_{50} values of oseltamivir against WT, S246N, H275Y, and S246N + H275Y strains were 0.30 nM, 0.96 nM, 195.02 nM, and 2146.96 nM, respectively. The IC_{50} values of zanamivir against WT, S246N, H275Y, and S246N + H275Y strains were 0.24 nM, 0.57 nM, 0.26 nM, and 1.25 nM, respectively. While the substitution alone did not confer a significant reduction in inhibition of neuraminidase, the combination with H275Y substitution resulted in a substantially greater reduction in inhibition of neuraminidase than either substitution alone.
- NA P139S (flu B): Sponsor states it is an artifact of passaging in MDCK cells Fujisaki et al, 2013. However, it was present in 2.7% of the clinical sample prior to amplification.
- HA N63K and N145D (H3N2): The EC_{50} values of peramivir, oseltamivir, and zanamivir were <0.001 μM, 0.01 μM, and <0.001 μM, respectively, against the WT virus (study report dd00048-pre-clinical-study-report). The EC_{50} values of peramivir, oseltamivir, and zanamivir were <0.001 μM, 0.01 μM, and <0.001 μM, respectively, against the double mutant virus.

Comment: We appreciate the suggestions that you have made to the resistance and cross-resistance tables. We agree with separating the substitutions that were observed in surveillance studies. We also agree that substitutions selected by oseltamivir and/or zanamivir should only be in the cross-resistance table (Table 5). However, we believe the following substitutions should still be included in the tables:

- NA S246N (H1N1): This substitution was originally identified in surveillance studies and reported as a neuraminidase inhibitor resistance substitution (Hurt et al., 2011). Influenza A virus (H1N1) with the NA S247N substitution has been detected in community specimens ranging from ~1% to ~30% in the US, Europe, Asian and Australia. While the substitution alone did not confer a significant reduction in inhibition of neuraminidase, the combination with H275Y substitution resulted in a substantially greater reduction in inhibition of neuraminidase than either substitution alone.
- NA P139S (flu B): We acknowledge that this substitution was expanded during passaging in MDCK cells (Fujisaki et al, 2013). However, this substitution was present at baseline prior to amplification, The IC_{50} values of peramivir, oseltamivir, and zanamivir were reduced by 322-fold, 10-fold, and 25-fold, respectively against the P139S strain. The authors also conclude that this substitution is resistance-associated. At a minimum, P139S is a resistance-associated polymorphism and should be included in the surveillance section.
- HA N63K and N145D (H3N2): The EC_{50} values of peramivir, oseltamivir, and zanamivir were <0.001 μM, 0.01 μM, and <0.001 μM, respectively, against the WT virus (study report dd00048-pre-clinical-study-report). The EC_{50} values of peramivir, oseltamivir, and zanamivir were 100 μM, >100 μM, and >100 μM, respectively, against the double mutant virus.

CONCLUSIONS

This submission contains the sponsor's revised label for peramivir. The sponsor has provided a reasonable proposal. However, there are a number of substitutions that should still be added to the label.

(Note: The sponsor has accepted the Division's recommendations on 11/14/14).

Takashi E. Komatsu, Ph.D., RAC
Clinical Virology Reviewer

CONCURRENCES

Date: _____

HFD-530/Clin Virology TL/J O'Rear

cc:
HFD-530/Original IND
HFD-530/Division File
HFD-530/RPM/Thompson

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/s/

TAKASHI E KOMATSU
11/17/2014

JULIAN J O REAR
11/17/2014

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 206426 **SDN:** 000 **DATE REVIEWED:** 8/20/14

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Eric F. Donaldson, Ph.D.

NDA #: (b) (4) **Supporting Document Numbers:** 000

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Eric F. Donaldson, Ph.D.

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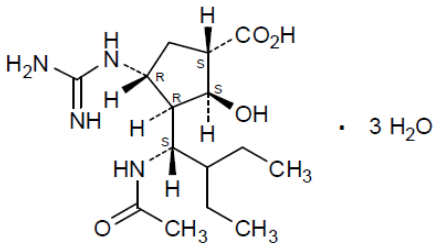
Related/Supporting Documents: IND 69038, IND (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4)

Product Name(s):

Proprietary: RAPIVAB
Non-Proprietary/USAN: Peramivir
Code Name/Number: RWJ-270201 (b) (4) BCX-1812

Chemical Names: (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-ethylbutyl]-4-(aminoiminomethyl)amino]-2-hydroxy-cyclopentanecarboxylic acid-trihydrate
Structure:



PERAMIVIR

Reference ID: 3613983

Molecular formula: $C_{15}H_{28}N_4O_4 \cdot 3H_2O$
Molecular weight: 382.45
Drug category: Antiviral
Indication(s): Treatment of acute uncomplicated influenza in patients 18 years and older
Dosage Form(s): 200 mg (10 mg/mL) in 0.9% sodium chloride solution
Route(s) of Administration: IV
Recommended Dosage: single 600 mg dose
Dispensed: Rx _X_ **OTC** ___ **(Discipline relevant)**

Abbreviations: AE, adverse event; AUC, area under the serum concentration; BID, twice daily; C_{max}, maximum serum concentration; CL, systemic clearance; EC, effective concentration; HA, hemagglutinin; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IC, inhibitory concentration; IP, intraperitoneal; IV, intravenous; mAb, monoclonal antibody; MCP-1, monocyte/macrophage chemoattractant protein; MDCK, Madin-Darby canine kidney; MIP, macrophage inhibitory protein; MOI, multiplicity of infection; NA, neuraminidase; NAI, neuraminidase inhibitor; NP, nuclear protein; OSE, oseltamivir; QD, once daily; RAT, rapid antigen test; RBV, ribavirin; RT-PCR, reverse transcription-polymerase chain reaction; $TCID_{50}$, 50% tissue culture infectious dose; TID, three times daily; T_{max}, time of maximum serum concentration; ZVR, zanamivir;

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 206426 **SDN:** 000 **DATE REVIEWED:** 8/20/14

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Eric F. Donaldson, Ph.D.

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VIROLOGY REVIEW

NDA: 206426 **SDN:** 000 **DATE REVIEWED:** 8/20/14

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Eric F. Donaldson, Ph.D.

EXECUTIVE SUMMARY

This new drug application (NDA) was submitted in support of peramivir (BCX1812, RAPIVAB) for the treatment of acute uncomplicated influenza in patients 18 years and older. There are currently two classes of approved drugs to treat uncomplicated influenza virus infection: adamantanes and neuraminidase inhibitors (NAIs). Two adamantanes are licensed for the treatment of influenza A virus infection (amantadine [Symmetrel®, NDAs 16020, 16023, 17118, and 18101; approved October 18, 1966] and rimantadine [Flumadine®, NDAs 19649 and 19650; approved September 17, 1993]) and two NAIs are licensed (zanamivir [Relenza®, NDA 21036, approved July 26, 1999] and oseltamivir [Tamiflu®, NDAs 21087 and 21246; approved October 27, 1999]). Peramivir is an influenza virus neuraminidase inhibitor.

The adamantanes, which inhibit the M2 ion channel, are only effective against influenza A viruses, as influenza B viruses do not produce this protein. These drugs are currently not recommended for use given the high degree of resistance among influenza A virus strains and their lack of efficacy against influenza B virus.

(b) (4) Zanamivir is administered QD (for prophylaxis) or BID (for treatment) via inhalation using a specialized device. A similar rational design approach was used to develop oseltamivir, which is administered as an oral prodrug formulation. Both zanamivir and oseltamivir have been licensed for use in many countries worldwide since 2000, with oseltamivir being more widely used due to its ease of oral administration. The intravenous formulation of peramivir described in this application provides treatment with a single dose.

The activity of peramivir was assessed in biochemical and cell culture assays. In biochemical studies using the neuraminidase inhibition assay as the endpoint, the median IC_{50} values of peramivir were 0.16 nM (n=44; range (b) (4) nM), (b) (4) nM (n= (b) (4); range (b) (4) nM), 0.13 nM (n=32; range 0.05-11 nM), (b) (4) nM (n=4; range (b) (4) nM), and (b) (4) nM (b) (4) range (b) (4) nM) against influenza A/H1N1, A/H2N2, A/H3N2, and A/H5N1 viruses, and influenza B virus, respectively. Against 2009 A/H1N1 influenza virus isolates, the IC_{50} values ranged from 0.03-0.5 nM (Gubareva et al., 2010). Of note, the activity of peramivir, like oseltamivir and zanamivir, is less against influenza B virus. The median EC_{50} values of peramivir using a plaque assay as the endpoint were 2.6 nM (n=13; range 0.09-21 nM), <1 nM (n=1), 0.08 nM (n=17; range <0.01- 1.9 nM), (b) (4) and 4.8 nM (n=11; range 0.06-120 nM) against influenza A/H1N1, A/H2N2, A/H3N2, and A/H5N1 viruses, and influenza B virus, respectively. As was the case in the biochemical studies, the activity of peramivir is less against influenza B virus. The relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, inhibition of influenza virus replication *in vivo*, and inhibition or reduction of infection-related symptoms in humans has not been established.

Selection in cell culture of virus resistant to peramivir was performed with 7 different influenza viruses; A/PR/8/34 (H1N1), A/WSN/33 (H1N1), A/Charlottesville/31/95 (H1N1), A/Singapore/1/57 (H2N2), X121 (a A/PR/8/34 [H1N1] reassortant virus with H3N2 surface glycoproteins), A/Shandong/09/92 (H3N2) and B/Yamagata/16/88. Reduced susceptibility to NAIs can arise through amino acid substitutions in either the neuraminidase (NA) and/or hemagglutinin (HA) (Blick et al., 1998). Of note, amino acid substitutions in HA were selected in cell culture by peramivir, in some cases in the absence of any resistance-associated amino acid substitutions in the NA. The HA G141E, D195N, and T197N amino acid substitutions developed in an (b) (4) developed the NA H275Y substitution (b) (4) This

Reference ID: 3613983

observation is consistent with an earlier report for zanamivir where the HA substitutions appeared before the NA substitutions (Blick et al., 1998).

The potential impact on antigenicity of peramivir resistance substitutions in HA was investigated. Antigenic sites reported in the literature were mapped onto the structures of HA1 of influenza A/H1 virus (Xu et al., 2010), A/H3 virus (Popova et al., 2012), and influenza B virus (Dreyfus et al., 2012). Resistance-associated substitutions were also mapped onto the HA1 to determine if substitutions occurred in the known antigenic sites. All of the peramivir resistance-associated substitutions that emerged in H1N1 and H3N2 influenza virus populations led to changes in antigenic sites. The resistance-associated substitutions that emerged in the presence of peramivir in influenza B virus also map to the HA surface. This observation indicates that patients who use peramivir and fail treatment may be at risk of propagating influenza viruses that are less susceptible to the influenza virus vaccine.

Cross-resistance to oseltamivir and zanamivir was observed for some peramivir resistance pathways. However, this analysis was incomplete as some pathways have not been evaluated. Some peramivir resistance pathways confer class cross-resistance and others are specific to oseltamivir or zanamivir. Peramivir shares more resistance pathways with oseltamivir than with zanamivir. Importantly, the HA N63K and N145D substitutions selected in cell culture in the absence of substitutions in NA and reducing susceptibility to peramivir, conferred cross-resistance to both oseltamivir and zanamivir. These results are concerning as the use of the neuraminidase assay to screen for resistance would not be expected to detect resistance developing in the viral hemagglutinin.

Clinical Virology analyses were conducted to evaluate the antiviral efficacy and virological resistance of IV or IM peramivir in subjects with uncomplicated influenza virus infection in 7 clinical trials: one pivotal non-IND Phase 2 study (0722T0621; i.v. formulation), one supportive Phase 2 IND study and one supportive Phase 3 IND study (BCX1812-211 and BCX1812-311; both i.m. formulation), as well as 4 other studies (0815T0631 [i.v. formulation], 0815T0632 [i.v. formulation], 0815T0633 [i.v. formulation], and BCX1812-212 [i.m. formulation]). Additionally, there were 3 studies conducted in hospitalized subjects (BCX1812-201 [i.v. formulation], BCX1812-301 [i.v. formulation], and BCX1812-303 [i.v. formulation]).

The inclusion criteria for most of the studies described above required a positive rapid antigen test (RAT) for influenza virus. RAT assays are limited in sensitivity, especially for some viruses and some vulnerable populations. Data in the literature support a RAT assay bias against elderly patients and indicate that enrolling subjects based on the RAT assay may lead to under-enrollment of older patients. Considering that elderly subjects in nursing homes would be good candidates for the use of i.v. peramivir, it is unfortunate that the studies enrolled few subjects in this age group precluding any meaningful conclusions with respect to efficacy. Additionally, the RAT assays used in these studies have less sensitivity against influenza B virus. Fewer subjects infected with influenza B virus were enrolled compared to surveillance data at 9 out of 11 sites. Given the limited enrollment of influenza B virus infected subjects and peramivir's reduced activity against influenza B virus compared to influenza A virus based on biochemical and cell culture data, conclusions about efficacy in subjects infected with influenza B virus cannot be drawn.

Study 0722T0621 (i.v.) was a pivotal Phase 2, double-blind, placebo-controlled, parallel group study of single-dose intravenous peramivir in subjects with acute uncomplicated influenza. Three-hundred subjects were enrolled at 75 study sites in Japan. This study met its primary endpoint of time to alleviation of symptoms for influenza A/H1N1 and A/H3N2 virus infection. Only 3 influenza B virus infected subjects were enrolled so an endpoint assessment cannot be made.

Results from Studies BCX1812-211 and BCX1812-311 were combined by the sponsor in a post hoc analysis due to the similarity of study design, endpoints, treatment regimens, and subject population. Study BCX1812-211 was a Phase 2 multinational, randomized study comparing the antiviral activity and safety of a 150 mg or 300 mg dose of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. This study enrolled 344 subjects at 151 study sites in the US, Canada, the United Kingdom, Australia, Hong Kong, New Zealand, and South Africa. Study BCX1812-311 was a Phase 3 randomized, double-blind study comparing the efficacy and safety of a 300 mg dose of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. This US-only study was terminated early in order to (b) (4) The analysis of the combined studies did not meet the overall primary endpoint of time to alleviation of symptoms for influenza. There was a dose/response significant reduction in time to alleviation of symptoms for A/H1N1 virus and a trend for A/H3N2 virus. There was a dose/response significant mean reduction in viral shedding between arms for A/H3N2 virus and a significant reduction at the lower dose for A/H1N1 virus. The number of subjects not shedding virus at day 3 for influenza A/H3N2 virus was statistically significant between peramivir 300 mg vs. placebo. There was no apparent activity in subjects infected with influenza B virus.

The resistance analyses that the sponsor conducted for the studies by Shionogi were limited. For study 0722T0621, the sponsor only sequenced the NA gene of the influenza viruses in specimens from subjects demonstrating pretreatment or post-treatment IC_{50} values for peramivir that were greater than a threshold of a mean IC_{50} value + 3 standard deviations (SDs) for NA inhibition activity. The utility of the phenotypic analyses as a screen for resistance is suspect given the selection bias of the assay for wildtype virus when viruses in subject samples are amplified in cell culture prior to testing and the assay itself which can show a bias in mixtures of wild-type and resistant virus. Additionally, the type of substrate used affects the sensitivity of the assay. Lastly, the use of the mean IC_{50} value + 3 SDs is arbitrary since a clinical cutoff for the NA assay has not been established. Another source of bias is the inability of the neuraminidase assay to detect resistance developing in the viral hemagglutinin. The sponsor has not genotyped the HA in their pivotal Phase 2 study and has only provided limited HA genotypic data from their supportive Phase 2 studies.

Only the NA H275Y amino acid substitution developed in influenza A/H1N1 virus from more than one subject. The virus from 9 subjects developed the NA H275Y amino acid substitution on-treatment. As expected, all of the isolates with the NA H275Y substitution had reduced susceptibility to peramivir and oseltamivir but not to zanamivir.

For subjects infected with influenza A/H3N2 virus, there were no amino acid substitutions that developed in more than one subject. The R292K neuraminidase resistance substitution conferring reduced susceptibility to peramivir, oseltamivir, and zanamivir developed in the virus from one subject. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.19 nM at baseline and 1.13 nM at Day 5. Another subject's virus developed the N294S neuraminidase resistance substitution, which confers resistance to peramivir, oseltamivir, and zanamivir. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.05 nM at baseline and 1.43 nM at Day 5.

For the few subjects infected with influenza B virus, there were no amino acid substitutions that developed in more than one subject. None of these amino acid substitutions conferred reduced susceptibility to peramivir, oseltamivir, or zanamivir.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 206426 **SDN:** 000 **DATE REVIEWED:** 8/20/14

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Eric F. Donaldson, Ph.D.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability: Approval of this original NDA for RAPIVAB is recommended with respect to Clinical Virology. RAPIVAB is recommended as a single IV regimen for the treatment of acute uncomplicated influenza in patients 18 (b) (4) years infected with influenza A virus.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, If Approvable:

- The applicant needs to submit the remainder of the clinical resistance data that were not included with the NDA. These include both the HA and NA data for studies BCX1812-201, BCX1812-211, and BCX1812-311.
- The applicant needs to determine the cross-resistance to oseltamivir and zanamivir for all of the HA peramivir resistance substitutions that have yet to be evaluated (A/H3N2 HA G78D, K189E). Additionally, cross-resistance to oseltamivir/zanamivir resistance substitutions needs to be evaluated (A/H1N1 NA R152K, I122K/T, G248R+I266V, Q312R+I427T, R371K, A/H3N2 NA E41G, I222L/V, Q226H, S247P, B NA D198Y, A246D/S/T, G420S).
- The applicant needs to evaluate the impact of the HA substitutions on the influenza vaccine.
  - Recombinant virus with the peramivir resistance substitutions in the HA and their parental virus should be evaluated against the serum samples from subjects vaccinated with the influenza virus vaccine.
  - Recombinant virus with the peramivir resistance substitutions in the HA and their parental virus should be evaluated against the baseline and end of treatment serum samples from subjects treated with peramivir.

2. Summary of OND Virology Assessments

2.1. Nonclinical Virology

Mechanism of Action

In biochemical studies using the neuraminidase inhibition assay as the endpoint, the median IC_{50} values of peramivir were 0.16 nM (n=44; range (b) (4) nM), (b) (4) nM (n (b) (4); range (b) (4)-1.77 nM), 0.13 nM (n=32; range 0.05-11 nM), (b) (4) nM (n (b) (4) range (b) (4) nM), and 0.99 nM (n=39; range (b) (4)-54.2 nM) against influenza A/H1N1, (b) (4) A/H3N2, and (b) (4) viruses, and influenza B virus, respectively. Against 2009 A/H1N1 influenza virus isolates, the IC_{50} values ranged from 0.03-0.5 nM (Gubareva et al., 2010). Of note, the activity of peramivir, like oseltamivir and zanamivir, is less against influenza B virus.

Antiviral Activity in Cell Culture

Peramivir was active in cell culture against numerous strains of influenza A and B viruses, as well as against different subtypes of avian influenza virus including H5N1, H5N3, and H7N7. MDCK cells were used as host cells. The median EC_{50} values of peramivir using a plaque assay as the endpoint were 2.6 nM (n=13; range 0.09-21 nM), <1 nM (n=1), 0.08 nM (n=17; range <0.01- 1.9 nM), (b) (4) and 4.8 nM (n=11; range 0.06-120 nM) against influenza A/H1N1, (b) (4) A/H3N2, and (b) (4) viruses, and influenza B virus, respectively. As was the case in the biochemical studies, the activity of peramivir is less against influenza B virus. The relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, inhibition of influenza virus replication *in vivo*, and inhibition or reduction of infection-related symptoms in humans has not been established.

Combination of Peramivir and Other Antiviral Agents in Cell Culture

Reference ID: 3613983

(b) (4)

Antiviral Activity in Animal Models
Peramivir has been evaluated for antiviral activity in mice (IV, IM, oral, and intranasal routes of administration), ferrets (IV, IM, and oral, administration), and cynomolgus monkeys (IV and oral administration). These prophylaxis and treatment studies indicated antiviral activity. Several combination regimens were studied in lethal influenza A virus mouse models. Combination treatment of peramivir with oseltamivir, RBV, rimantadine, or (b) (4) (IND (b) (4) resulted in higher survival; in addition, improvements were generally seen in the number of days to death, prevention of weight loss, and/or oxygen saturation levels.

Resistance Analyses
Selection of resistance to peramivir in cell culture was performed with 7 different influenza viruses; A/PR/8/34 (H1N1), A/WSN/33 (H1N1), A/Charlottesville/31/95 (H1N1), A/Singapore/1/57 (H2N2), X121 (a A/PR/8/34 [H1N1] reassortant virus with H3N2 surface glycoproteins), A/Shandong/09/92 (H3N2) and B/Yamagata/16/88. Reduced susceptibility to NA inhibitor(s) can potentially arise through amino acid substitutions in either the NA and/or HA. Of note, some of the amino acid substitutions in the HA that were selected in the sponsor's studies were in the absence of any resistance-associated amino acid substitutions in the NA. Additionally, the HA N63K and N145D substitutions, selected in the absence of NA substitutions, reduced susceptibility to peramivir and conferred cross-resistance to both oseltamivir and zanamivir. These results are concerning as the use of the neuraminidase assay would not be expected to detect resistance developing in the viral hemagglutinin. The HA G141E, D195N, and T197N amino acid substitutions developed at (b) (4) developed the NA H275Y substitution (b) (4) This observation is consistent with an earlier report for zanamivir where the HA substitutions appeared before the NA substitutions (Blick et al., 1998).

Mapping of HA Peramivir Resistance Substitutions to Antigenic Sites
Antigenic sites reported in the literature were mapped onto the structures of HA1 of influenza A/H1 virus (Xu et al., 2010), A/H3 virus (Popova et al., 2012; Huang et al., 2012; Cai et al., 2014), and influenza B virus (Dreyfus et al., 2012). Resistance-associated substitutions were also mapped onto the HA1 to determine if substitutions occurred in the known antigenic sites. All of the peramivir resistance-associated substitutions that emerged in H1N1 and H3N2 influenza virus populations led to changes in antigenic sites. The resistance-associated substitutions that emerged in the presence of peramivir in influenza B virus also map to the HA surface. This observation indicates that patients who use peramivir and fail treatment may be at risk of propagating influenza viruses that are less susceptible to the influenza virus vaccine.

Cross-Resistance
Peramivir shares more resistance pathways with oseltamivir than with zanamivir. However, there are substitutions that confer cross-resistance to zanamivir but not oseltamivir (NA E119G, NA Q136K in influenza A/H1N1 virus and (b) (4) in influenza B virus). Cross-resistance to oseltamivir and zanamivir should be determined for all of the novel peramivir resistance-associated substitutions.

2.2. Clinical Virology

Clinical Virology analyses were conducted to evaluate the antiviral efficacy and virological resistance of IV or IM peramivir in subjects with uncomplicated influenza virus infection in 7 clinical trials: one pivotal non-IND Phase 2 study (0722T0621; i.v. formulation), two supportive IND Phase 2 and Phase 3 studies (BCX1812-211 and BCX1812-311; both i.m. formulation), as well as 4 other studies (0815T0631 [i.v. formulation], 0815T0632

[i.v. formulation], 0815T0633 [i.v. formulation], and BCX1812-212 [i.m. formulation]). Additionally, there were 3 studies conducted in hospitalized subjects (BCX1812-201 [i.v. formulation], BCX1812-301 [i.v. formulation], and BCX1812-303 [i.v. formulation]).

The Division recommended that the sponsor enroll subjects based upon clinical symptoms and confirm the diagnosis with an RT-PCR assay. However, for most of the studies described above, the sponsor required for inclusion a positive rapid antigen test (RAT) for influenza virus. RAT assays are limited in sensitivity, especially for some viruses and some vulnerable populations. Data in the literature support a RAT assay bias against elderly patients and that enrolling based on the RAT assay may lead to under-enrollment of older patients (Steininger et al., 2008; Gooskens et al., 2008). Considering that elderly subjects in nursing homes would be good candidates for the use of i.v. peramivir, it is unfortunate that there were insufficient numbers of subjects over 65 years of age enrolled to make any meaningful conclusions with respect to efficacy. Additionally, the RAT assays that were used in their studies have less sensitivity to detect influenza B virus (Chartrand et al., 2012). The use of RAT assays as inclusion criteria may have led to fewer subjects infected with influenza B virus being enrolled compared to surveillance data in 9 out of 11 sites (Table 5). Given that peramivir is less active against influenza B virus based on biochemical and cell culture data, it will be important to assess efficacy specifically in subjects infected with influenza B virus.

Study 0722T0621 (i.v.) was a Phase 2, double-blind, placebo-controlled, parallel group study of single-dose intravenous peramivir in subjects with acute uncomplicated influenza. This study was the pivotal study and met its primary endpoint of time to alleviation of symptoms for influenza A/H1N1 and A/H3N2 virus infection. The study enrolled 300 subjects at 75 study sites in Japan. The influenza A/H1N1 virus with the NA H275Y substitution that confers resistance to peramivir was not circulating during the study. The combined peramivir group (300 mg and 600 mg) showed a significant shortening of the time to alleviation of symptoms in comparison to the placebo group. There was a statistically significant benefit in time to alleviation of symptoms, change in viral shedding from baseline at day 3, and number of subjects not shedding virus at day 3 for influenza A/H3N2 virus. A dose/response in symptom relief was not seen for influenza A/H1N1 virus despite a significant reduction in viral shedding. There were only 3 subjects infected with influenza B virus.

Results from Studies BCX1812-211 and BCX1812-311 were combined by the sponsor in a post hoc analysis due to the similarity of study design, endpoints, treatment regimens, and subject population. Study BCX1812-211 was a Phase 2 multinational, randomized study comparing the antiviral activity and safety of a 150 mg or 300 mg dose of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. This study enrolled 344 subjects at 151 study sites in the US, Canada, the United Kingdom, Australia, Hong Kong, New Zealand, and South Africa. Study BCX1812-311 was a Phase 3 randomized, double-blind study comparing the efficacy and safety of a 300 mg dose of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. This US-only study was terminated early (b) (4) The influenza A/H1N1 virus with NA H275Y substitution was not circulating during either of the studies. In the integrated analysis, the median time to alleviation of symptoms was reduced for subjects who received 150 mg (114.1 hours) or 300 mg (113.2 hours) of peramivir, compared with subjects who received placebo (134.8 hours). However, this reduction was not significant. There was a dose/response significant reduction in time to alleviation of symptoms for A/H1N1 virus and a trend for A/H3N2 virus. There was a dose/response significant reduction in viral shedding for A/H3N2 virus and a significant reduction at the lower dose for A/H1N1 virus. The number of subjects not shedding virus at day 3 for influenza A/H3N2 virus was statistically significant between peramivir 300 mg vs. placebo. There was no apparent activity in subjects infected with influenza B virus.

The resistance analyses that the sponsor conducted for the studies by Shionogi were limited. For study 0722T0621, the sponsor only sequenced the NA gene of the influenza viruses in specimens from subjects demonstrating pretreatment or post-treatment IC_{50} values for peramivir that were greater than a threshold of the mean IC_{50} value + 3 standard deviations (SDs) for NA inhibition activity. The utility of the phenotypic analyses as a screen for resistance is suspect given the selection bias of the assay for wildtype virus when viruses in subject samples are amplified in cell culture prior to testing and the assay itself which can show a bias in mixtures of wild-type and resistant virus (Wetherall et al., 2003; Deyde et al., 2009; Yen et al., 2013). Additionally, NAI resistance appears to arise relatively quickly following treatment with oseltamivir (e.g. 2 days after start of treatment; Hu et al., 2013). Additionally, the type of substrate used affects the sensitivity of the assay (Wetherall et al., 2003). Lastly, the use of the mean IC_{50} value + 3 SDs is arbitrary since a clinical cutoff for the NA assay has not been established. Another source of bias is the inability of the neuraminidase assay to detect resistance developing in the viral hemagglutinin. The sponsor has not genotyped the HA in their pivotal Phase 2 study and has only provided some HA genotypic data from their supportive Phase 2 studies. Amino acid substitutions in the HA conferring reduced susceptibility to peramivir have been selected in cell culture studies.

Only the NA H275Y amino acid substitution developed in more than one subject for subjects infected with influenza A/H1N1 virus. There were 9 subjects who developed the NA H275Y amino acid substitution on-treatment (0722T0621.AL1.080-1, 0722T0621.BI1.135-6, 0722T0621.BQ1.148-4, 0722T0621.CH1.163-5, 0722T0621.AP1.086-5, BCX1812-211.662.007, BCX1812-211.671.004, BCX1812-301.575.002, and BCX1812-303.143.003). Subjects BCX1812-301.575.002 and BCX1812-303.143.003 were from the study in hospitalized subjects. Additionally, there were 3 subjects who had the NA H275Y amino acid substitution at baseline (0722T0621.AF1.005-1, BCX1812-211.069.028, and BCX1812-301.202.039) and one subject who had the NA H275Y substitution at Day 3 but the baseline sample was not genotyped (0722T0621.CM1.172-4). As expected, all of the isolates with the NA H275Y substitution had reduced susceptibility to peramivir and oseltamivir but not to zanamivir.

For subjects infected with influenza A/H3N2 virus, there were no amino acid substitutions that developed in more than one subject. The NA R292K amino acid substitution conferring reduced susceptibility to peramivir, oseltamivir, and zanamivir developed in one subject. Subject BCX1812-211.461.052 had a baseline viral load of 4.3 $\log_{10}$ $TCID_{50}$/mL at baseline, 2.3 $\log_{10}$ $TCID_{50}$/mL at Day 3 and <LLOQ at Day 5. The NA R292K amino acid substitution was observed on Day 3. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.19 nM at baseline and 1.13 nM at Day 5. Subject BCX1812-211.004.012 had a viral load of 5.75 $\log_{10}$ $TCID_{50}$/mL at baseline, 1.5 $\log_{10}$ $TCID_{50}$/mL at Day 3 and 1.5 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA N294S, which confers reduced susceptibility to peramivir, oseltamivir, and zanamivir, was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.05 nM at baseline and 1.43 nM at Day 5.

For subjects infected with influenza B virus, there were no amino acid substitutions that developed in more than one subject. None of these amino acid substitutions conferred reduced susceptibility to peramivir, oseltamivir, or zanamivir.

3. Administrative

3.1. Reviewer's Signatures

Takashi E. Komatsu, Ph.D., RAC
Clinical Virology Reviewer

3.2. Concurrence

_____
HFD-530/Clin.Virol.TL/J. O'Rear, Ph.D.

CC:
HFD-530/NDA # 206426
HFD-530/Division File
HFD-530/RPM/E. Thompson

OND VIROLOGY REVIEW

1. Introduction and Background

1.1. Important milestones in product development

Peramivir (BCX1812, RAPIVAB) is an influenza virus neuraminidase inhibitor in an intravenous formulation for the treatment of acute uncomplicated influenza in patients 18 years and older. The Original IND 69038 was submitted on November 23, 2005. Several non-clinical and clinical studies have been conducted using oral, intramuscular, and intravenous formulations under IND's 69038 (oral, intravenous formulations) (b) (4) . Clinical study reports for Phase 2 Study 0722T0621, using an intravenous formulation, Phase 2 Study BCX1812-211, using an intramuscular formulation, and Phase 3 Study BCX1812-311, also using an intramuscular formulation, were submitted to support the indication for this Original NDA. Clinical study reports for 0815T0631 (Phase 3, intravenous formulation), 0816T0632 (Phase 3, intravenous formulation), 0918T0633 (Phase 3, intravenous formulation), BCX1812-212 (Phase 2, intramuscular formulation), BCX1812-201 (Phase 2, intravenous formulation), BCX1812-301 (Phase 3, intravenous formulation), and BCX1812-303 (Phase 3, intravenous formulation) were submitted as additional studies. A Type C telecom meeting with the sponsor was held on 6/25/2013 to address Clinical Virology issues and a pre-NDA face-to-face meeting with the sponsor was held on 6/28/2013. At the time of the pre-NDA meeting, an agreement with the sponsor was reached on the Clinical Virology data to be submitted with the NDA.

1.2. Overview of Influenza

Influenza is a respiratory viral infection that causes severe illness in 3–5 million people worldwide each year with up to 500,000 deaths (WHO, Influenza Fact Sheet, 2009). From the 1970s through 2007, the annual deaths in the US attributable to influenza have ranged from 3,000 to 50,000 (CDC, MMWR 2010). On average, more than 200,000 people with seasonal influenza virus infections are hospitalized each year with more than 24,000 deaths. This number is likely an underestimate, as many persons who die from influenza may die due to complications from bacterial super-infections or cardiovascular events, in addition to a direct result of the virus itself. Influenza virus infection causes fevers, chills, myalgias, and upper respiratory symptoms. When severe, it can cause lower respiratory tract infections and can lead to acute respiratory distress syndrome (ARDS). As a result of the high attack rate, extensive resources and health care expenditures are dedicated yearly to the detection, prevention, and treatment of influenza.

There are three types of influenza virus: A, B and C, which differ in their host range and severity (Ferraris and Lina, 2008). Influenza A and B viruses are most commonly associated with infections in humans and can cause severe disease (Paul-Glezen et al., 2013), whereas influenza C virus infection is rarely diagnosed and typically causes only mild illness in adults and adolescents, although it has been associated with severe disease in young children (Paul-Glezen et al., 2013; Calvo et al., 2006).

Influenza viruses belong to the Orthomyxoviridae family and have an enveloped, ribonucleoprotein core containing a negative sense, segmented RNA genome with eight (in the case of influenza A and B viruses) or seven (in the case of influenza C virus) segments (Chen and Holmes, 2008). The total genome size is approximately ~13 kb. For influenza A virus, the 8 segments encode 11 proteins: PA (Segment 6), PB1 (Segment 7), PB1-F2 (Segment 7), PB2 (Segment 8), HA (Segment 1), NP (Segment 3), NA (Segment 2), M1 (Segment 4), M2 (Segment 4), NS1 (Segment 5), and NEP (NS2) (Segment 5). The size of the different segments and proteins are: PA (~81 kD) = ~2178 nucleotides (nt), PB1 (~86 kD) = ~2310 nt, PB2 (~84 kD) = ~2256 nt, HA (~65 kD) = ~1755 nt, NP (~62 kD) = ~1680 nt, NA (~52 kD) = ~1401 nt, M1 (~28 kD) = ~759 nt, M2 (~11 kD) = ~294 nt, NS1 (~24 kD) = ~660 nt, and NEP (~13 kD) = ~366 nt. Although the structures of influenza A and B viruses are very similar, they can be distinguished by their specific nucleoprotein and matrix proteins (Ferraris and Lina, 2008).

Influenza A virus is divided into many subtypes according to the two envelope glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Seventeen different HA and 9 different NA subtypes have been identified (Laver et al., 1999; Fouchier et al., 2005; Zhu et al., 2013) and different combinations of these 2 proteins are possible. Influenza A virus subtypes H1N1pdm09 and H3N2 are currently circulating seasonally in humans (Paul-Glezen et al., 2013), whereas all influenza A virus subtypes circulate in wild water birds (Alexander, 2000). Influenza B viruses typically only infect humans, apart from a single detection of the virus in seals (Paul-Glezen et al., 2013; Osterhaus et al., 2000), and formed a homogeneous group until the 1970s, after which they diverged into two genetically and antigenically distinct lineages: B/Victoria/2/87-like viruses and B/Yamagata/16/88-like viruses (Chen and Holmes, 2008). Although the clinical presentation of influenza B virus infection has been reported to be similar to that of influenza A virus and with both causing severe disease in children on occasions (Paul-Glezen et al., 2013; Lo et al., 2013), influenza B viruses have attracted comparatively less research attention than influenza A viruses.

Influenza viruses bind through HA onto sialic acid sugars on the surfaces of epithelial cells (Wagner et al., 2002) (Figure 1, from Basler, 2007). After HA is cleaved by a trypsin-like protease, the cell imports the virus by endocytosis (Steinhauer, 1999). Once inside the vacuole within the cell, the acidic conditions in the endosome causes part of the hemagglutinin protein to fuse the viral envelope with the endosome's membrane. The integral membrane protein M2 acts as an ion channel allowing protons to move through the viral envelope and acidify the core of the virus. Internal acidification of the influenza virion disrupts protein-protein interaction and releases the viral genomic RNA molecules, accessory proteins and the viral encoded RNA-dependent RNA polymerase, composed of PA, PB1, and PB2 subunits, into the cytoplasm (Lakadamyali et al., 2003; Bouvier and Palese, 2008). These core proteins and viral genomic RNA form a complex that is transported into the cell nucleus, where the RNA-dependent RNA polymerase begins transcribing complementary positive-sense viral mRNA (Cros and Palese, 2003). The polymerase transcribes viral mRNAs using a cap-snatching mechanism, a strategy unique to the Orthomyxoviridae and Bunyaviridae families of Mononegavirales. Host-cell pre-mRNAs are bound via their 5' cap to the PB2 subunit and cleaved at nucleotide 10–13 by the endonucleolytic activity of the PB1 subunit (Plotch et al., 1981; Li et al., 2001). These capped oligoribonucleotides prime synthesis of viral mRNAs, which are polyadenylated at their 3' ends by stuttering of the polymerase at an oligo-U motif at the 5' end of the template (Poon et al., 1999), rendering them translation competent. Capped RNA recognition by PB2 is stimulated by the binding of the 5' end of the viral RNA template to PB1 (Cianci et al., 1995). The location of the cap binding site on PB2 is controversial; various cross-linking and mutagenesis experiments have implicated different regions including amino acid residues 242–252 (N-site) (Honda et al., 1999), 533–577 (C-site) (Li et al., 2001; Honda et al., 1999) and a more central site involving residues F363 and F404 (Fechter et al., 2005), which were proposed to sandwich the methylated guanosine, as occurs in other cap binding proteins (Fechter and Brownlee, 2005).

The positive sense viral RNA either is exported into the cytoplasm as mRNA for translation or remains in the nucleus where it serves as template for the synthesis of viral genomic RNA segments. Newly synthesized viral envelope proteins are either secreted through the Golgi apparatus onto the cell surface (HA, NA, M2) or transported back into the nucleus to bind viral genomic RNA and form new viral genome particles. Synthesis and folding of viral core proteins occur entirely in the cytosol. NP and the RNA polymerase components interact with newly synthesized viral RNA to form ribonucleoproteins (RNPs). The M1 protein starts to interact with the C-terminal domains of HA and NA on the cell plasma membrane, forming patches with a high density of HA and NA, excluding cellular plasma membrane proteins. Subsequently, newly formed RNPs interact actively with the M1 lining at these patches. This interaction also prevents re-entry of RNPs into the nucleus. Other viral proteins have multiple actions in the host cell, including degrading cellular mRNA and using the released nucleotides for viral genomic RNA synthesis and also inhibiting translation of host-cell mRNAs (Kash

et al., 2006). Negative-sense viral genomic RNAs that form the genomes of future viruses, RNA-dependent RNA polymerase, and other viral proteins are assembled into a virion. Hemagglutinin and neuraminidase molecules cluster into a bulge in the cell membrane. The viral genomic RNA and viral core proteins are transported from the nucleus and enter this membrane protrusion. The mature virus buds off from the cell in a sphere of host phospholipid membrane, acquiring hemagglutinin and neuraminidase with this membrane coat (Nayak et al., 2004). As before, the viruses adhere to the cell through hemagglutinin; the mature viruses detach once their neuraminidase has cleaved sialic acid residues from the host cell. Virus production eventually leads to host cell death.

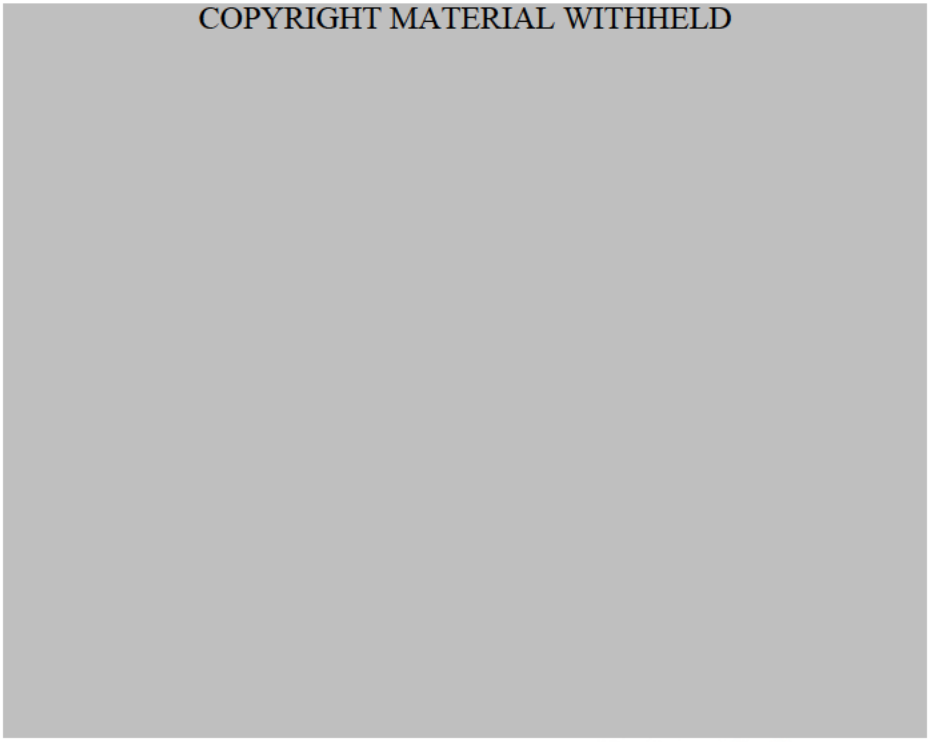

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Figure 1: Replication Cycle of Influenza Virus (from von Itzstein, 2007).

Because of the absence of RNA proofreading enzymes, the RNA-dependent RNA polymerase that copies the viral genome makes an error roughly every 10 thousand nucleotides, which is the approximate length of the influenza viral RNA genome. This error-prone synthesis causes antigenic drift, which is a slow change in the antigens on the viral surface over time (Drake, 1993). For example, in the current H7N9 outbreak, evolution within the human host could lead to easier transmission between individuals (Bao et al., 2013). The separation of the genome into eight separate segments of viral genomic RNA allows mixing or reassortment of viral genomic RNAs if more than one type of influenza virus infects a single cell. When new HA or NA subtypes are introduced via mixing in a mixed infection, or within other species (avian or swine), a new subtype is created–this is called antigenic shift and is responsible for pandemics (Cheng et al., 2012). In the twenty-first century, there has been one pandemic: the 2009 swine H1N1 strain.

The neuraminidase is a mushroom-shaped homotetrameric glycoprotein with a stalk domain anchored to the viral membrane and a globular head that contains a catalytic site. While the HA protein is responsible for virus attachment to the sialic acid receptors on the host cell, the catalytic activity of the NA cleaves off the terminal N-acetyl neuraminic acid (Neu5Ac) on these 2,3 and 2,6 sialic acid moieties. Therefore, the enzymatic activity of the influenza virus NA plays a key role in releasing progeny virions from the host cell and also in facilitating

viral spread throughout the upper airways by cleaving off the sialic acid on the mucin of respiratory mucus. Given its catalytic function, the structure of the NA active site is highly conserved among influenza A and B viruses, and hence constitutes an attractive target for antiviral therapy. The crystallographic data of NAs from N2 (Colman et al., 1983), N9 (Baker et al., 1987) and B (Burmeister et al., 1992) viral backgrounds contributed to the design and synthesis of a series of compounds able to mimic the natural substrate of the NA enzyme and compete for the binding to the active site. Since these neuraminidase inhibitors (NAIs) are based on the structure of the 2,3-didehydro analog of the N-acetyl-neuraminic acid, they possess higher binding affinity than the Neu5Ac thus preventing the cleavage of the natural substrate. As a result, progeny virions fail to be released from the sialic acid receptors and aggregate on the surface of the infected cell, hampering the spread of infection to other non-infected cells.

NA releases viral progeny from host cells by catalyzing the cleavage of sialic acid moieties from host cell receptors and newly synthesized viruses (Burmeister et al., 1992). The NA also prevents the new viruses from aggregating on the surface of the target cell or with each other (Laver et al., 1999; McKimm-Breschkin, 2000) and enables viruses to penetrate the mucin layer of the respiratory tract (McKimm-Breschkin, 2000). The NA protein is a good drug target because it plays an integral role in viral replication, and the active site of NA is highly conserved for all types and subtypes of influenza A and B viruses. The NA active site comprises eight catalytic residues that make direct contact with the substrate sialic acid, and a further 11 framework residues that stabilize the shape of the active site (Colman et al., 1983).

1.3. Current Antiviral Drugs for Influenza

There are two classes of approved drugs to treat influenza: adamantanes and neuraminidase inhibitors (NAIs). The adamantanes are only effective against influenza A viruses, as they inhibit the M2 ion channel, which is not coded by influenza B viruses. Adamantanes inhibit hydrogen ion influx and thereby prevent the virus from uncoating (Takeda et al., 2002). Two adamantanes are licensed for the treatment of influenza A virus infection (amantadine [Symmetrel®, NDAs 16020, 16023, 17118, and 18101; approved October 18, 1966] and rimantadine [Flumadine®, NDAs 19649 and 19650; approved September 17, 1993]). However, given the high degree of resistance among influenza A virus strains to these drugs, they are currently not recommended for use and as noted above, are ineffective against influenza B virus (Cheng et al., 2012; McKimm-Breschkin, 2013; Schirmer and Holodniy, 2009; Deyde et al., 2009; Bright et al., 2005).

Modeling the interactions between conserved neuraminidase residues (8 catalytic residues that make direct contact with the sialic acid and 11 framework residues that stabilize the shape of the active site) and sialic acid led to the development of the first NAI, zanamivir (zanamivir [Relenza®, NDA 21036, approved July 26, 1999]), whose structure is analogous to sialic acid (von Itzstein et al., 1993). The design of zanamivir provides additional interactions with the NAI active site compared to sialic acid, making it an inhibitor of the NA enzyme in cell culture (Woods et al., 1993). Zanamivir is administered via inhalation using a specialized device. A similar rational design approach was used to develop oseltamivir (oseltamivir [Tamiflu®, NDAs 21087 and 21246; approved October 27, 1999]), which is administered as an oral prodrug formulation (Laver et al., 1999). Both zanamivir and oseltamivir have been licensed for use in many countries worldwide since 2000 (Ikematsu et al., 2012), with oseltamivir being more widely used due to its ease of oral administration (Cooper et al., 2003).

Peramivir (BCX-1812, RWJ-270201), a neuraminidase inhibitor, is a cyclopentane derivative with a negatively-charged carboxylate group, a positively-charged guanidino group and a lipophilic side chain (Babu et al., 2000). Due to its low bioavailability, this drug needs to be delivered parenterally (Bantia et al., 2006; Yun et al., 2008). Peramivir has demonstrated activity in cell culture and in animal models against various influenza A and B viruses, including the A (H1N1) pdm09 virus and highly pathogenic A (H5N1) viruses (Babu et al., 2000;

Boltz et al., 2008; Kitano et al., 2011). Peramivir was first developed for oral administration and showed activity *in vivo* in both mice and ferrets (Bantia et al., 2001; Sidwell et al., 2001; Sweet et al., 2002). However, oral peramivir did not demonstrate a statistically significant clinical benefit in controlled trials of prophylaxis and treatment, despite the fact that it was associated with significant reduction in viral titers. These results have been attributed to a low oral bioavailability in humans (Barroso et al., 2005).

To improve the bioavailability, subsequent studies have been performed using intravenous (IV) or intramuscular (IM) injections of peramivir. Preclinical studies in mice and ferrets demonstrated that parenteral administrations of peramivir rapidly produced high plasma concentrations (Bantia et al., 2006; Yun et al., 2008) and that IM administration was associated with a higher survival rate than oral administration in mice (100% versus 50%) (Bantia et al., 2006). In clinical studies, the use of IV peramivir at a single 300 or 600 mg dose was associated with clinical benefits that were comparable to those provided by oseltamivir at a dose of 75 mg twice a day (Kohno et al., 2011). In addition, the overall incidence of adverse effects in the 300 mg IV peramivir group was significantly lower compared to the oseltamivir one. These results contributed to the approval of IV peramivir for adults in Japan and South Korea in 2010. The availability of IV NAIs offers an alternative route of administration, which is especially important for patients who cannot take oral or inhaled medication, such as mechanically-ventilated patients. The IV administration of peramivir (600 mg daily for 5 days) resulted in a peak plasma concentration of 118.18 μM (45.2 μg/ml) (Kohno et al., 2011). Peramivir is approximately 90% eliminated as an unchanged drug by the kidneys with an apparent half-life of 7.7–20.8 h in adults with normal renal function.

For the 2012–2013 season, oseltamivir resistance within the US was reported to be approximately 0% for H3N2 and <1% for 2009 H1N1 influenza A viruses and influenza B virus strains in circulation (CDC, Influenza Fact Sheet, 2013). However, in 2008–2009, prevalence of circulating influenza A/H1N1 virus expressing the NA H275Y substitution conferring resistance to oseltamivir had been quite high and widespread in the US, Europe, and other countries (Thorlund et al., 2011; Meijer et al., 2009; Dharan et al., 2009). The prevalence of influenza A/H1N1 virus resistant to oseltamivir was not the case during the 2009 pandemic. Additionally, it was once thought that oseltamivir resistance mediated by the H275Y (N1 numbering) substitution in NA led to the decreased viral fitness and transmissibility previously seen in cell culture phenotypic models and ferret models (Ives et al., 2002; Herlocher et al., 2004). However, during the 2007 season, H1N1 virus containing the NA H275Y substitution increased and eventually became the dominant strain in circulation (Baranovich et al., 2011; Lackenby et al., 2008). An additional concern was that resistance in this strain may have arisen without any drug pressure or exposure, indicating that the mutated strain was more fit than susceptible strains (Baranovich et al., 2011). It has been shown that decreased viral fitness associated with NA H275Y substitution can be mediated by NA V234M and NA R222Q substitutions (Bloom et al., 2010). However, many NA H275Y strains have unimpaired transmission. Within the H3N2 strains, the majority of resistance is mediated by NA E119V and NA R292K substitutions; to date, these appear to result in decreased viral fitness and require higher titers in experimental models to cause infection in ferrets (Baranovich et al., 2011).

During the 2009 pandemic, the novel H1N1 virus demonstrated susceptibility to oseltamivir. Although NAI-resistant strains were reported, the majority of those strains was found in immunocompromised hosts and was thought to have arisen secondarily to increased exposure to oseltamivir and prolonged shedding. Studies demonstrated that transmission of oseltamivir-resistant strains may be slightly less efficient than that of the wild-type virus, but resistant strain viral titers tend to be comparable to that of the wild-type virus in cell culture (Baranovich et al., 2011). In ferret and guinea pig animal models, transmissibility of resistant strains is comparable to that in the wild type, though this is somewhat controversial as other studies have found less efficient transmission of resistant strains (Govorkova, 2013; Duan et al., 2011). For avian H5N1 virus, experimental models have shown that substitutions like H275Y and N294S within NA can lead to decreased

enzymatic function, but viral fitness appears to be similar to that of wild-type influenza virus, which is of concern given the high pathogenicity in humans of this avian influenza virus subtype (Baranovich et al., 2011). For the emergent H7N9 subtype, there has not been documentation of transmission between persons (McKimm-Breschkin, 2013). However, NAI resistance appears to arise relatively quickly following treatment with oseltamivir (e.g. 2 days after start of treatment; Hu et al., 2013). The NA gene resistance substitution was documented as R292L and was associated with patients who deteriorated quickly, maintained high viral loads, and required extracorporeal membrane oxygenation (ECMO) support (Hu et al., 2013). Additionally, resistance to neuraminidase inhibitors conferred by the NA R292K substitution in the human influenza H7N9 virus was not detected using the phenotypic assay (Yen et al., 2013) which further illustrates the limitations of these assays.

Given the limited number of treatment options for influenza, and that there is increasing concern for resistance, the use of combination therapy has been proposed as a means to increase efficacy. In addition, combination therapy may help in instances in which treatment is started later or in hosts with compromised immune systems. Some initial combinations that have been evaluated have been combinations of NAIs themselves. The antiviral activity of oseltamivir–zanamivir combination therapy compared to that of oseltamivir or zanamivir monotherapy was evaluated in mice infected with influenza A/H3N2 or A/H1N1/pdm09 viruses (Pizzorno et al., 2014). There was no additional benefit of combination therapy over zanamivir alone, though there was a significant benefit compared to oseltamivir alone. In a placebo-controlled study, patients presenting with influenza during the 2008–2009 influenza season were randomized to receive oseltamivir monotherapy with an inhaled placebo (176 patients), oseltamivir/zanamivir combination therapy (192 patients), or a zanamivir plus an oral placebo pill (173 patients) (Duval et al., 2010). The outcome was defined as influenza viral copy number at 48 hours after treatment. The dual therapy was associated with higher viral copy numbers than oseltamivir monotherapy, potentially because zanamivir caused decreased oseltamivir binding. In another small study, dual therapy with oseltamivir and zanamivir was compared to oseltamivir alone during the 2009 pandemic; there did not appear to be a difference in efficacy as measured by influenza viral load and symptoms (though the study was underpowered, with only 24 patients) (Escuret et al., 2012). The results of these oseltamivir/zanamivir combination studies illustrate the shortcomings of using combination therapy with two drugs from the same class.

1.4. Materials and Methods

This section summarizes clinical virology procedures that were used for the Phase 2 and Phase 3 studies.

1.4.1. Studies Conducted by (b) (4)

Diagnostic Viral Culture (0722t0621-vir-study-report)

Madin-Darby canine kidney (MDCK) cell monolayers were infected with the virus recovered from the throat and nasal swabs. Cells were observed every 1 to 3 days for ≤2 weeks until a cytopathic effect (CPE) was observed. Supernatant from CPE positive cells was collected and spread onto newly prepared MDCK cell monolayers for virus propagation. These cells were observed every 2 or 3 days and the supernatant was collected when CPE was observed. A part of the supernatant was mixed with 1/100 volume of 10% NP-40 (final concentration of 0.1%) to inactivate the virus. The inactivated virus was used as the NA source for the NA inhibition assay. An aliquot from each influenza viral specimen was also used in a titration assay for determination of $TCID_{50}$ value.

Determination of Influenza Virus Type by Real-Time RT-PCR (0722t0621-vir-study-report)

A real-time RT-PCR assay for influenza A and B viruses was performed on all baseline samples (screening visit).

(b) (4)

(b) (4)

[redacted]

Determination of Influenza A Virus Subtype by Real-Time RT-PCR (0722t0621-vir-study-report)
A subtyping polymerase chain reaction (RT-PCR) was performed to assess the hemagglutinin 1, 3, or 5 (H1, H3, or H5) subtype. Determination of influenza A virus H1, H3, and H5 subtype was performed by real-time RT-PCR using the primers and probes described below.

(b) (4)

[redacted]

Neuraminidase Enzyme Phenotyping Assay (0722t0621-vir-study-report)
An NAI assay was performed on virus in MDCK culture supernatant to assess the activity of peramivir, oseltamivir, and zanamivir. For the neuraminidase enzyme assay, 2-(4-methlyumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) was used as substrate. Assays were performed for all subjects on paired specimens collected at baseline and at the last influenza virus positive culture or at the last culture for which a NAI assay could be performed. Influenza A/PR/8/34 virus strain (American Type Culture Collection) served as the internal assay control.

Neuraminidase Genotyping Assay (0722t0621-vir-study-report)
The NA gene of the influenza viruses in specimens from subjects demonstrating pretreatment or post-treatment IC_{50} values for peramivir that were greater than a threshold of a mean IC_{50} value + 3 standard deviations (SDs) for NA inhibition activity of peramivir defined for the pretreatment isolates and for the post-treatment (the last isolated virus on Day 2 to 5) isolates, respectively, was characterized by nucleotide sequence analysis. When the applicable virus was from a pretreatment specimen, the last-isolated virus from the same subject was also selected for sequencing to investigate whether genotype changes occurred in the course of treatment. When the IC_{50} value of peramivir increased more than 3 SD higher in the course of treatment, the applicable virus pair (i.e., pretreatment and post-treatment specimens) was also selected for sequencing. The utility of the phenotypic analyses as a screen for resistance is suspect given the selection

bias of the assay for wildtype virus when viruses in subject samples are amplified in cell culture prior to testing and the assay itself which can show a bias in mixtures of wild-type and resistant virus (Wetherall et al., 2003; Deyde et al., 2009; Yen et al., 2013). Another source of bias is the inability of the neuraminidase assay to detect resistance developing in the viral hemagglutinin (Tai et al., 1998; Ison et al., 2006). Additionally, the type of substrate used affects the sensitivity of the assay (Wetherall et al., 2003). Finally, the use of 3 SD as a cutoff is arbitrary and has not been associated with clinical resistance. For these reasons, the Division recommends that phenotypic assays not be used to identify which samples to conduct further resistance analyses and that subject samples be evaluated by genotypic assay directly without an intervening culture step. Site-directed mutant virus or plaque purified resistant virus should be used to assess phenotypic resistance to peramivir and cross-resistance to other NAIs. Another issue with the studies conducted by (b) (4) is that they only genotyped the NA gene and not the HA gene. Resistance to neuraminidase inhibitors have been identified in the HA (described in more details below).

The following reference standards, defined by the International Society for Influenza and other Respiratory Viral Diseases (ISIRV Antiviral Group, 2012: Panel of influenza A and B viruses for assessment of neuraminidase inhibitor susceptibility. Accessed at: http://www.isirv.org/site/index.php/reference-panel), were used for alignment:

- A/H1N1 Influenza Research Database (IRD): JF972564 Influenza A virus (b) (4)
- A/H3N2: Influenza Research Database (IRD): CY064960 Influenza A virus (b) (4)
- B: Influenza Research Database (IRD): DQ174088 Influenza B virus (b) (4)

1.4.2. Studies Conducted by (b) (4)

Diagnostic Viral Culture (BCX1812-211-311-vir-study-report)

Madin-Darby canine kidney (MDCK) cell monolayers were infected with the virus recovered from the nasal swabs. Cells were observed every 1 to 3 days for ≤2 weeks until a cytopathic effect (CPE) was observed. Supernatant from CPE positive cells was collected and spread onto newly prepared MDCK cell monolayers for virus propagation. These cells were observed every 2 or 3 days and the supernatant collected when CPE was observed. A part of the supernatant was mixed with 1/100 volume of 10% NP-40 (final concentration of 0.1%) to inactivate the virus. The inactivated virus was used as the NA source for the NA inhibition assay. An aliquot from each influenza viral specimen was also used in a titration assay. The LOD/LLOQ of the cell culture virus titration assay is 0.75 $\log_{10}$ $TCID_{50}$/ml.

Determination of Influenza Virus Type by Real-Time RT-PCR (BCX1812-211-311-vir-study-report)

A real-time RT-PCR assay for influenza A and B viruses was performed on all Baseline samples (screen/baseline visit). This assay was also used to determine the titer of the virus.

(b) (4)

(b) (4)

- The limit of detection (LOD): 1.58 $\log_{10}$ copies/mL for influenza A virus and 1.49 $\log_{10}$ copies/mL for influenza B virus.
- The lower limit of quantitation (LLOQ): 2.6 $\log_{10}$ copies/mL for influenza A virus and 3.0 $\log_{10}$ copies/mL for influenza B virus.

Determination of Influenza A Virus Subtype by Real-Time RT-PCR (BCX1812-211-311-vir-study-report)
A subtyping polymerase chain reaction (RT-PCR) was performed to assess the hemagglutinin 1, 3, or 5 (H1, H3, or H5) subtype. Determination of influenza A virus H1, H3, and H5 subtype was performed by real-time RT-PCR using the primers and probes described below.

(b) (4)

Neuraminidase Enzyme Phenotyping Assay (BCX1812-211-311-vir-study-report)
An NAI assay was performed on virus MDCK culture supernatant to assess the activity of peramivir, oseltamivir, and zanamivir. For the neuraminidase enzyme assay, 2-(4-methlyumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) was used as substrate. Assays were performed for all subjects on paired specimens collected at baseline and at the last influenza virus positive culture or at the last culture for which an NA enzyme assay could be performed. Influenza strain A/PR/8/1934 virus (or A/Ned/300/00) served as internal assay controls for influenza A virus; influenza strain B/Lee/1940 virus (or B/Ned/022/95) served as internal assay controls for influenza B virus subtypes.

Genotyping Assays (BCX1812-211-311-vir-study-report)
(b) (4) was extracted directly from clinical samples using the (b) (4) Nucleic Acid Isolation kit and used to generate cDNA using (b) (4) and the following NA and HA primers:

1. Influenza A/H1N1 and A/H3N2 viruses: (b) (4)
2. Influenza B virus (mix of): (b) (4)

Amplification of the NA and HA genes was performed by nested RT-PCR using Hotstar Taq PCR. Forward and reverse primers utilized for the first and second rounds of amplification are provided below.

(b) (4)

(b) (4)

[redacted]

(b) (4)

Reference standards used for alignment are shown below. The standards were chosen based on strains circulating during the time period covered by the clinical studies.

- Influenza A virus (A/California/07/2009[H1N1]) (b) (4)
- Influenza A virus (A/California/07/2009[H1N1]) (b) (4)
- Influenza A virus (A/Brisbane/10/2007[H3N2]) NA
- Influenza A virus (A/Brisbane/10/2007[H3N2]) HA
- Influenza A virus (A/reassortant/NYMC X-187(Victoria/210/2009 x Puerto Rico/8/1934)[H3N2])_NA (CY121722)

- Influenza A virus (A/reassortant/NYMC X-187(Victoria/210/2009 x Puerto Rico/8/1934)[H3N2])_HA (CY121720)
- Influenza B virus (B/Brisbane/60/2008) (b) (4)
- Influenza B virus (B/Brisbane/60/2008) (b) (4)

1.5. Prior FDA Virology reviews

The Original IND 69038 and subsequent IND submissions through SDN 092 (3/2009) were reviewed by Clinical Virology Reviewer Jules O'Rear, Ph.D. IND submissions after SDN 092 were reviewed by Clinical Virology Reviewer Takashi E. Komatsu, Ph.D. The Original IND (b) (4) and subsequent IND submissions through SDN 085 (6/2009) were reviewed by Clinical Virology Reviewer Jules O'Rear, Ph.D. IND submissions after SDN 085 were reviewed by Clinical Virology Reviewer Takashi E. Komatsu, Ph.D.

2. Nonclinical Virology

2.1. Biochemical Studies (Study Numbers S-021812-EB-117-N, DD00005, P97-1812-001, EDMS-USRA-3424162, S-021812-EB-111, S-021812-EB-116-N, PMV-EB-037-N, DD99044, DD01030, Gubareva et al., 2001, Kitano et al., 2011, S-021812-EB-060-N, Boltz et al., 2008, DD01044, CDC 2009, Gubareva et al., 2010, PMV-EB-033-N)

In biochemical studies using the neuraminidase inhibition assay as the endpoint, the median IC_{50} values of peramivir were 0.16 nM (n=44; range 0.09-13.87 nM), 0.17 nM (n=11; range 0.06-1.77 nM), 0.13 nM n=32; range 0.05-11 nM), 0.04 nM (n=4; range 0.23-0.6 nM), and 0.99 nM (n=39, range 0.4-54.2 nM) against influenza A/H1N1, A/H2N2, A/H3N2, and A/H5N1 viruses, and influenza B virus, respectively (Table 1). Against 2009 A/H1N1 influenza virus isolates, the IC_{50} values ranged from 0.03-0.5 nM (Gubareva et al., 2010). The test method used in each of these reports was predominantly a standard NA assay that monitored cleavage of a fluorescent substrate (2-(4-methlyumbelliferyl)-α-D-N-acetylneuraminic acid [MUNANA]) using tissue culture supernatants containing the virus or protein obtained from the dissolution of protein crystals as the source of NA. Of note, the activity of peramivir, like oseltamivir and zanamivir, is less against influenza B virus.

Table 1: Activity of Peramivir against Influenza A and B Viruses in Neuraminidase Inhibition Assays

Report Number	Influenza Virus Subtype[a]	Number of Virus Strains Tested	Range of Mean IC_{50} Value (nM)[b]		
			Peramivir	Oseltamivir	Zanamivir
S-021812-EB-117-N	H1N1	1	0.54–0.61	Not tested	Not tested
DD00005	H1N1	2	0.2–1.8	0 9–1.4	1.1–3
P97-1812-001	H1N1	6[c]	0.09–0.81	0.69–2.24	0.30–0.80
	H1N9	1[c]	1.3	2.1	1.6
	H2N2	4	0.17–1.39	0 01–1.45	0.76–1.78
	H6N2	1	1 08	0.84	1.07
	H3N2	4	0.14–0.83	0 21–0.56	0.68–2.32
	B	8	0.60–10.78	5 00–24.3	1.53–17.0
EDMS-USRA-3424162	H1N1	4	0.09–0.37	0.69–2.24	0.30–0.80
	H2N2	2	0.17–0.18	0 21–0.25	0.76–0.99
	H3N2	3	0.14–0.29	0 21–0.24	0.68–2.32
	B	4	0.82–1.53	7.70–10.36	1.53–3.31
S-021812-EB-111	H1N1	12[c]	0.50–0.94	0 94–1.61	1.43–3.21
	H2N2	3	1.39–1.77	0 82–1.04	4.72–6.42

Report Number	Influenza Virus Subtype[a]	Number of Virus Strains Tested	Range of Mean IC_{50} Value (nM)[b]		
			Peramivir	Oseltamivir	Zanamivir
	H3N2	19	0.92–1.72	0 54–1.16	2.80–9.07
	B	14	1.82–54.2	4 39–18.0	7.12–13.7
S-021812-EB-116-N	H1N1	4	1.4–2.0	5.7–9.1	2.6–9.1
	H2N2	1[e]	0.66	1.3	4.2
	H3N2	6[e]	0.54–11.0	1.0–14	2.0–14
	B	5	6.8–17	28–79	14–40
PMV-EB-037-N	A	8[c]	0.31–1.10	0 34–1.31	Not done
	B	6	0.4–1.92	1.47–6.07.	Not done
DD99044	H1N1	5	0.26–0.43	0 30–0.60	0.73–1.05
	H3N2	6	0.47–0.87	0 27–0.45	1.85–3.13
	B	10	1.0-1.7	5.3–18.3	1.9–3.6
DD01030	H1N1	5	0.13–0.58	0.71–2.07	0.52–1.32
	H3N2	40	0.3–1.09	0 32–1.97	0.85–4.76
	B	24	0.52–1.66	4.93–18.59	2.19–9.53
Gubareva et al., 2001	H1N1	5	0.26–0.43	0.45–0.60	0.73–1.05
	H3N2	6	0.47–0.87	0 27–0.45	1.85–3.13
	B	8	1.08–1.95	5.33–18.33	2.00–3.10
Kitano et al., 2011	B	1	1.8	5.9	Not done
	B	1	4.3	13.1	Not done
S-021812-EB-060-N	Avian H5N1	3	0.23–0.40	5 00–8.07	0.99–1.77
	Avian H7N1	1	0 26	7.63	1.72
	Avian H7N7	1	0.12	1.10	4.88
Boltz et al., 2008	Avian H5N1	1	0.6	0.3	0.9
DD01044	Avian[d]	18	0.9–4.3	1.9–69.2	2.2–30.1
CDC 2009	2009 H1N1	13	0.06–0.26	0 28–1.41	0.30–1.34
	H1N1	1	0.16	0.61	0.56
	H1N1	1	13.87	200.73	0.8
Gubareva et al., 2010	2009 H1N1	1440	0.03–0.5	0.05–2.41[f]	0.04–1.24[g]
PMV-EB-033-N	H1N1	1	0 50	1.40	Not done
	H2N2	1	0 94	0.70	Not done
	Avian H6N1	1	0.62	2.09	Not done
	Avian H9N2	1	1.12	0.86	Not done
	Avian H2N3	1	0.69	0.99	Not done
	Avian H5N3	1	0 90	1.03	Not done
	Avian H8N4	1	0 91	1.22	Not done
	Avian H10N4	1	0 51	1.22	Not done
	Avian H4N5	1	0 24	0.99	Not done
	Avian H12N5	1	0 30	1.02	Not done
	Avian H11N6	1	0 82	1.21	Not done
	Avian H13N6	1	0.69	1.19	Not done
	Avian H7N7	1	0 85	0.86	Not done
	Avian H10N7	1	1.18	2.46	Not done

Report Number	Influenza Virus Subtype[a]	Number of Virus Strains Tested	Range of Mean IC_{50} Value (nM)[b]		
			Peramivir	Oseltamivir	Zanamivir
	Avian H3N8	1	0 39	3.38	Not done
	Avian H6N8	1	0 35	2.19	Not done
	Avian H11N9	2	0.39–0.45	1.13–1.18	Not done
(b) (4) (CDC)	Avian H7N9	3	0.04-0.09	0.23-0.33	0.37-0.54
Dr. (b) (4), WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia[h]	Avian H7N9	1	0 24	0.41	1.06

a. All strains are seasonal unless listed as pandemic (2009 A/H1N1)
b. Where the data range was not provided in the report, the mean IC_{50} value alone is presented.
c. The report had a discrepancy in the number of strains tested between various sections of the report. The values from the report tabulations are presented here.
d. Included H6N1, H1N1, H7N2, H9N2, H5N3, H2N3, H8N4, H14N5, H6N5, H13N6, H4N6, H7N7, H10N7, H4N8, H10N8, H11N9.
e. Strain A/Kumamoto/Y5/67 is incorrectly listed as subtype H3N2 in Table 1 of report S-021812-EB-116.
f. Oseltamivir was tested against 3335 isolates.
g. Zanamivir was tested against 3335 isolates.
h. Laninamivir was also tested, with a mean IC_{50} value of 1.39 nM.

2.2. Cell Culture Studies

2.2.1. Antiviral Activity in Cell Culture (Study Numbers S-021812-EB-115-N, S-021812-EB-108-N, P97-1812-001, Smee et al., 2001, DD01037, PMV-EB-034-N, PMV-EB-035-N, Boltz et al., 2008)

Peramivir was active in cell culture against numerous strains of influenza A and B viruses, as well as against different subtypes of avian influenza virus including H5N1, H5N3, and H7N7. MDCK cells were used as host cells. The median EC_{50} values of peramivir using a plaque assay as the endpoint were 2.6 nM (n=13; range 0.09-21 nM), <1 nM (n=1), 0.08 nM (n=17; range <0.01- 1.9 nM), 0.3 nM (n=1), and 4.8 nM (n=11; range 0.06-120 nM) against influenza A/H1N1, A/H2N2, A/H3N2, and A/H5N1 viruses, and influenza B virus, respectively (Table 2). Of note, the activity of peramivir, like oseltamivir and zanamivir, is less against influenza B virus. The relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, inhibition of influenza virus replication *in vivo*, and inhibition or reduction of infection-related symptoms in humans has not been established.

Table 2: Activity of Peramivir against Influenza A and B Viruses in Cell Culture Assays

Report Number	Influenza Virus Subtype	No. of Virus Strains Tested	Endpoint	Range of Mean EC_{50} Value (nM)[a]		
				Peramivir	Oseltamivir	Zanamivir
S-021812-EB-115-N	H1N1	2	Plaque counts by neutral red staining	0.93-15	60-140	5.8-37
	H3N2	1		0.36	0.78	4.6
	B	2		25–26	31–42	21–29
S-021812-EB-108-N	H1N1	3	Plaque counts by neutral red staining	0.48-18	7.5–360	13–130
	H3N2	4		0.06–1.9	5.3–260	3.6–59
	B	3		4.8–120	30–720	7.9–150
P97-1812-001	H2N2	1	Plaque counts by crystal violet staining	<1.0	<1.0	<1.0
	B	1		95.0	467	67
Smee et al., 2001	H1N1	5	Neutral red uptake	0.09–21	0.17– >100	0.22– >100

Report Number	Influenza Virus Subtype	No. of Virus Strains Tested	Endpoint	Range of Mean EC_{50} Value (nM)[a]		
				Peramivir	Oseltamivir	Zanamivir
	H3N2	12		<0.01–0.19	<0.01–0.50	<0.01–0.65
	B	5		0.06–3.2	0.11–3.0	0.03–1.3
	Avian H5N1	2		0.01–0.02	0.22–0.26	0.20–0.22
DD01037[b]	H1N1	9	Cytotoxicity by MTT[c]	3.1–>100	Not tested	Not tested
	H2N2	1		1.4	Not tested	Not tested
	H3N2	10		0.5–28.7	Not tested	Not tested
	B	9		All >100	Not tested	Not tested
	Avian H5N3	1		24.1	Not tested	Not tested
PMV-EB-034-N	H1N1	1	Cell viability by WST-8 reagent	>100	>100	Not tested
	H3N2	1		13	45	Not tested
	B	4		All >100	All >100	Not tested
PMV-EB-035-N[d]	H1N1	4	Cytopathic effect by visual light microscopy	13–>100	All >100	All >100
	H3N2	2		72– >100	81– >100	Both >1000
	B	4		110–490	280– >1000	130–470
Boltz et al., 2008	H5N1	1	Plaque size reduction assay	0.3	0.5	0.7

a. Where data range was not provided in the report, the mean EC_{50} value alone is presented.
b. Comparator drug was ribavirin.
c. MTT = 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide.
d. Report PMV-EB-035-N only provided EC_{90} value data.

2.2.2. Cytotoxicity of Peramivir (Study Numbers DD00004, DD00008, DD00025, DD99003)

The cell culture cytotoxicity of peramivir was evaluated in MDCK cells grown in the presence of drug for 3 days. The CC_{50} value in MDCK cells was >1 mM using the MTT assay as endpoint, giving a therapeutic index of >290,000, >1,000,000, >2,630,000, >3,330,000, and >26,000 against influenza A/H1N1, A/H2N2, A/H3N2, and A/H5N1 viruses, and influenza B virus, respectively. The CC_{50} value in transformed human bronchial epithelium cell line BEAS-2B cells grown in presence of drug for 4 days using optical density of cultures in a microtiter assay was >300 μM. The CC_{50} value in MDCK, human lung carcinoma cells, human epidermoid carcinoma cells, [redacted] (b) (4) grown in presence of drug for 3 days using changes in cell appearance when viewed microscopically and measuring [redacted] (b) (4) for all cell lines tested.

2.2.3. Combination of Peramivir and Other Antiviral Agents in Cell Culture (Study Numbers Smee et al., 2010, Govorkova et al., 2004, DD00044)

In MDCK cells infected with influenza A/NWS/33 (H1N1) virus, combination of peramivir and oseltamivir was not antagonistic (Smee et al., 2010). The combination of rimantadine with peramivir was not antagonistic against A/New Caledonia/20/99 (H1N1) and A/Panama/2007/99 (H3N2) virus (Govorkova et al., 2004). The

combination of peramivir and RBV was not antagonistic against influenza A/Shangdong/09/93 (H3N2) virus (study report DD00044).

2.3. Antiviral Activity in Animal Models

Peramivir has been evaluated for antiviral activity in mice (IV, oral, IM, and intranasal routes of administration), ferrets (IV, oral, and IM administration), and cynomolgus monkeys (oral and IV administration). The majority of studies (more than 40 reports and publications) used oral administration of peramivir. However, several studies used the IV route of administration.

2.3.1. Antiviral Activity in Mouse Models of Influenza

2.3.1.1. Antiviral Activity of Intravenous Administered Peramivir in Lethal Mouse Influenza Models (Study Numbers S-021812-EB-103-N, S-021812-EB-104-N, S-021812-EB-105-N, S-021812-EB-106-N, PMV-EB-039-N, S-021812-EB-123-N, S-021812-EB-082-N, NIA-527)

Several studies showed that a single IV dose of peramivir was active in the mouse infection model with seasonal influenza A virus, influenza B virus, or avian (H5N1) influenza virus (Table A3) when dosed prior to or immediately after the time of viral inoculation. The mice for the majority of these studies were conducted by Shionogi who followed the mice for only 14 days (~2x MTD) after intranasal virus inoculation so the interpretation of the results of these studies is limited. The sponsor should have assessed the mice for 4-5x the mean time to death of placebo treated animals to assess the possibility that peramivir only delayed death. Five of these studies, compared the activity (measured as survival) of a single IV dose of peramivir with single or multiple oral doses of oseltamivir or multiple IV doses of zanamivir. Treatment began immediately after viral inoculation. Mice were infected with influenza A/PR/8/34 (H1N1), A/Kumamoto/Y5/67 (H2N2), A/Victoria/3/75 (H3N2), B/Maryland/1/59 or B/Lee/40 viruses. A single dose of peramivir administered IV was comparable in activity, as measured by survival, to oseltamivir dosed orally or IV BID for 5 days or zanamivir administered IV BID for 5 days. When each drug was dosed for 5 days, peramivir administered IV at doses of 10 or 30 mg/kg/day produced better survival rates than oral oseltamivir or zanamivir administered IV at the same daily doses. Intravenous peramivir at all doses resulted in increased survival compared with controls.

The studies summarized in Table A3 demonstrate that peramivir is active in the mouse models of influenza virus infections and that the peramivir activity is comparable to or better than that of oseltamivir administered orally or IV, or zanamivir administered IV. A single IV dose of peramivir administered before or at the time of viral inoculation was active in treating mice infected with seasonal influenza A virus, influenza B virus, or avian influenza virus (H5N1).

Several studies were conducted in a mouse model of infection with influenza A/WS/33 virus (H1N1) to evaluate the antiviral activity of IV peramivir administered from 24 to 96 hours after viral inoculation (Table A4). Two of these studies also evaluated the activity of oral oseltamivir. In each study, a single IV dose of peramivir was active in increasing survival in mice after inoculation with seasonal influenza A virus. Treatment with peramivir from 24 to 72 hours after viral inoculation prevented mortality. However, peramivir was not able to prevent mortality when treatment was initiated >72 hours after infection.

2.3.1.2. Antiviral Activity of Intramuscular or Intranasal Administered Peramivir in Lethal Mouse Influenza Models (Study Numbers NIA-562, P09-1812-008, BCA-48, P04-1812-001, NIA-518, P09-1812-009)

The sponsor also examined the activity of an IM or intranasal injection of peramivir using experimental infections with seasonal or pandemic influenza A virus strains. The mice for the majority of these studies were followed for ~4x the mean time to death of placebo treated animals after influenza virus inoculation. The results were similar to those observed in experiments using oral or intravenous peramivir administration.

2.3.1.3. Antiviral Activity of Orally Administered Peramivir in Lethal Mouse Influenza Models (Study Numbers DD99057, EDMS-USRA-270526, Bantia et al., 2001, EDMS-USRA-3251731, EDMS-USRA-3251543, EDMS-USRA-3251811, EDMS-USRA-3251808, DD00012, DD99046, P04-1812-001, EDMS-USRA-3252053, Sidwell et al., 2001, DD00013, DD01044)

Mice were intranasally inoculated with virus 4 hours after the first treatment with peramivir or vehicle; peramivir was administered orally once daily or BID for 5 days. For experimental infections, challenge was done with influenza A virus at a titer that generally resulted in a 40% to 100% mortality rate in the control untreated group of mice (Table A1). One or more of the following virus strains were used: A/Shangdong/09/93 (H3N2), A/Turkey/Mass/76XA/Beijing 32/92 (H6N2), A/Bayern/57/93 (H1N1), A/NWS/33 (H1N1) or A/Victoria/3/75 (H3N2). In these studies, inoculation of the animals with virus occurred 4 hours after oral dosing with either peramivir (0.01-100 mg/kg/day, depending on the study) or oseltamivir (0.01–10 mg/kg/day, depending on the study) and are thus only supportive of prophylaxis. Some studies also used RBV at 75 mg/kg/day as a positive control. Peramivir was well tolerated at doses up to 100 mg/kg/day in control animals. Activity against influenza A viruses, measured by survival, was observed with peramivir doses as low as 0.1 mg/kg/day. Two avian viruses, A/HK/156/97 virus (H5N1) and A/quail/HK/G1/97 virus (H9N2), were also tested in this mouse model. Activity of peramivir was observed at doses as low as 0.1 mg/kg/day. Activity was also demonstrated in influenza B virus mouse infection models using B/Hong Kong/5/72 or B/Lee/40 virus. In the case of influenza B viruses, activity, measured by survival, was observed with peramivir doses as low as 1.0 mg/kg/day. Other parameters evaluated in some of the studies that demonstrated activity of peramivir included increased mean days to death and oxygen saturation, as well as decreased body weight loss, lung score, lung weight, and lower virus titers in the lungs and brain.

Several studies examined the antiviral activity of peramivir in a mouse influenza treatment model in which dosing with peramivir was initiated up to 60 hours following viral inoculation (Table A2). Mice were inoculated with avian influenza A/Hong Kong/156/97 (H5N1) virus or with one of the following influenza A virus strains: A/Turkey/Mass/76XA/Beijing 32/92 [R] (H6N2), A/NWS/33 (H1N1), A/PR/8/34 (H1N1) or A/Shangdong/09/93 (H3N2). In the majority of these studies, animals dosed with peramivir at 10 mg/kg/day up to 60 hours after initiation of infection showed increased survival compared with the animals treated with vehicle control (70% to 100% lethality in vehicle control mice). Peramivir administered orally at doses ranging from 1 to 100 mg/kg/day, prevented mortality in mice with experimental influenza virus infection. In general, peramivir was active against influenza A virus when dosing began up to 36 hours after viral inoculation, and was active when dosing began up to 60 hours after viral inoculation in all but one study. Oral administration of peramivir to mice improved survival at daily doses as low as 1 mg/kg, depending on the viral strain and dosing regimen. The activity of peramivir was compared with that of oseltamivir in 2 studies. There was no statistical difference in survival in peramivir-treated animals compared with oseltamivir-treated animals, regardless of the time of treatment initiation.

There was a significant improvement in lung scores, lung viral titer, prevention of body weight loss, and mean arterial oxygen saturation (when measured) in these studies compared with the vehicle treatment group.

2.3.1.4. Antiviral Activity of Orally or Intravenous Administered Peramivir in a Lethal Mouse Model Using Immunocompromised Mice (Study Numbers DD00016, DD00043, DD01034, S-021812-EB-131-N, Kitano et al., 2013)

Several studies were conducted to evaluate the activity of peramivir in influenza virus-infected immunocompromised mice, using either the cyclophosphamide (CP)-induced immunosuppression model or SCID mice (Table A5). The mice for the majority of these studies were not assessed at 4-5x the mean time to death of placebo treated animals after intranasal virus inoculation so the interpretation of the results is limited. Peramivir was moderately active against influenza virus infection in totally immunocompromised SCID mice. Oral or IV peramivir was more active than oral oseltamivir in CP-treated mice using a 5- or 20-day treatment regimen. The clinical relevance of these findings is unclear.

2.3.1.5. Activity of Peramivir in Combination with Other Antiviral Agents in Lethal Mouse Influenza Models (Study Numbers DD00040, NIA-956, P09-1812-004, P09-1812-005, Tarbet et al., 2012)

Several combination regimens were studied in lethal influenza A virus mouse models. Combination treatment of peramivir with favipiravir, oseltamivir, RBV, or rimantadine resulted in higher survival compared to either drug alone (for each drug combination studies) indicating a higher genetic barrier; in addition, improvements were generally seen in the number of days to death, prevention of weight loss, and/or oxygen saturation levels.

2.3.1.6. Evaluation of Virulence in Viruses Exposed to Peramivir (Study Numbers DD01022, DD00021)

The virulence of influenza A viruses with reduced susceptibility to peramivir generated by passage in MDCK cells was similar to or less than that of WT viruses, based on lung titer, lung consolidation, and/or survival in 6 studies in adult mice. Study DD01022 used A/PR/8/34 P-15 R2A virus, which had HA amino acid substitutions R208K and D129S; and Study DD00021 used A/Singapore/1/57 virus (H2N2) which had amino acid substitutions in both the HA (G130/135D H2/H3) and NA (R292K). It should be noted that there are other pathways not evaluated and that the fitness of virus expressing the NA H275Y oseltamivir resistance substitution is variable and can be virulent.

Conflicting results for influenza B virus were observed. This study used influenza B/Yamagata/15/88 variant virus having HA amino acid substitutions T139N, G141E, and D195N as well as influenza B/Yamagata/15/88 virus having HA amino acid substitutions T139N, G141E, R162M, D195N and Y319H, and NA amino acid substitution H274Y. In 1 experiment, results indicated that influenza B/Yamagata/15/88 virus having HA amino acid substitutions T139N, G141E, R162M, D195N and Y319H, and NA amino acid substitutions H274Y had increased virulence compared with WT. When this experiment was repeated, no deaths were observed in both the WT and resistant virus infected mice so no conclusions could be drawn about virulence. Additionally, viral titers were higher compared to WT in mice infected with influenza B/Yamagata/15/88 variant virus having HA amino acid substitutions T139N, G141E, and D195N.

2.3.1.7. Correlation of Pharmacodynamic and Pharmacokinetic Properties of Peramivir (Study Numbers Drusano et al., 2001, S-021812-EB-053-N)

Studies were conducted using the mouse model of influenza in order to delineate which aspect of the peramivir concentration-time curve affects outcome. In the Drusano et al. (2001) publication, mice intranasally inoculated with the influenza A/Shangdong/09/93 virus (H3N2) were used for the evaluation of peramivir pharmacokinetic-pharmacodynamic relationships. Animals were dosed once orally (by gavage) with peramivir (3.33, 5, 10, or 20 mg/kg). Plasma was obtained at 10 time points over 24 hours from 6 animals at each time point for each

regimen (240 animals). Plasma samples were assayed for peramivir by a liquid chromatography-mass spectroscopy procedure. All 3 exposure covariates (peak concentration, trough concentration, and AUC) significantly affected the time to death. However, the AUC was the dynamically linked variable. In another study (S-021812-EB-053-N), mice were intranasally inoculated with an influenza A/WS/33 virus (H1N1) and dosed with IV peramivir (0.25, 0.5, 1, 2, 4, or 8 mg/kg/day) 48 hours after viral inoculation according to one of the following regimens over a period of 12 hours: one dose, 2 equally divided doses at a 6-hour interval, or 3 equally divided doses at 3-hour intervals. Plasma samples were assayed for peramivir by a liquid chromatography-mass spectroscopy procedure. Consistent with the other study, the AUC parameter correlated best with activity of peramivir in mice infected with influenza virus.

2.3.2. Antiviral Activity of Orally or Intraveneously Administered Peramivir in Nonlethal Ferret Models of Influenza (Study Numbers DD01042, Kitano et al., 2011, Yun et al., 2008)

Oral peramivir was examined for antiviral activity against H3N2 influenza virus (H3N2 clone 7a of the influenza A/PR/8/34-A/England/939/69 reassortant virus) in experimental infection in ferrets (study report DD01042). This study was not a lethal model. Ferrets (4 per group) were infected intranasally with 10^6 $TCID_{50}$ value of clone 7a). Drugs were given orally 2 hr prior to virus inoculation and then at 12, 24, 36, 48, 60 and 72 hr p.i. Following virus inoculation, nasal washings were taken and temperatures recorded every 6-12 hr at 12, 24, 30, 36, 48, 54, 60, 72, 78, 84 and 96 hr p.i. At peramivir dose of 10 mg/kg there was no statistically significant reduction in viral titers in nasal washes or in the inflammatory response compared with untreated ferrets, although there was little fever and a modest reduction in mean lung viral titers. Peramivir at 30 or 100 mg/kg caused a significant reduction in peak virus titers in nasal washes, total virus shedding, and nasal inflammatory responses compared with untreated animals.

Intraveneously administered peramivir was examined for antiviral activity against influenza B/Kadoma/1/2005 virus in experimental infection in ferrets (Kitano et al., 2011). This study was not a lethal model. Treatment began 1 day after intranasal infection with virus at 300 $TCID_{50}$ value for groups of 4 (drug-treated) or 6 (vehicle control) ferrets. Animals were treated with either a single IV dose of peramivir at 30 or 60 mg/kg, once daily IV peramivir at 30 mg/kg/day for 3 days, oral oseltamivir at 30 or 60 mg/kg/day BID for 3 days, or oral vehicle control (0.5% methyl cellulose) BID for 3 days. Evaluations included pharmacokinetics, virus titers obtained from nasal swabs, body temperature, and effects on the production of anti-HA antibodies. Nocturnal body temperature and body weights were compared with pre-treatment values, and nasal clinical signs (discharge, sneezing and mouth breathing) were also evaluated. The activity of a single IV dose of 60 mg/kg/day peramivir in ferrets inoculated with an influenza B virus strain was generally comparable to or exceeded that resulting from multiple doses of 30 or 60 mg/kg/day oral oseltamivir BID or of 30 mg/kg/day IV peramivir for 3 days, based on nasal wash viral titer, protein concentration, and number of inflammatory cells. Systemic signs of illness, including rise in body temperature, loss of body weight, and nasal clinical signs, were also improved with peramivir treatment.

Intramuscularly administered peramivir was examined for antiviral activity against influenza A/Vietnam/1203/04 virus (H5N1) in experimental infection in ferrets (Yun et al., 2008). The study was a lethal model though the ferrets were not assessed for 4-5x the mean time to death of placebo treated animals after virus inoculation so the interpretation of the results is limited. Ferrets (8-10 ferrets per group) were inoculated intranasally influenza A virus (H5N1; A/Vietnam/1203/04) at ~10^3 $TCID_{50}$ value. Treated animals received once daily IM peramivir 30 mg/kg/day, while control animals either were treated with vehicle (drug diluent) starting at 1 hour post-infection and daily from Day 1 through Day 4 post-infection or were untreated. The study design was for prophylaxis treatment so the clinical relevance is limited. Increased survival was observed in peramivir-treated animals compared with control animals (trial 1: 86% vs 43% [P = 0.09]; trial 2: 75% vs 11% [P = 0.004]; trial 3: 70% vs

30% [P = 0.037]). In trials 2 and 3, peramivir also mitigated virus-induced disease, based on a lower occurrence of encephalitis and paralysis in peramivir-treated animals versus control animals. Encephalitis occurred in 25% to 50% of peramivir-treated animals compared with 50% to 70% of control animals. Paralysis occurred in only 1 peramivir-treated animal (trial 3); 44% to 60% of control animals experienced paralysis. There were also reduced virus titers in the lungs and brains of the peramivir treated ferrets.

2.3.3. Antiviral Activity in a Nonlethal Cynomolgus Monkey Model of Influenza (Study Numbers DD00041, Kitano et al., 2011)

The antiviral activity of peramivir was studied in adolescent cynomolgus monkeys (20 per group) that were inoculated intratracheally with influenza A/Netherlands/18/94 virus (H3N2) (10^7 $TCID_{50}$ value) and then treated orally with peramivir for up to 5 days. A vehicle control was utilized. Drug treatment was initiated 24 hours post infection. While the time to onset of symptoms is unclear in this animal model, the study design is likely supportive of prophylaxis. Peramivir doses in descending order (given as days of treatment x mg peramivir per 2.5 kg body weight) were 5 x 14; 1 x 28 + 2 x 14; 3 x 14; 5 x 7 (i.e., 5.6 mg/kg/day for 5 days; 11.2 mg/kg for 1 day + 5.6 mg/kg for 2 days; 5.6 mg/kg/day for 3 days; 2.8 mg/kg/day for 5 days). Endpoints evaluated included the virus specific antibody response measured in a hemagglutination inhibition assay and quantification of virus titers in biological samples other than blood. All monkeys showed a virus specific serum antibody response on Day 11 that was still detectable on Day 28. Antiviral activity of peramivir was observed in cynomolgus monkeys with experimental influenza virus infection due to influenza A/H3N2 virus based on reduced viral shedding compared to untreated controls. Similar results were observed in cynomolgus macaque monkeys inoculated intranasally with B/SendaiH/1051/2007 virus (Kitano et al., 2011).

2.4. Resistance Analyses (Study Numbers DD00048, Baz et al., 2007, Nedyalko et al., 2002, P98-1812-001, DD00020, DD00047)

Selection of resistance to peramivir in cell culture was performed with 7 different influenza viruses; A/PR/8/34 (H1N1), X121 (a reassortant virus with H3N2 surface glycoproteins), B/Yamagata/16/88, A/Singapore/1/57 (H2N2), A/Shandong/09/92 (H3N2), A/Charlottesville/31/95 (H1N1) and A/WSN/33 (H1N1). Cells were passaged in the presence of increasing concentrations of the peramivir. Reduced susceptibility to NA inhibitor(s) can potentially arise through amino acid substitutions in either the NA and/or HA. Influenza virus strains with reduced susceptibility to peramivir by passaging in MDCK cells produced viruses with amino acid substitutions in HA or NA alone, in both HA and NA, or with a deletion in RNA segment 6 that encodes for NA (Table 3).

Table 3: Amino Acid Substitutions Selected in Cell Culture

Study Number	Influenza Virus and Source	Protein	Emergent Amino Acid Substitution	EC_{50} value for Peramivir (µM)	EC_{50} value for WT control (µM)	EC_{50}/IC_{50} value for Oseltamivir (µM)	EC_{50}/IC_{50} value for Zanamivir (µM)
DD00048[a]	A/PR/8/34 (H1N1) Passage 4 in presence of peramivir	HA	R208K	>10	Not Done		
	A/PR/8/34 (H1N1) Passage 10 in presence of peramivir[b]	HA	R208K D129S[c]	>100	<0.001	EC_{50} value = 1 µM	EC_{50} value = 100 µM
	A/PR/8/34 (H1N1) Passage 13 in presence of peramivir	HA	R208K D129S[c]	>100	0.05		

Study Number	Influenza Virus and Source	Protein	Emergent Amino Acid Substitution	EC_{50} value for Peramivir (µM)	EC_{50} value for WT control (µM)	EC_{50}/IC_{50} value for Oseltamivir (µM)	EC_{50}/IC_{50} value for Zanamivir (µM)
Baz et al., 2007	A/WSN/33 (H1N1) recombinant virus Passage 18 in presence of peramivir	NA	H275Y	Not stated	Not stated		
Nedyalkova et al., 2002	A/Charlottesville/31/95 (H1N1) Passage 18 in presence of peramivir	NA[e]	N58D I211T[c]	>300[h]	0.3		
P98-1812-001	H2N2 A/Singapore/1/57 Passage D11 in presence of peramivir	HA	G130D	10–100	<0.001		
	H2N2 A/Singapore/1/57 Passage D15 in presence of peramivir[g]	HA NA	HA: G130D NA: R292K[c]	302	<0.001	IC_{50} value = 253 µM	IC_{50} value >1000 µM
DD00048[a]	X121 (H3N2) Passage 10 in presence of peramivir (Isolate R2)	HA	G78D	100	<0.01		
	X121 (H3N2) Passage 15 in presence of peramivir[f]	HA	N63K, N145D[c]	100	<0.001	EC_{50} value >100 µM	EC_{50} value >100 µM
DD00020	A/Shangdong/09/92 (H3N2) Passage 10 in presence of peramivir	HA	K189E	32	0.1		
DD00047[d]	A/Shangdong/09/92 (H3N2) Passage 10 in presence of peramivir	HA	K189E	1.8	0.002		
DD00048[a]	B/Yamagata/16/88 Passage 3 in presence of peramivir	HA	G141E D195N T197N[c]	10	0.1		
	B/Yamagata/16/88 Passage 15 in presence of peramivir	HA	G141E D195N T197N T139N R162M Y319H[c]	>100	0.5		
		NA	H275Y				

a. This study also described the A/Shandong/09/92 (H3N2) variant isolated at Passage 10 in the presence of peramivir.
b. EC_{50} values for oseltamivir and zanamivir were 1 and 100 µM, respectively.
c. All amino acid substitutions are present in the same virus
d. Viruses used in this study were obtained from mice infected with virus that had demonstrated decreased susceptibility to peramivir in cell culture before use to inoculate mice.
e. This variant showed an accumulation of defective RNA molecules for NA. The peramivir IC_{50} values for NA inhibition were 0.1 and 0.3 nM for mutant and WT virus, respectively.
f. EC_{50} values for oseltamivir and zanamivir were both >100 µM.
g. IC_{50} values for oseltamivir and zanamivir were 253 and >1000 µM, respectively.

Amino acid substitutions in HA observed in different virus subtypes in the cell culture studies presented no specific pattern. 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. Of note, some of the amino acid substitutions in the HA that were selected in the sponsor's studies (Table 3) were in the absence of any resistance-associated amino acid substitutions in the NA. Additionally, the HA N63K and N145D substitutions conferred cross-resistance to both oseltamivir and zanamivir. These results are concerning as the use of the neuraminidase assay as a screen for resistant virus would not be expected to detect resistance developing in the viral hemagglutinin. Cross-resistance to oseltamivir and zanamivir should be determined for all of the amino acid substitutions described in Table 3.

In addition to HA and NA amino acid substitutions, reduced susceptibility to peramivir in cell culture has also been shown to be caused by deletions in RNA segment 6, which encodes NA. One such variant strain was generated from a clinical isolate of influenza A/Charlottesville/31/95 virus (H1N1) after 18 passages in MDCK cells in the presence of peramivir. This variant demonstrated reduced susceptibility to peramivir, oseltamivir, and zanamivir based on measurements of plaque size, while activity against NA enzyme was unaffected (Nedyalkova et al., 2002). The authors state that there was an internal ~300 amino acid deletion (missing nucleotide 128-1026).

In plaque reduction assays, peramivir was active against influenza A/WSN/33 virus (H1N1), but not against A/Charlottesville/31/95 virus (H1N1) (lacks NA activity). A reassortant virus, in which the HA gene of an influenza A/WSN/33 virus was substituted with the HA gene of the A/Charlottesville/31/95 virus, showed reduced susceptibility to peramivir in a plaque reduction assay. This study found that sialylation of oligosaccharide chains in the vicinity of the HA receptor-binding site likely provides a compensatory mechanism for the lack of NA activity and allows emergence of mutants that lack NA (Gubareva et al., 2002).

Recombinant H1N1 virus carrying the NA H275Y amino acid substitution conferred decreased susceptibility to peramivir and oseltamivr, but not to zanamivir using the plaque reduction assay in MDCK cells. The recombinant H1N1 virus carrying the NA E119Q amino acid substitution demonstrated decreased susceptibility only to oseltamivir but not to peramivir or zanamivir (Abed et al., 2004). A recombinant variant virus with reduced susceptibility to peramivir due to the NA H275Y amino acid substitution resulted in the same resistance phenotype and replicative properties as the virus containing the NA H275Y amino acid substitution obtained after 18 passages in MDCK cells in the presence of peramivir (Baz et al., 2007).

An influenza A/H1N1/09 virus was passaged in cell culture in increasing concentrations of either zanamivir or a combination of zanamivir and oseltamivir. The NA enzyme inhibition assays showed that a recombinant A/H1N1/09 virus carrying the NA N146S amino acid substitution had an approximately 2-fold decrease in susceptibility for both oseltamivir and zanamivir, but not for peramivir, while the D198G NA amino acid substitution conferred a 10-fold reduction in susceptibility to oseltamivir, peramivir, and zanamivir. The clinical relevance of a 2-fold decrease in susceptibility in this assay is unclear. A recombinant A/H1N1/09 virus carrying the D198G and H275Y NA amino acid substitution was resistant to oseltamivir and peramivir, but not to zanamivir (McKimm-Breschkin et al., 2012).

The NA Y155H amino acid substitution identified in plaque purified virus from clinical isolate A1/Hokkaido/15/02 conferred reduced susceptibility in enzyme inhibition assays to peramivir (30-fold), as well

as to oseltamivir and zanamivir (>100-fold) compared with WT NA (McKimm-Breschkin et al., 2013). The isolate was identified during NAI susceptibility screening of global isolates through the Neuraminidase Inhibitor Susceptibility Network (Monto et al., 2006). One virus had both the NA Y155H amino acid substitution and an HA D225G amino acid substitution that rescued the small-plaque phenotype of the Y155H virus and reduced receptor binding and drug susceptibility in cell culture and binding assays. Another virus with NA VI14I and HA D225N amino acid substitutions affected susceptibility in the enzyme inhibition assay and receptor binding, respectively, but to lesser extents than the Y155H and D225G amino acid substitutions.

Clinical isolates with reduced susceptibility to peramivir have been reported among influenza A virus strains, including H1N1 and 2009/H1N1, as well as among influenza B virus strains (summarized in Table A6). Amino acid substitutions that were identified include NA Q136K and NA H275Y for viruses of the N1 NA subtypes, NA R292K for viruses of the N9 subtype, and NA R152K and NA D198E for influenza B viruses. The NA Q136K substitution was present only in viruses grown in MDCK cells but not in matching original clinical specimens which were available for testing, indicating that these variants were the result of cell culture selection or were present at a very low frequency in the clinically derived viruses. Of note, the HA G141E, D195N, and T197N amino acid substitutions developed in earlier passage (passage 3) before the strain developed the NA H275Y substitution (passage 15). This observation is consistent with an earlier report for zanamivir where the HA substitutions appeared before the NA substitutions (Blick et al., 1998). The influenza A/H3N2 HA K189 position is reported to be an antigenic site (Huang et al., 2012; Cai et al., 2014).

2.5. Activity of Peramivir in Lethal Mouse Models of Influenza against Viruses with Amino Acid Substitutions in Neuraminidase or Hemagglutinin (Study Number DD00020, DD00047, S-021812-E B-134-N, EPB09-1812-001, Abed et al., 2012)

Several studies were conducted in which mice were treated with peramivir before or at the time of viral inoculation with virus having amino acid substitutions in NA or HA proteins that confer reduced susceptibility to peramivir (Table A7). Routes of peramivir administration included oral, IV, and IM. In these studies, peramivir was active against viruses with the HA K189E or the NA H275Y amino acid substitutions. The activity of peramivir against the WT virus infection was better than that for the virus with NA H275Y amino acid substitution.

2.6. Cross-Resistance to Approved Drugs (Over 30 studies, see Table A6)

Several studies were conducted to evaluate the cross-resistance of peramivir to approved drugs (Table A6). The N1 NA H275Y substitution conferred cross resistance to oseltamivir but not zanamivir. The N2 NA R292K conferred cross resistance to both oseltamivir and zanamivir. The amino acid substitutions that conferred cross-resistance to oseltamivir and/or zanamivir are summarized in Table 4. Of note, there are substitutions that confer cross-resistance to oseltamivir but not zanamivir and others that confer cross-resistance to zanamivir but not oseltamivir.

Table 4: Oseltamivir and Zanamivir Resistance-Associated Substitutions with Cross-Resistance to Peramivir

	Type/ Subtype		
Protein	**A/H1N1**	**A/H3N2**	**B**
HA	D129S, R208K	N63K, N145D	-
NA	E119G/V, Q136K, D151G*/N*, Y155H, D198G, I222K/R*/V, S246N, H275Y, N294S	E119G/I*/V, T148I, D151A/E/G/N/V, I222V*, R292K*, N294S	E105K*, E116A/D/G/V, Q138R*, P139S*, G140R*, R149K*, D198E*/N*/Y, I222T/V, H275Y*, R292K, R371K

Red – cross resistant to oseltamivir

Blue – cross resistant to zanamivir
Purple – cross resistant to oseltamivir and zanamivir
*Amino acid substitution observed *in vivo*

2.7. Mapping of Peramivir Resistance-Associated Substitutions on the HA (b) (4) Structure.

Structural bioinformatics was employed to determine if resistance-associated amino acid substitutions that evolved in H1N1 and H3N2 influenza A viruses in cell culture in the presence of peramivir (Table 3) could potentially interfere with epitopes important for immunity, such as vaccine epitopes. To perform these analyses, the structures of the influenza A H1N1 (b) (4) and H3N2 (b) (4) HA proteins were downloaded from the protein databank (PDB) and analyzed using (b) (4) .

Antigenic sites related to vaccine epitopes have not been definitively defined for the influenza A and B virus strains that have been included in vaccine formulations. However, several publications have identified putative epitopes on the surface of the HA in the head domain and performed bioinformatics analyses, X-ray crystallography studies, and in some cases site-directed mutagenesis to determine that these sites are important for antibody recognition and binding. The antigenic sites identified for influenza A viruses H1N1 and H3N2 were mapped onto the appropriate structures and then the peramivir resistance-associated substitutions that arose for each flu type were also mapped onto the structures to determine if these amino acids substitutions occurred proximal to or within these putative epitopes. The resistance-associated substitutions for each of the HA types were as follows:

1. H1: D129S and R208K
2. H3: N63K, G78D, N145D, and K189E
3. Influenza B virus HA: T139N, G141E, R162M, D195N, T197N, and Y319H
4. ***NOTE:*** *All of these substitutions which confer reduced susceptibility to peramivir, were selected in cell culture and are not the result of host immune selective pressure. Most of these substitutions were selected in the absence of substitutions in NA (D129S and R208K in H1; N63K, G78D, N145D, and K189E in H3; G141E, D195N, T197N in influenza B virus).*

For the H1N1 influenza A virus, putative antigenic sites that were conserved between influenza A viruses H1N1-1918 and H1N1-2009 have been identified (Xu at al., 2010) based on comparison of HA amino acid sequences and HA structures from these related viruses. In this paper, the co-crystal structures of the influenza A virus H1N1-1918 HA in complex with 2D1, an antibody that was isolated from a survivor of the 1918 Spanish flu that neutralizes both influenza A viruses H1N1-1918 and H1N1-2009 was reported (Xu at al., 2010). Interestingly, the crystal structure of the hemagglutinin protein from influenza virus A/California/04/2009 H1N1 revealed that its antigenic structure, particularly within the Sa antigenic site, was nearly identical in structure and amino acid composition as the Sa antigenic sites of influenza A H1N1 viruses that circulated early in the 20th century. The epitope identified in this study was conserved in both pandemic viruses, and the authors speculated that the antigenic similarity between the 2009 and 1918-like influenza A viruses provides an explanation for the age-related immunity to observed for the most recent influenza A H1N1 virus pandemic.

Given the conservation of these antigenic sites among influenza A H1N1 viruses spanning 91 years, these antigenic sites were mapped onto the H1N1 HA structure to see if any peramivir resistance-associated substitutions mapped to these epitopes (Figure 2).

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Figure 2: Crystal Structure, Phylogeny, and Antigenic Variation in Influenza A 2009 H1N1 HA (from Xu at al., 2010).
(A) Phylogenetic tree of selected H1 HAs in swine and human. (B) Antigenic structure of CA04 HA from the 2009 H1N1 pandemic virus. A trimer complex is shown in surface representation with the antigenic sites highlighted: Sa site in magenta, Sb site, cyan; Ca site, orange; and Cb site, blue. Sa and Sb sites are located near the receptor-binding site. The Ca site straddles the subunit interface in the trimer. (C) Sequence alignment of membrane-distal domains from representative H1 HAs (38). Antigenic epitopes are color-coded as in (B).

The two peramivir resistance-associated substitutions that arose in H1 (D129S and R208K) occurred in the Sa and Ca antigenic sites, respectively (Figure 3). Mapping these epitopes and amino acid positions onto the H1 HA structure showed that the Sa antigenic site lies on the H1 HA on the external surface close to the receptor binding site, while the Ca antigenic site is internal and hidden from the surface. Moreover, the D129S substitution that maps to the surface of the H1 HA likely alters the binding site, particulary given that it occurs at a surface exposed residue and alters the charge of that residue from aspartic acid (D), which has a negative charge to serine (S), which is neutrally charged (Figure 4).

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Figure 3: Sites of Peramivir Resistance in the Antigenic Sites of H1N1 (alignment from Xu at al., 2010).
The green arrows indicate positions of peramivir resistance in H1N1, including position 129 in the Sa antigenic site and position 208 in the Ca antigenic site.

The resistance-associated substitution at position 129 (D129S) occurs on the surface of H1 in the Sa epitope, wherease observation of the R208K substitution required that the structure be rotated 60-degrees upward on the X-axis to reveal its relatively restricted position in the Cb epitope (Figure 4).

(b) (4)

Figure 4: Mapping of Peramivir Resistance on H1N1 HA (DAVP Analysis).
Top panel shows a top down view of the surface with green marking position 129. The bottom panel shows a side view of the H1N1 HA trimer (rotated 60 degrees upward) with K208 marked in green. Sa, magenta; Sb, cyan; Ca, orange; Cb, blue.

Both peramivir resistance-associated substitutions that arose in influenza A virus H1N1 alter predicted epitopes that have been implicated as important sites for antibody recognition and binding, indicating that it is possible that peramivir could select for mutant influenza A virus H1N1 strains that are less effectively neutralized by current vaccine strains or the memory immune response of those who have suffered from previous influenza A virus H1N1 infections.

For influenza A virus H3N2, there were four peramivir resistance-associated substitutions that arose in the H3 protein: N63K, G78D, N145D, and K189E. Five antigenic sites have been identified for influenza virus A H3N2, known as sites A-E and most of these are exposed on the HA surface near the sialic acid receptor binding site (Figure 5, Popova et al., 2012).

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Figure 5: Five Antigenic Sites of Influenza A Virus H3N2 (from Popova et al., 2012).
Five antigenic sites A–E are mapped on the HA1 surface of H3N2 influenza viruses. Antigenic site A (red color) and antigenic site B (blue color) are localized on the top of HA around the receptor binding pocket.

The five antigenic sites (A-E) were mapped on the influenza virus A H3N2 HA protein (b) (4) and then the four peramivir resistance-associated sites that arose in the H3 leading to reduced susceptilbity to peramivir were also mapped onto the structure to determine if these sites occurred proximal to or within these antigenic sites (Figure 6).

The four influenza A virus H3N2 peramivir resistance-associated substitutions mapped to three of the five conserved epitopes, with N63K and G78D mapping to the E antigenic site, N145D mapping to the immunodominant A antigenic site, and K189E mapping to the B antigenic site (Figure 6).

All four peramivir resistance-associated substitutions that arose in influenza A virus H3N2 alter predicted epitopes that have been implicated as important sites for antibody recognition and binding of the H3 HA, indicating that it is possible that peramivir could select for mutant influenza A virus H3N2 strains that are less effectively neutralized by current vaccine strains or the memory immune response of those who have suffered from previous influenza A virus H3N2 infections.

(b) (4)

Figure 6: Mapping of Peramivir Resistance on H3N2 HA (DAVP Analysis).
Top panel shows a top down view of the surface with yellow marking peramivir resistance-associated positions (b) (4), which map to the A and B antigenic sites, respectively. The bottom panel shows a side view of the H3N2 HA trimer (rotated 60 degrees upward) with peramivir resistance-associated positions (b) (4) shown in yellow and both mapping to the E antigenic site. A antigenic site, red; B antigenic site, blue; C antigenic site, green; D antigenic site, magenta; E antigenic site, cyan.

3. Clinical Virology

Clinical Virology analyses were conducted to evaluate the antiviral efficacy and virological resistance of IV or IM peramivir in subjects with uncomplicated influenza virus infection in 7 clinical trials: one pivotal non-IND Phase 2 study (0722T0621; i.v. formulation), two supportive IND Phase 2 and Phase 3 studies (BCX1812-211 and BCX1812-311; both i.m. formulation), as well as 4 other studies (BCX1812-212 [i.m. formulation], 0815T0631 [i.v. formulation], 0815T0632 [i.v. formulation], and 0815T0633 [i.v. formulation]). Additionally, there were 3 studies conducted in hospitalized subjects (BCX1812-201 [i.v. formulation], BCX1812-301 [i.v. formulation], and BCX1812-303 [i.v. formulation]) that are described in detail in the Appendix.

3.1. Limitations of the Influenza Diagnostic RAT Assays

The Division recommended that the sponsor enroll subjects based upon clinical symptoms and confirm the diagnosis with an RT-PCR assay. However, for most of the studies described above, the sponsor required for inclusion a positive rapid antigen test (RAT) for influenza virus. RAT assays are limited in sensitivity, especially for some viruses and some vulnerable populations. A study of influenza virus rapid-test results (using the QuickVue Influenza A+B test) among military service personnel and their dependents showed that the sensitivity of this rapid test for pandemic H1N1 influenza virus detection was 51% with a 95% confidence interval of 35% to 67% (Faix et al., 2009). The sensitivity of the test for seasonal H1N1 was 63% and for seasonal H3N2 31%, and the specificity of the test for each of the three viruses was 99%. However, earlier studies on seasonal influenza virus have reported a median sensitivity of 27% (range 19%-32%) for the same assay (Uyeki et al., 2009). Therefore, the Division typically recommends that sponsors enroll subjects based upon clinical criteria and follow up testing with a real-time RT-PCR assay and cell culture for confirmation of

influenza virus infection in pivotal studies (FDA Guidance for Industry – Influenza: Developing Drugs for Treatment and/or Prophylaxis [April 2011]). Concerns about the use of their diagnostic assays have been communicated to the sponsor including communications on 10/24/06 and 7/13/09.

The RAT assays usually have good specificity and positive predictive value, but poor sensitivity and negative predictive value (comparing to cell culture or RT-PCR assays). The sensitivity of the assays varies across populations, but it is higher in children than in adults and for influenza A virus infection than for influenza B virus infection (Chartrand et al., 2012) (Note: even for influenza A viruses, the sensitivity can vary). The sensitivity of RAT assays is generally better with higher viral shedding. The sponsor enrolled fewer subjects ≥65 years old despite having inclusion criteria that would have allowed such subjects in most of their studies. There were 13/624 [2.1%] who were ≥65 years old across studies 0722T0621, BCX1812-211/311, and BCX1812-212. There were 19/1099 [1.7%] who were ≥65 years old in study 0815T0631. The sensitivities of 4 RAT assays (BinaxNOW is FDA-approved) decreases with increasing patient age (Steininger et al., 2008). The estimated sensitivity for influenza A/H3N2 virus detection in nasopharyngeal swabs was 17-56% in children 1 year of age and decreased to 8-22% in patients 80 years of age (logistic regression). Additionally, Gooskens et al. (2008) demonstrated that in a nursing home setting a sensitivity of 38% for the Directigen RAT test (FDA-approved) using nasopharyngeal swabs. Overall, these results support a RAT assay bias against elderly patients and that enrolling based on the RAT assay may lead to under-enrollment of older patients. Considering that elderly subjects in nursing homes would be good candidates for the use of i.v. peramivir, it is unfortunate that the studies enrolled very few subjects in this age group to make any meaningful conclusions with respect to efficacy.
Inclusion criteria for studies:

0722T0621 (i.v.): Age: 20 years old - 65 years old.
0815T0631 (i.v.): Age ≥20 years.
BCX1812-211 (i.m.): Age ≥18 years.
BCX1812-311 (i.m.): Age ≥18 years.
BCX1812-212 (i.m.): Age ≥18 years.

The sponsor also enrolled fewer subjects infected with influenza B virus compared to surveillance data in 9 out of 11 sites (Table 5). The RAT assays that were used in their studies have less sensitivity against influenza B virus (Table 6) and **their use as inclusion criteria may have played a role in less influenza B virus infected subjects being enrolled.** Given that peramivir is less active against influenza B virus based on biochemical and cell culture data, it will be important to assess efficacy specifically in subjects infected with influenza B virus.

Table 5: Breakdown of Subjects Enrolled in the Studies by Influenza Virus Type/Subtype

Study	Study Dates	Study Location(s)	Circulating Influenza Virus Info (RT-PCR and/or Cell Culture)	Oseltamivir resistance Info	Rates Enrolled in Study
Shionogi Study 0722T0621	13Dec07-18Apr 08	Japan	**Japan:** Estimated 81.4% H1N1, 11.2% H3N2, 6.8% B	**Japan:** Oseltamivir resistance freq.= 1.6-2.6%, no H275Y identified	**Japan:** 73% H1N1 (215/296), 23% H3N2 (70/296),3% A (8/296), 1% B (3/296)

Study	Study Dates	Study Location(s)	Circulating Influenza Virus Info (RT-PCR and/or Cell Culture)	Oseltamivir resistance Info	Rates Enrolled in Study
0815T0631	15Nov08- 15May09	Japan, South Korea, Taiwan	**Japan:** Estimated 55% H1N1, 24% H3N2, 21% B **South Korea:** 64% H1N1, 34.8% H3N2, 1.3% B **Taiwan:** H1N1 was dominant (~80%)	**Japan:** 100% of H1N1 surveillance samples were oseltamivir resistant **South Korea:** 100% of H1N1 surveillance samples were oseltamivir resistant **Taiwan:** ~100% of H1N1 surveillance samples were oseltamivir resistant	**Japan:** 56% H1N1 (431/769), 26% H3N2 (200/769), 5% A (40/769), 9% B (69/769) **South Korea:** 16% H1N1 (17/105), 70% H3N2 (73/105), 7% A (7/105), 0% B (0/105), +8 Unknown **Taiwan:** 67% H1N1 (168/252), 26% H3N2 (65/252), 4% A (11/252), 1% B (3/252)
BCX1812-211	23Jan07-05Sep07	US, Canada, UK, Australia, Hong Kong, New Zealand, South Africa	**US:** 49% H1N1, 30% H3N2. 21% B **New Zealand:** 31.1% H1N1, 43.7% H3N2, 25.2% B **Australia:** 34.4% H1N1, 58.7% H3N2, 6.9% B **South Africa** had mainly A, equal H1N1 and H3N2 circulation	**US:** 0.7% of H1N1 had Oseltamivir resistance **Australia:** est. 1% oseltamivir resistance	**US:** 23% H1N1 (23/99), 47% H3N2 (47/99), 27% B (27/99) **New Zealand:** 33% H1N1 (11/34), 53% H3N2 (18/34), 14%, B (5/34) **Australia:** 28% H1N1 (24/85), 65% H3N2 (55/85), 3.5% A (3/85), 3.5% B (3/85) **South Africa:** 36% H1N1 (25/69), 32% H3N2 (22/69), 3% A (2/69), 29% B (20/69)
BCX1812-311	07Jan08-15Feb08	US	**US:** 18% H1N1, 53% H3N2, 29% B	**US:** ~11% of US H1 viruses were Osel. Resistant. Global rise in Oseltamivir resistance in 2007-2008 flu season (due to H275Y mutation). All H3N2 and flu B were oseltamivir sensitive	**US:** 26% H1N1 (80/314), 61% H3N2 (190/314), 13% B (40/314)
BCX1812-212	02Jul08-02Apr09	US (N~161), Australia (N~19), New Zealand (N~26), South Africa (N~128)	**US:** 57% H1N1, 9% H3N2, 34% B **Australia:** 11.8% H1N1, 21.7% H3N2, 66.5% B **New Zealand:** 0.6% H1N1, 30.8% H3N2, 59.8% B **South Africa:** Season was mainly H1N1	**US:** 99.4% of surveillance sample H1N1 resistant to Oseltamivir, all H3N2 and flu B were Osel.-sensitive **Australia:** 93% of H1N1 isolates tested were Oseltamivir resistant **New Zealand:** 2/2 H1N1 samples were Oseltamivir resistant **South Africa:** 100% of H1N1 surveillance isolates tested positive for oseltamivir resistance	**US:** 8% H1N1 (13/160), 86% H1N1 H275Y (137/160), 6% H3N2 (10/160), 0% B (0/160) **Australia:** 5% H1N1 (1/20), 15% H1N1 H275Y (3/20), 55% H3N2 (11/20), 25% B (5/20) **New Zealand:** 0% H1N1 (0/26), 0% H1N1 H275Y (0/26), 38% H3N2 (10/26), 62% B (16/26) **South Africa:** 5% H1N1 (14/291), 79% H1N1 H275Y (230/291), 5% H3N2 (15/291), 11% B (31/291)

(Source: Kawai et al., 2009; Ujike et al., 2010; Kawakami et al., 2008; Furuse et al., 2009; Tashiro et al., 2009; Sheu et al., 2008; Baranovich et al., 2010; Japan WHO report, Sep07-Mar08; Japan CDC flu subtype data 1997-2008; CDC 2006-07 US and worldwide flu season summary; CDC MMWR: US and Worldwide influenza activity May-Sep 2007; CDC 06-07 US Oseltamivir resistance data; Australia Annual report 2007; New Zealand Annual Report 2007; South Africa IAV/IBV Data 2006-2010; CDC 2007-08 US flu season summary; Global map of Oseltamivir-resistant H1N1 1July08; CDC 2008-09 US flu season summary; WHO 2008/2009 influenza report: Northern hemisphere; CDC MMWR US and Worldwide, May-Sep 2008; WHO 2008

influenza summary: southern hemisphere; Australia Annual Report, 2008; New Zealand Annual Report 2008; South Africa IAV/IBV Data 2006-2010; Taiwan CDC 2008-2009 summary; WHO 2008/2009 influenza report: Northern hemisphere; Yang et al., 2011; Choi et al., 2011)

Table 6: RAT Assay Used and their Performance Characteristics

Study Number	Rapid Antigen Test Products Provided to Sites by Biocryst	Performance Information
(Shionogi Study 0722T0621)	Fujirebio ESPLINE® Influenza A&B-N Capilia® Flu A+B Rapid Testa® Flu Stick Rapid Testa® Flu II	**ESPLINE Influenza A&B-N:** Sensitivity: A-96.8%, B-87.9% – Nasal Swab A-85.3%, B-71.6% – Nasopharyngeal swab Specificity: A-97.4%%, B-99.4% – Nasal Swab A-100%, B-99.2% – Nasopharyngeal swab **Capilia Flu A+B:** Sensitivity: A-94%, B-100% – Nasal swab A-87%, B-92% – Pharyngeal swab Specificity: A-95%, B-99% – Nasal swab A-95%, B-99% – Pharyngeal swab **Rapid Testa Flu Stick** Sensitivity: A-94.3%, B-85.2% – Nasal swab Specificity: A-100%, B-100% – Nasal swab **Rapid Testa Flu II** Sensitivity: A-94.3%, B-87% – Nasal swab Specificity: A-96.8%, B-100% – Nasal swab
(Shionogi Study 0815T0631)	Same as Study 0722T0621	

Study Number	Rapid Antigen Test Products Provided to Sites by Biocryst	Performance Information
BCX-1812-201, BCX-1812-211, BCX-1812-301, BCX-1812-303	Quidel QuickVue® Influenza A+B test (all sites)	Sensitivity: A-94%, B-70% – Nasal swab A-83%, B-62% – Nasopharyngeal swab Specificity: A-90%, B-97% – Nasal swab A-89%, B-98% – Nasopharyngeal swab
BCX-1812-311	. Quidel QuickVue Influenza A+B test (all sites) . Alere BinaxNOW® Influenza A+B test (all sites) Note: Sites were instructed to use the QuickVue test, and were permitted to repeat the test with the BinexNOW test if the QuickVue test was negative. The actual test used as the basis for confirming eligibility for each subject was not recorded.	Quidel QuickVue Influenza A+B: See above BinaxNOW Influenza A & B: Sensitivity: A-70%–89%, B-50%–69% Specificity: A-90%–99%, B-94%–100%
BCX-1812-212	Quidel QuickVue Influenza A+B test (all sites)	Sensitivity: A-94%, B-70% – Nasal swab A-83%, B-62% – Nasopharyngeal swab Specificity: A-90%, B-97% – Nasal swab A-89%, B-98% – Nasopharyngeal swab

3.2. Study 0722T0621

This study was a Phase 2, double-blind, placebo-controlled, parallel group study of single-dose intravenous peramivir in subjects with acute uncomplicated influenza. This study was the pivotal study. This study enrolled 300 subjects at 75 study sites in Japan. The influenza A/H1N1 virus with NA H275Y substitution was not circulating during the study. Randomized and treated subjects received a single intravenous dose of peramivir 300 mg (n = 99), peramivir 600 mg (n = 97), or placebo (n = 100), administered by intravenous infusion over 30 minutes. Efficacy evaluations included subject self-assessments of body temperature, severity of influenza symptoms, and ability to perform daily activities. All assessments were recorded in a diary provided to the subject. Other assessments included virologic tests (virus subtypes [A-H1/H3/H5, B], viral titer [$TCID_{50}$ value], and NA-inhibitory activity [IC_{50} value]); CRP, IL-6 and TNF-α sampling; serum antibody titration (serum antibodies against influenza virus types A and B); and influenza-related complications (sinusitis, otitis media, bronchitis, and pneumonia).

The primary endpoint of this study was the time to alleviation of symptoms (time to disappearance of influenza symptoms/duration of influenza), defined as the time from the start of investigational product dosing to the time point at which all of the 7 influenza symptoms (cough, sore throat, headache, nasal congestion, feverishness or chills, aches or pains of the muscle or joints, and fatigue) as recorded in the Subject Diary showed a score of "0: none" or "1: mild," and when this condition persisted for at least 21.5 hours (24 hours - 10%).

This study met its primary endpoint of time to alleviation of symptoms. The combined peramivir group (300 mg and 600 mg) showed a significant shortening of the time to alleviation of symptoms (one-sided P value: 0.0010) in comparison to the placebo group (please see the review of the statistical reviewer). The time to alleviation of symptoms was analyzed based on influenza virus type/subtype (Table 7). The median time to alleviation of symptoms in subjects infected with influenza A/H1N1 virus was 52.02 hours (n=74), 61.83 hours (n=69), and 81.44 hours (n=72) in the 300 mg group, 600 mg group, and placebo group, respectively. The median time to alleviation of symptoms in subjects infected with influenza A/H3N2 virus was 76.10 hours (n=21), 50.45 hours (n=25), and 81.00 hours (n=24) in the 300 mg group, 600 mg group, and placebo group, respectively. The median time to alleviation of symptoms in subjects infected with influenza B virus was 104.63 hours (n=2) and 65.58 hours (n=1) in the 300 mg group and 600 mg group, respectively. There were no subjects in the placebo arm who were infected with influenza B virus. Additionally, for influenza A/H3N2 virus, there was a trend towards better outcome at the 600 mg dose.

Table 7: Time to Alleviation of Symptoms and Baseline NA Activity

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
Time to Onset of Symptoms		Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)
H1N1													
0 to 24 hours		N=47				N=37				N=32			
	Median	46.00	1.24	2 02	3.15	60.90	1.19	2 04	3.28	81.44	1.19	2.07	3 26
	Average	78.97	1.50	2 67	4.24	89.81	1.36	2 28	3.61	91 80	1.48	2.43	3 86
24+ to 48 hours		N=27				N=32				N=40			
	Median	57.08	1.10	1 80	3.00	64.46	0.98	1.71	2.53	80.48	1.15	2.01	3.10
	Average	84.81	1.24	2 08	3.35	87.97	1.56	3 97	3.05	112.75	1.31	2.12	3 38
Total		N=74				N=69				N=72			
	Median	52.02	1.18	1 86	3.06	61.83	1.12	1 96	2.96	81.44	1.18	2.01	3 20
	Average	81.10 (P<0.003)	1.39	2.43	3.88	88.96 (P=0.13)	1.45	3 04	3.36	103.44	1.38	2.25	3 59
H3N2													

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
Time to Onset of Symptoms		Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)
0 to 24 hours		N=9				N=11				N=13			
	Median	66.83	1.21	0 87	2.95	44.03	1.35	0 97	3.21	169.50	1.44	1.06	3 53
	Average	97.52	1.46	1 06	3.23	48.57	1.47	1 06	3.54	162.64	1.41	0.99	3 35
24+ to 48 hours		N=12				N=14				N=11			
	Median	76.79	1.35	1 02	3.31	61.82	1.42	1 00	3.49	67 33	1.35	1.00	3.19
	Average	73.71	1.40	0 98	3.27	70.70	1.60	1.13	3.67	83 32	1.55	1.20	3 55
Total		N=21				N=25				N=24			
	Median	76.10	1.34	0 98	3.26	50.45	1.39	0 98	3.44	81 00	1.36	1.03	3 37
	Average	83 92 (P<0 04)	1.42	1 02	3.26	60 96 (P<0.001)	1.54	1.10	3.61	126.29	1.47	1.09	3.44
A													
0 to 24 hours		N=2				N=2				N=3			
	Median	96.16	1.32	2 37	3.82	44.88	1.14	1.49	2.82	126.00	1.00	1.35	2.48
	Average	96.16	1.32	2 37	3.82	44.88	1.14	1.49	2.82	168.42	1.00	1.35	2.48
24+ to 48 hours										N=1			
	Median									315.35			
	Average									315.35			
Total		N=2				N=2				N=4			
	Median	96.16	1.32	2 37	3.82	44.88	1.14	1.49	2.82	220.68	1.00	1.35	2.48
	Average	96.16	1.32	2 37	3.82	44.88	1.14	1.49	2.82	205.15	1.00	1.35	2.48
B													
0 to 24 hours		N=1				N=1							
	Median	104 02	3.00	8 94	9.92	65.58	2.81	7 31	8.66				
	Average	104 02	3.00	8 94	9.92	65.58	2.81	7 31	8.66				
24+ to 48 hours		N=1											
	Median	105 25	2.62	8 26	8.56								
	Average	105 25	2.62	8 26	8.56								
Total		N=2				N=1							
	Median	104 63	2.81	8 60	9.24	65.58	2.81	7 31	8.66				
	Average	104 63	2.81	8 60	9.24	65.58	2.81	7 31	8.66				

The median change in virus titer (log_{10} $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -3.00 log_{10} $TCID_{50}$/mL, -2.85 log_{10} $TCID_{50}$/mL, and -2.70 log_{10} $TCID_{50}$/mL in the 300 mg group, 600 mg group, and placebo group, respectively (Table 8). However, the differences in the means between the treatment arms and placebo arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was higher in both the 300 mg (42/74, 56.8%) and 600 mg (49/69, 71.0%) groups than in the placebo group (34/72, 47.2%). The difference between the 600 mg group and placebo was statistically significant (P <0.004). The median change in virus titer from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -2.00 log_{10} $TCID_{50}$/mL, -2.30 log_{10} $TCID_{50}$/mL, and -1.85 log_{10} $TCID_{50}$/mL in the 300 mg group, 600 mg group, and placebo group, respectively. The difference in the means between the 600 mg group and placebo was statistically significant (P <0.02). The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was higher in both the 300 mg (18/21, 85.7%) and 600 mg (21/25, 84.0%) groups than in the placebo group (13/24, 54.2%). The difference between the 300 mg group (P <0.02), 600 mg group (P <0.02), and placebo were statistically

significant. The median change in virus titer from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was -2.00 log_{10} $TCID_{50}$/mL and -4.00 log_{10} $TCID_{50}$/mL in the 300 mg group and 600 mg group, respectively. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) in the 300 mg and 600 mg group was 1/2 (50%) and 1/1 (100%), respectively. Few subjects in any of the treatment groups had detectable virus on Visit 5 (Day 9).

Table 8: Viral Load ($TCID_{50}$)

Time to Onset of Symptoms		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		$TCID_{50}$ (log_{10}/ml)											
		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9
H1N1													
0 to 24 hours		N=47				N=37				N=32			
	Median	4 80	-3.00	-3.70	-3.70	4 80	-3.00	-3.70	-3.70	4.40	-2.30	-3.15	-3.30
	Average	4 64	-2.70	-3 38	-3.53	4.74	-3.06	-3 36	-3.61	4.41	-2.31	-3.14	-3.31
	# Subjects with Undetectable	0 (0%)	20 (42.6%)	40 (85.1%)	46 (97.9%)	0 (0%)	23 (62 2%)	32 (86.5%)	37 (100%)	0 (0%)	12 (37.5%)	28 (87.5%)	32 (100%)
24+ to 48 hours		N=27				N=32				N=40			
	Median	4.10	-2.70	-3 00	-3.00	4.10	-2.30	-2.70	-3.00	4.40	-2.85	-3.30	-3.30
	Average	4 22	-2.76	-3 03	-3.12	4.18	-2.76	-2 95	-3.05	4.49	-2.76	-3.32	-3.39
	# Subjects with Undetectable	0 (0%)	22 (81.5%)	26 (96.3%)	27 (100%)	0 (0%)	26 (81 3%)	29 (90.6%)	31 (100%)	0 (0%)	22 (55%)	34 (85%)	39 (97.5%)
Total		N=74				N=69				N=72			
	Median	4 80	-3.00	-3 30	-3.70	4.40	-2.85	-3.15	-3.30	4.40	-2.70	-3.30	-3.30
	Average	4.49	-2.72 (P=0.26)	-3 25	-3.38	4.48	-2.93 (P=0 09)	-3.17	-3.35	4.46	-2.56	-3.24	-3.36
	# Subjects with Undetectable	0 (0%)	42 (56.8%) (P=0 2)	66 (89.2%)	73 (98.6%)	0 (0%)	49 (71%) (P<0.004)	61 (88.4%)	68 (98.6%)	0 (0%)	34 (47.2%)	62 (86.1%)	71 (98.6%)
H3N2													
0 to 24 hours		N=9				N=11				N=13			
	Median	3.40	-2.30	-2 30	-2.30	3.10	-2.00	-2 00	-2.00	3.40	-1.40	-2.30	-2.30
	Average	3 32	-1.86	-2 03	-2.22	3.46	-2.35	-2 35	-2.26	3 35	-1.45	-2.09	-2.25
	# Subjects with Undetectable	0 (0%)	7 (77.8%)	8 (88.9%)	9 (100%)	0 (0%)	11 (100%)	11 (100%)	10 (+1 NA) (100%)	0 (0%)	5 (38.5%)	11 (84.6%)	13 (100%)
24+ to 48 hours		N=12				N=14				N=11			
	Median	2 80	-1.50	-1.70	-1.70	3 60	-2.50	-2 50	-2.50	4.10	-2.00	-3.00	-3.00
	Average	2.72	-1.45	-1 62	-1.62	3 64	-2.40	-2 54	-2.54	3 52	-1.96	-2.35	-2.42
	# Subjects with Undetectable	0 (0%)	11 (91.7%)	12 (100%)	12 (100%)	0 (0%)	10 (71.4%)	14 (100%)	14 (100%)	0 (0%)	8 (72.7%)	10 (90.9%)	11 (100%)
Total		N=21				N=25				N=24			
	Median	3.10	-2.00	-2 00	-2.00	3.40	-2.30	-2 30	-2.30	3.40	-1.85	-2.30	-2.30
	Average	2 98	-1.62 (P=0.43)	-1 80	-1.88	3 56	-2.38 (P<0 02)	-2.46	-2.43	3.43	-1.69	-2.21	-2.33
	# Subjects with Undetectable	0 (0%)	18 (85.7%) (P<0.02)	20 (95.2%)	21 (100%)	0 (0%)	21 (84%) (P<0 02)	25 (100%)	24 (+1 NA) (100%)	0 (0%)	13 (54.2%)	21 (87.5%)	24 (100%)
A													
0 to 12 hours		N=2				N=2				N=3			
	Median	3.75	-2.65	-2 65	-2.65	1 60	-0.50	0.15	-0.50	4.10	-1.40	-3.00	-3.00
	Average	3.75	-2.65	-2 65	-2.65	1 60	-0.50	0.15	-0.50	4.10	-1.90	-3.00	-3.00
	# Subjects with Undetectable	0 (0%)	2 (100%)	2 (100%	2 (100%)	0 (0%)	2 (100%)	1 (50%)	2 (100%)	0 (0%)	1 (33%)	3 (100%)	3 (100%)
24+ to 48 hours										N=1			
	Median									5.40	-4.00	-4.30	-4.30
	Average									5.40	-4.00	-4.30	-4.30

Time to Onset of Symptoms		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		$TCID_{50}$ (log_{10}/ml)											
		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9
	# Subjects with Undetectable									0 (0%)	0 (0%)	1 (100%)	1 (100%)
Total		N=2				N=2				N=4			
	Median	3.75	-2.65	-2 65	-2.65	1 60	-0.50	0.15	-0.50	4.45	-2.20	-3.35	-3.35
	Average	3.75	-2.65	-2 65	-2.65	1 60	-0.50	0.15	-0.50	4.43	-2.43	-3.33	-3.33
	# Subjects with Undetectable	0 (0%)	2 (100%)	2 (100%)	2 (100%)	0 (0%)	2 (100%)	1 (50%)	2 (100%)	0 (0%)	1 (25%)	4 (100%)	4 (100%)
B													
0 to 24 hours		N=1				N=1							
	Median	2.10	-1.00	0 30	-1.00	5.10	-4.00	-4 00	-4.00				
	Average	2.10	-1.00	0 30	-1.00	5.10	-4.00	-4 00	-4.00				
	# Subjects with Undetectable	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)				
24+ to 48 hours		N=1											
	Median	5.10	-3.00	-4 00	-4.00								
	Average	5.10	-3.00	-4 00	-4.00								
	# Subjects with Undetectable	0 (0%)	0 (0%)	1 (100%)	1 (100%)								
Total		N=2				N=1							
	Median	3 60	-2.00	-1 85	-2.50	5.10	-4.00	-4 00	-4.00				
	Average	3 60	-2.00	-1 85	-2.50	5.10	-4.00	-4 00	-4.00				
	# Subjects with Undetectable	0 (0%)	1 (50%)	1 (50%)	2 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)				

Several investigators have tried to establish a correlation between cytokines and disease during influenza. Several independent studies documented an early rise of IFN-α, TNF-α, IL-1 α and β and IL-6 in bronchoalveolar lavage (BAL) fluids or lung homogenates, in temporal association with symptom formation and lung pathology (Vacheron et al., 1990; Hennet et al., 1992; Peper and Van Campen, 1995; Kurokawa et al., 1996). In humans, nasal lavage fluids were shown to contain elevated levels of (pro)inflammatory cytokines and chemokines, such as IFN- α, IL-6, TNF- α, IL-8, MIP-1 α, MIP-1β and MCP-1 (Gentile et al., 1998; Hayden et al., 1998; Fritz et al., 1999; Skoner et al., 1999). The sponsor looked at several biomarkers for inflammation.

The median change in C-Reactive Protein (CRP) levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -0.23 mg/L, -0.17 mg/L, and -0.14 mg/L in the 300 mg group, 600 mg group, and placebo group, respectively (Table 9). The differences in the means between the treatment arms and placebo arm were not statistically significant. The median change in CRP levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -0.41 mg/L, -0.38 mg/L, and -0.01 mg/L in the 300 mg group, 600 mg group, and placebo group, respectively. The differences in the means between the treatment arms and placebo arm were not statistically significant. The median change in CRP levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was 0.44 mg/L and -0.07 mg/L in the 300 mg group and 600 mg group, respectively.

Table 9: CRP

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		CRP (mg/L)											
Time to Onset of Symptoms		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14
H1N1													
0 to 24 hours		N=47				N=37				N=32			

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		CRP (mg/L)											
Time to Onset of Symptoms		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14
	Median	0.93	-0.08	-0.57	-0.88	0.77	-0.12	-0.58	-0.72	1.17	-0.16	-0.83	-1.06
	Average	1.49	-0.10	-0.95	-1.43	1 60	0.05	-1.05	-1 51	1.25	-0.29	-0.91	-1.04
24+ to 48 hours		N=27				N=32				N=40			
	Median	0.97	-0.32	-0.73	-0.80	1 66	-0.23	-1.11	-1 55	1.18	-0.11	-0.50	-1.07
	Average	1.40	-0.52	-1.14	-1.28	1 96	-0.64	-1.58	-1 85	1.63	-0.09	-0.41	-1.47
Total		N=74				N=69				N=72			
	Median	0.93	-0.23	-0.63	-0.88	1.11	-0.17	-0.86	-0 93	1.18	-0.14	-0.59	-1.07
	Average	1.46	-0.25	-1.02	-1.37	1.77	-0.27	-1.29	-1 67	1.46	-0.18	-0.63	-1.28
H3N2													
0 to 24 hours		N=9				N=11				N=13			
	Median	1.46	0.05	-0.88	-1.41	1.19	0.34	-0.33	-0 95	1.13	-0.29	-0.70	-0.82
	Average	2.27	0.03	-1.25	-2.21	1.48	-0.04	-0.55	-1.44	2.19	-0.24	-0.94	-1.81
24+ to 48 hours		N=12				N=14				N=11			
	Median	1.72	-1.00	-1.51	-1.67	1.77	-0.54	-1.23	-1 63	1.03	0.17	-0.44	-0.98
	Average	2.33	-1.18	-2.03	-2.25	2 84	-1.10	-2.20	-2 67	1.41	0.87	-0.59	-1.33
Total		N=21				N=25				N=24			
	Median	1.57	-0.41	-1.02	-1.52	1 35	-0.38	-1.09	-1 33	1.08	-0.01	-0.61	-0.91
	Average	2.30	-0.66	-1.70	-2.23	2 24	-0.63	-1.47	-2.16	1.83	0.27	-0.78	-1.59
A													
0 to 24 hours		N=2				N=2				N=3			
	Median	2.64	-1.45	-2.34	-2.59	1.17	-0.64	-0.94	-1.11	1.40	-0.33	-0.89	-0.68
	Average	2.64	-1.45	-2.34	-2.59	1.17	-0.64	-0.94	-1.11	1.22	-0.16	-0.60	-0.77
24+ to 48 hours										N=1			
	Median									1.55	-0.40	-1.25	-1.50
	Average									1.55	-0.40	-1.25	-1.50
Total		N=2				N=2				N=4			
	Median	2.64	-1.45	-2.34	-2.59	1.17	-0.64	-0.94	-1.11	1.47	-0.37	-0.90	-0.95
	Average	2.64	-1.45	-2.34	-2.59	1.17	-0.64	-0.94	-1.11	1.31	-0.22	-0.76	-0.95
B													
0 to 24 hours		N=1				N=1							
	Median	0.21	1.05	0.17	-0.16	0 20	-0.07	-0.15	-0.15				
	Average	0.21	1.05	0.17	-0.16	0 20	-0.07	-0.15	-0.15				
24+ to 48 hours		N=1											
	Median	0.25	-0.17	-0.20	-0.20								
	Average	0.25	-0.17	-0.20	-0.20								
Total		N=2				N=1							
	Median	0.23	0.44	-0.02	-0.18	0 20	-0.07	-0.15	-0.15				
	Average	0.23	0.44	-0.02	-0.18	0 20	-0.07	-0.15	-0.15				

The median change in IL-6 levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -8.48 pg/mL, -9.94 pg/mL, and -6.76 pg/mL in the 300 mg group, 600 mg group, and placebo group, respectively (Table 10). The difference in the means between the 600 mg group and placebo was statistically significant (P <0.05). The median change in IL-6 levels from the time of Screening up

to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -11.75 pg/mL, -12.45 pg/mL, and -4.85 pg/mL in the 300 mg group, 600 mg group, and placebo group, respectively. The differences in the means between the treatment arms and placebo arm were not statistically significant. The median change in IL-6 levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was 3.15 pg/mL and -2.6 pg/mL in the 300 mg group and 600 mg group, respectively.

Table 10: IL-6

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		IL-6 (pg/mL)											
Time to Onset of Symptoms		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14
H1N1													
0 to 24 hours		N=47				N=37				N=32			
	Median	12 87	-8.86	-10.45	-12 26	11 94	-10.25	-10.31	-10.98	12.19	-7 32	-9.34	-9 33
	Average	15 08	-11.41	-12 50	-14.10	50 50	-46.37	-47.76	-48.93	12.48	-8 09	-10 53	-10.79
24+ to 48 hours		N=27				N=32				N=40			
	Median	9.66	-7.50	-7.16	-8.65	11 61	-9 63	-10.69	-11.01	12.27	-6 20	-8.92	-10.83
	Average	13 35	-10.72	-11 32	-12.14	63 63	-45.01	-50.34	-52.41	16.44	-9 06	-10.71	-14.36
Total		N=74				N=69				N=72			
	Median	11 36	-8.48	-9.47	-10 63	11 86	-9 94	-10.50	-10.98	12.27	-6.76	-9.14	-10.65
	Average	14.45	-11.15	-12 06	-13 38	56 59	-45.75	-48.94	-50.54	14.68	-8 63	-10 63	-12.77
H3N2													
0 to 24 hours		N=9				N=11				N=13			
	Median	16.78	-15.87	-15 94	-16.14	13.43	-11.75	-11.70	-11.55	13.02	-6 58	-10 34	-10.47
	Average	22.49	-20.32	-20.74	-21.74	15 62	-12.71	-13.57	-11.93	19.13	-10.96	-15 28	-16.02
24+ to 48 hours		N=12				N=14				N=11			
	Median	13 24	-9.21	-11 39	-10 87	17.72	-14.61	-11.96	-15.22	14.96	-3.76	-11.18	-11.31
	Average	15 68	-12.66	-14.42	-14 52	18 32	-14.74	-14.42	-16.86	19.63	-7 97	-14.76	-17.53
Total		N=21				N=25				N=24			
	Median	14 06	-11.75	-12 95	-12.77	14.40	-12.45	-11.70	-12.41	13.22	-4 85	-10.76	-10.89
	Average	18 60	-15.94	-17.13	-17 62	17.13	-13.85	-14.05	-14.80	19.36	-9 59	-15 04	-16.71
A													
0 to 24 hours		N=2				N=2				N=3			
	Median	11 54	-9.31	-8.79	-10 97	8.02	-6 95	-3.79	-7.08	15.98	-7 99	-14 02	-15.43
	Average	11 54	-9.31	-8.79	-10 97	8.02	-6 95	-3.79	-7.08	17.46	-7 84	-13 30	-12.66
24+ to 48 hours										N=1			
	Median									12.14	-10.26	-11 58	-11.68
	Average									12.14	-10.26	-11 58	-11.68
Total		N=2				N=2				N=4			
	Median	11 54	-9.31	-8.79	-10 97	8.02	-6 95	-3.79	-7.08	14.06	-9.13	-12 80	-13.56
	Average	11 54	-9.31	-8.79	-10 97	8.02	-6 95	-3.79	-7.08	16.13	-8.45	-12 87	-12.41
B													
0 to 24 hours		N=1				N=1							
	Median	3.41	5.84	-2.18	-3.16	4.04	-2 60	-3.01	-3.38				
	Average	3.41	5.84	-2.18	-3.16	4.04	-2 60	-3.01	-3.38				
24+ to 48 hours		N=1											
	Median	1.95	0.45	-1.57	-1.62								

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		IL-6 (pg/mL)											
Time to Onset of Symptoms		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14
	Average	1.95	0.45	-1.57	-1.62								
Total		N=2				N=1							
	Median	2.68	3.15	-1.88	-2.39	4.04	-2 60	-3.01	-3.38				
	Average	2.68	3.15	-1.88	-2.39	4.04	-2 60	-3.01	-3.38				

The median change in TNF-α levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was 0.00 pg/mL, 0.00 pg/mL, and 0.00 pg/mL in the 300 mg group, 600 mg group, and placebo group, respectively (Table 11). The differences in the means between the treatment arms and placebo arm were not statistically significant. The median change in TNF-α levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -0.20 pg/mL, 0.00 pg/mL, and 0.05 pg/mL in the 300 mg group, 600 mg group, and placebo group, respectively. The difference in the means between the 300 mg group and placebo was statistically significant (P <0.01). The median change in TNF-α levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was 0.10 pg/mL and -0.20 pg/mL in the 300 mg group and 600 mg group, respectively.

Table 11: TNF-α

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		TNF-α (pg/mL)											
Time to Onset of Symptoms		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14
H1N1													
0 to 24 hours		N=47				N=37				N=32			
	Median	1.60	-0 05	-0 35	-0.35	1.40	0 00	-0.20	-0.40	1.60	0 00	-0.10	-0.40
	Average	1.64	-0 04	-0 38	-0.35	1.56	0 02	-0.27	-0.38	1.73	0 06	-0.26	-0.41
24+ to 48 hours		N=27				N=32				N=40			
	Median	1.70	0 00	-0.40	-0.40	1.75	-0.10	-0.40	-0.20	1.70	0 05	-0.15	-0.45
	Average	1.80	-0 09	-0 38	-0.34	1.83	-0 01	-0.34	-0.31	1.82	0 02	-0.16	-0.43
Total		N=74				N=69				N=72			
	Median	1.60	0.00	-0.40	-0.40	1.50	0.00	-0 30	-0 30	1.70	0.00	-0.10	-0.40
	Average	1.70	-0.06	-0.38	-0 35	1.68	0.01	-0 30	-0 34	1.78	0.04	-0 21	-0.42
H3N2													
0 to 24 hours		N=9				N=11				N=13			
	Median	1.40	0 00	-0 20	-0.40	1.60	-0.10	-0.40	-0.40	1.80	0 00	-0.30	-0.40
	Average	1.52	-0 02	-0.17	-0.33	1.71	-0.15	-0.41	-0.41	2.05	0 09	-0.23	-0.48
24+ to 48 hours		N=12				N=14				N=11			
	Median	1.95	-0 30	-0 55	-0.65	1.80	0.15	-0.40	-0.30	1.70	0.10	-0.10	-0.40
	Average	1.93	-0 38	-0 67	-0.57	1.94	0 04	-0.26	-0.27	1.70	0.15	-0.06	-0 25
Total		N=21				N=25				N=24			
	Median	1.60	-0.20	-0.40	-0 50	1.70	0.00	-0.40	-0 35	1.75	0.05	-0 20	-0.40
	Average	1.76	-0.22	-0.45	-0.47	1.84	-0.04	-0 32	-0 33	1.89	0.12	-0.15	-0.38
A													
0 to 24 hours		N=2				N=2				N=3			
	Median	1.80	-0 35	-0 60	-0.40	1.95	-0.75	-0.75	-1.00	2.50	-0.40	-0.20	-0.70
	Average	1.80	-0 35	-0 60	-0.40	1.95	-0.75	-0.75	-1.00	2.43	-0.43	-0.27	-0 53

		Peramivir 300 mg				Peramivir 600 mg				Placebo			
		TNF-α (pg/mL)											
Time to Onset of Symptoms		BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14	BL	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 14
24+ to 48 hours										N=1			
	Median									1.40	0.00	-0 30	-0.40
	Average									1.40	0.00	-0 30	-0.40
Total		N=2				N=2				N=4			
	Median	1.80	-0.35	-0.60	-0.40	1.95	-0.75	-0.75	-1 00	2.30	-0.30	-0 25	-0.55
	Average	1.80	-0.35	-0.60	-0.40	1.95	-0.75	-0.75	-1 00	2.18	-0.33	-0 28	-0.50
B													
0 to 24 hours		N=1				N=1							
	Median	1.70	0 20	0 20	0.00	2.30	-0 20	-0.50	-0.50				
	Average	1.70	0 20	0 20	0.00	2.30	-0 20	-0.50	-0.50				
24+ to 48 hours		N=1											
	Median	0.70	0.00	0.00	-0 20								
	Average	0.70	0.00	0.00	-0 20								
Total		N=2				N=1							
	Median	1.20	0.10	0.10	–0.10	2.30	-0.20	-0 50	-0 50				
	Average	1.20	0.10	0.10	–0.10	2.30	-0.20	-0 50	-0 50				

Overall, for study 0722T0621, there was a statistically significant benefit in time to alleviation of symptoms, change in viral shedding from baseline at day 3, and number of subjects not shedding virus at day 3 for influenza A/H3N2 virus. A dose/response in symptom relief was not seen for influenza A/H1N1 virus despite a significant reduction in viral shedding. There were only 3 subjects infected with influenza B virus.

3.3. Studies BCX1812-211/311 Combined

Results from Studies BCX1812-211 and BCX1812-311 were combined by the sponsor in a post hoc analysis due to the similarity of study design, endpoints, treatment regimens, and subject population.

Study BCX1812-211 was a Phase 2 multinational, randomized study comparing the antiviral activity and safety of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. This study enrolled 344 subjects at 151 study sites in the US, Canada, the United Kingdom, Australia, Hong Kong, New Zealand, and South Africa. Subjects were randomized 1:1:1 ratio to receive 1 of 3 treatments: peramivir 150 mg, peramivir 300 mg, or placebo. At Screening, eligible subjects had anterior nasal swabs collected for testing by Rapid Antigen Test (RAT) for influenza A and B viruses. If this test was positive and the subject was enrolled, additional specimens were obtained for culture of influenza virus, RT-PCR assay, and further virologic testing. A negative initial RAT could be repeated within 1 hour of the negative result. A second negative RAT would exclude the subject from enrollment. All subjects enrolled in the study were instructed to record the following information in a Study Diary: oral temperature measurements with an electronic thermometer (provided by the Sponsor for the study) every 12 hours, twice-daily assessments of the seven influenza symptoms, and once-daily assessments of the subject's time lost from work or usual activities and productivity compared to normal using a 0-10 point visual analog scale. Daily subject assessment of injection site discomfort for the first 5 days was also recorded. Following administration of study drug, anterior nasal (bilateral) and posterior pharyngeal swabs were collected on Days 2, 3, 5, and 9 for influenza viral culture and real-time RT-PCR assay. The influenza A/H1N1 virus with NA H275Y substitution was not circulating during the study.

Study BCX1812-311 was a Phase 3 randomized, double-blind study comparing the efficacy and safety of a 300 mg dose of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. This US-only study was terminated early in order to utilize a higher concentration product in subsequent studies. At the time of study termination, 103 sites had been initiated and 82 of the planned 600 subjects were enrolled at 37 study sites. All subjects were centrally randomized to treatment with 300 mg of peramivir or placebo in a ratio of 2:1, and randomization was stratified according to smoking status and RAT result for influenza A or B viruses. Study drug was administered as bilateral 2 mL intramuscular injections (total of 4 mL injected in equally divided doses). Anterior nose (bilateral) and posterior pharynx specimens (swabs) were collected at Day 1 (pretreatment), 3, 5, and 9 for quantitative virologic assessments. Specimens from all subjects yielding influenza virus were assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result on culture) as well as other virologic assessments (e.g. RT-PCR, genotypic testing). The primary endpoint was time to alleviation of symptoms. The influenza A/H1N1 virus with NA H275Y substitution was not circulating during the study.

In the integrated analysis, the median time to alleviation of symptoms was reduced for subjects who received 150 mg (114.1 hours) or 300 mg (113.2 hours) of peramivir, compared with subjects who received placebo (134.8 hours) (please see the review of the statistical reviewer). The time to alleviation of symptoms was analyzed based on influenza virus type/subtype (Table 12). The median time to alleviation of symptoms in subjects infected with influenza A/H1N1 virus was 135.65 hours (n=32), 113.23 hours (n=37), and 173.33 hours (n=35) in the 150 mg group, 300 mg group, and placebo group, respectively. The difference in the means between the 300 mg group and placebo was statistically significant (P <0.02). The median time to alleviation of symptoms in subjects infected with influenza A/H3N2 virus was 90.43 hours (n=50), 90.29 hours (n=90), and 118.15 hours (n=69) in the 150 mg group, 300 mg group, and placebo group, respectively. The differences in the means between the treatment arms and placebo arm were not statistically significant. The median time to alleviation of symptoms in subjects infected with influenza B virus was 99.83 hours (n=19), 124.25 hours (n=31), and 114.5 hours (n=28) in the 150 mg group, 300 mg group, and placebo group, respectively. The differences in the means between the treatment arms and placebo arm were not statistically significant. There was no clear trend that was observed with respect to initiation of treatment relative to the time to onset of symptoms and the time to alleviation of symptoms for any of the influenza virus type/subtype.

Table 12: Time to Alleviation of Symptoms and Baseline NA Activity

		Peramivir 150 mg				Peramivir 300 mg				Placebo			
Time to Onset of Symptoms		Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)
H1N1													
0 to 24 hours		N=14				N=9				N=11			
	Median	140.70	0.12	1 00	1 01	175.25	0.04	2.16	0.97	167.34	0.10	0.94	0.26
	Average	131.44	0.18	2 61	5 20	154.96	0.96	4.14	2.12	161.98	0.17	1.14	0.39
24+ to 48 hours		N=18				N=28				N=24			
	Median	134.18	0 05	1 03	0 51	109.91	0.20	0.72	0.50	179.33	0 32	1.66	0.70
	Average	151.37	0 69	8.16	0 59	121.50	0.45	1.28	1.04	176.74	0 55	3.58	5.56
Total		N=32				N=37				N=35			
	Median	135.65	0 08	1 02	0.76	113.23	0.12	0.72	0.55	173.33	0 25	1.19	0.57
	Average	142.65 (P=0 08)	0.41	5.11	3.13	129.64 (P<0.02)	0.61	2.18	1.38	172.40	0.42	2.74	3.78
H3N2													
0 to 24 hours		N=14				N=28				N=22			
	Median	77 50	0.14	0.40	0 53	114.00	0.14	0.30	0.34	117.38	0.15	0.36	0.25

		Peramivir 150 mg				Peramivir 300 mg				Placebo			
Time to Onset of Symptoms		Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)
	Average	119.39	0 21	0.46	0.48	111.52	0.13	0.60	0.33	129.22	0.16	0.57	0.31
24+ to 48 hours		N=36				N=62				N=47			
	Median	99.77	0.15	0.40	0.41	88.00	0.10	0.45	0.51	118.92	0.14	0.43	0.42
	Average	138.54	0.18	0 57	0.45	131.37	0.15	0.55	0.79	146.64	0 29	1.47	0.73
Total		N=50				N=90				N=69			
	Median	90.43	0.15	0.40	0.43	90.29	0.14	0.44	0.45	118.15	0.14	0.41	0.37
	Average	133.18 (P=0.3)	0.19	0 54	0.46	125.19 (P=0.1)	0.14	0.56	0.69	141.26	0 25	1.18	0.60
A													
0 to 24 hours		N=1								N=1			
	Median	149.50	1 33	10.09	8 34					168.43	0 01	1.59	0.41
	Average	149.50	1 33	10.09	8 34					168.43	0 01	1.59	0.41
24+ to 48 hours		N=2				N=2				N=2			
	Median	219.24	2 64	18.63	4 84	72.13	1.48	15.45	2.33	222.13	0 03	0.82	1.50
	Average	219.24	2 64	18.63	4 84	72.13	1.48	15.45	2.33	222.13	0 03	0.82	1.50
Total		N=3				N=2				N=3			
	Median	149.50	1 33	10.09	8 34	72.13	1.48	15.45	2.33	168.43	0 02	1.21	0.96
	Average	195.99	2 20	15.78	6 00	72.13	1.48	15.45	2.33	204.23	0 02	1.21	0.96
B													
0 to 24 hours		N=5				N=6				N=8			
	Median	81 97	1 55	19.45	5 91	139.54	1.99	19.04	5.45	127.43	1 31	10.48	2.99
	Average	159.54	1.76	17.68	5 62	139.57	3.05	32.36	5.49	138.78	2 07	10.40	3.03
24+ to 48 hours		N=14				N=25				N=20			
	Median	100.32	3 60	19.54	5.46	110.75	1.84	14.63	2.67	114.46	1.77	15.42	2.74
	Average	130.35	4 27	21.19	5 31	124.77	2.27	15.81	3.36	130.10	2.40	18 93	4.32
Total		N=19				N=31				N=28			
	Median	99 83	3.11	19.45	5.46	124.25	1.84	14.63	2.94	114.50	1.40	14 55	2.77
	Average	138 03 (P=0.4)	3 64	20.31	5 39	127.63 (P=0.4)	2.42	18.96	3.77	132.58	2 31	16 68	3.98

The median change in virus titer (log_{10} $TCID_{50}$/mL) from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -2.51 log_{10} $TCID_{50}$/mL, -2.89 log_{10} $TCID_{50}$/mL, and -3.14 log_{10} $TCID_{50}$/mL in the 150 mg group, 300 mg group, and placebo group, respectively (Table 13). The differences in the means between the treatment arms and placebo arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was higher in both the 150 mg (19/32, 59.4%) and 300 mg (18/37, 48.6%) groups than in the placebo group (15/35, 42.9%). The differences between the treatment arms and placebo arm were not statistically significant. The median change in virus titer from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -3.13 log_{10} $TCID_{50}$/mL, -3.01 log_{10} $TCID_{50}$/mL, and -2.38 log_{10} $TCID_{50}$/mL in the 150 mg group, 300 mg group, and placebo group, respectively. The difference in the means between the 150 mg group ($P < 0.01$), 300 mg arm ($P < 0.01$), and placebo were statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was higher in both the 150 mg (23/50, 46%) and 300 mg (58/90, 64.4%) groups than in the placebo group (27/69, 39.1%). The difference between the 300 mg arm and placebo was statistically significant ($P < 0.002$). The median change in virus titer from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was -1.75 log_{10} $TCID_{50}$/mL, -2.38 log_{10} $TCID_{50}$/mL, and -2.25 log_{10} $TCID_{50}$/mL in the 150 mg group, 300 mg group, and placebo, respectively. The differences in the means between the treatment arms

and placebo arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) in the 150 mg (8/19, 42.1%), 300 mg (11/31, 35.5%) group was comparable to placebo (10/28, 35.7%). The differences between the treatment arms and placebo arm were not statistically significant. Few subjects in any of the treatment groups had detectable virus on Visit 5 (Day 9).

Table 13: Viral Load ($TCID_{50}$)

Time to Onset of Symptoms		Peramivir 150 mg					Peramivir 300 mg					Placebo				
		$TCID_{50}$ (log_{10}/mL)														
		BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9
H1N1																
0 to 24 hours		N=14					N=9					N=11				
	Median	4.38	-2.63	-3.25	-2.89	-3.64	4.75	-2.75	-3.50	-3.76	-3.76	4.75	-1.63	-2.50	-3.76	-4.26
	Average	4.04	-2.38	-2.79	-2.85	-3.24	4.11	-2.36	-3.23	-3.06	-3.31	4.84	-1.43	-2.75	-3.81	-4.06
	# Subjects with Undetectable	0 (0%)	4 (28.6%)	5 (35.7%)	9 (64.3%)	13 (92.9%)	1 (11.1%)	1 (11.1%)	5 (55.6%)	6 (66.7%)	8 (88.9%)	0 (0%)	0 (0%)	0 (0%)	5 (45.5%)	9 (+1 NA) (90%)
24+ to 48 hours		N=18					N=28					N=24				
	Median	3.25	-1.51	-2.26	-2.26	-2.26	3.75	-2.00	-2.75	-3.01	-3.01	4.25	-1.75	-3.26	-3.51	-3.51
	Average	3.08	-1.74	-2.05	-2.27	-2.30	3.84	-2.00	-2.83	-3.05	-3.08	4.05	-2.00	-2.98	-3.31	-3.31
	# Subjects with Undetectable	2 (11.1%)	9 (50%)	14 (77.8%)	16 (88.9%)	17 (94.4%)	0 (0%)	1 (3.6%)	13 (46.4%)	21 (75%)	26 (+1 NA) (96.3%)	0 (0%)	1 (4.2%)	17 (70.8%)	21 (87.5%)	24 (100%)
Total		N=32					N=37					N=35				
	Median	3.50	-2.00	-2.51	-2.51	-2.64	3.75	-2.38	-2.89	-3.01	-3.01	4.50	-1.75	-3.14	-3.51	-3.64
	Average	3.50	-2.02	-2.37 (P<0.04)	-2.52	-2.71	3.91	-2.13	-2.93 (P=0.4)	-3.05	-3.14	4.30	-1.80	-2.91	-3.46	-3.53
	# Subjects with Undetectable	2 (6.3%)	13 (40.6%)	19 (59.4%) (P=0.2)	25 (78.1%)	30 (93.8%)	0 (0%)	2 (5.4%)	18 (48.6%) (P=0.6)	27 (73%)	34 (+1 NA) (94.4%)	0 (0%)	1 (2.9%)	15 (42.9%)	26 (74.3%)	33 (+1 NA) (97.1%)
H3N2																
0 to 24 hours		N=14					N=28					N=22				
	Median	4.38	-2.13	-2.89	-3.14	-3.51	4.00	-2.75	-3.26	-3.14	-3.26	4.50	-1.00	-2.26	-3.26	-3.76
	Average	4.34	-2.07	-2.81	-3.27	-3.55	4.08	-2.66	-3.20	-3.11	-3.23	4.22	-1.25	-2.61	-3.26	-3.51
	# Subjects with Undetectable	0 (0%)	2 (14.3%)	7 (50%)	9 (64.3%)	13 (92.9%)	1 (3.6%)	4 (14.3%)	17 (60.7%)	20 (71.4%)	24 (85.7%)	0 (0%)	1 (4.5%)	8 (36.4%)	13 (59.1%)	18 (+1 NA) (85.7%)
24+ to 48 hours		N=36					N=62					N=47				
	Median	4.50	-2.25	-3.25	-3.64	-3.76	3.75	-2.25	-3.00	-3.01	-3.01	3.75	-1.50	-2.50	-3.01	-3.01
	Average	4.28	-1.99	-3.06	-3.29	-3.36	3.82	-2.10	-2.87	-2.97	-3.07	3.77	-1.37	-2.35	-2.85	-2.87
	# Subjects with Undetectable	1 (2.8%)	6 (16.7%)	16 (44.4%)	27 (75%)	31 (86.1%)	0 (0%)	11 (17.7%)	41 (66.1%)	51 (82.3%)	57 (91.9%)	2 (4.3%)	3 (6.4%)	19 (40.4%)	39 (83%)	40 (85.1%)
Total		N=50					N=90					N=69				
	Median	4.50	-2.25	-3.13	-3.51	-3.76	4.00	-2.25	-3.01	-3.01	-3.26	4.00	-1.50	-2.38	-3.01	-3.26
	Average	4.30	-2.02	-2.99 (P<0.01)	-3.29	-3.41	3.90	-2.23	-2.97 (P<0.01)	-3.01	-3.12	3.91	-1.33	-2.43	-2.98	-3.07
	# Subjects with Undetectable	1 (2%)	8 (16%)	23 (46%) (P=0.5)	36 (72%)	44 (88%)	1 (1.1%)	15 (16.7%)	58 (64.4%) (P<0.002)	71 (78.9%)	81 (90%)	2 (2.9%)	4 (5.8%)	27 (39.1%)	52 (75.4%)	58 (+1 NA) (85.3%)
A																
0 to 24 hours		N=1										N=1				
	Median	4.25	0.00	-1.00	-3.51	-3.51						1.00	2.25	1.00	-0.26	-0.26
	Average	4.25	0.00	-1.00	-3.51	-3.51						1.00	2.25	1.00	-0.26	-0.26
	# Subjects with Undetectable	0 (0%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)						0 (0%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)
24+ to 48 hours		N=2					N=2					N=2				
	Median	3.38	-0.75	-1.88	-2.63	-2.64	3.00	-0.88	-2.13	-2.26	-4.51	2.25	-0.25	-0.51	-1.51	-1.51
	Average	3.38	-0.75	-1.88	-2.63	-2.64	3.00	-0.88	-2.13	-2.26	-4.51	2.25	-0.25	-0.51	-1.51	-1.51

Time to Onset of Symptoms		Peramivir 150 mg					Peramivir 300 mg					Placebo				
		$TCID_{50}$ (log_{10}/mL)														
		BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 5	Δ from BL Day 9
	# Subjects with Undetectable	0 (0%)	0 (0%)	1 (50%)	1 (50%)	2 (100%)	1 (50%)	1 (50%)	1 (50%)	2 (100%)	1 (+1 NA) (100%)	0 (0%)	0 (0%)	1 (50%)	2 (100%)	2 (100%)
Total		N=3					N=2					N=3				
	Median	4.25	0.00	-1.00	-3.51	-3.51	3.00	-0.88	-2.13	-2.26	-4.51	1.75	1.00	0.00	-1.01	-1.01
	Average	3.67	-0.50	-1.59	-2.92	-2.93	3.00	-0.88	-2.13	-2.26	-4.51	1.83	1.00	0.00	-1.09	-1.09
	# Subjects with Undetectable	0 (0%)	0 (0%)	1 (33.3%)	2 (66.7%)	3 (100%)	1 (50%)	1 (50%)	1 (50%)	2 (100%)	1 (+1 NA) (100%)	0 (0%)	0 (0%)	1 (33.3%)	3 (100%)	3 (100%)
B																
0 to 24 hours		N=5					N=6					N=8				
	Median	4.25	-1.50	-3.50	-3.51	-3.51	4.13	-0.76	-2.13	-2.88	-3.39	3.75	-1.38	-1.25	-2.63	-2.88
	Average	3.70	-1.25	-2.20	-2.96	-2.96	4.04	-1.05	-1.96	-2.92	-3.30	3.34	-1.25	-1.53	-2.44	-2.51
	# Subjects with Undetectable	1 (20%)	1 (20%)	3 (60%)	4 (80%)	5 (100%)	0 (0%)	1 (16.7%)	3 (50%)	4 (66.7%)	6 (100%)	1 (12.5%)	1 (12.5%)	2 (25%)	6 (75%)	7 (87.5%)
24+ to 48 hours		N=14					N=25					N=20				
	Median	3.63	-0.25	-1.50	-2.76	-2.88	3.75	-1.01	-2.38	-2.76	-2.89	4.38	-1.25	-2.38	-3.39	-3.64
	Average	3.75	-0.64	-1.52	-2.90	-3.04	3.75	-1.03	-2.07	-2.88	-2.97	4.10	-1.16	-2.37	-3.22	-3.36
	# Subjects with Undetectable	1 (7.1%)	2 (14.3%)	5 (35.7%)	11 (78.6%)	12 (+1 NA) (92.3%)	0 (0%)	2 (8%)	8 (32%)	19 (76%)	24 (+1 NA) (100%)	2 (10%)	3 (15%)	8 (40%)	17 (85%)	20 (100%)
Total		N=19					N=31					N=28				
	Median	4.00	-0.50	-1.75	-2.76	-3.01	3.75	-1.00	-2.38	-2.76	-2.89	4.13	-1.25	-2.25	-3.25	-3.26
	Average	3.74	-0.80	-1.70 (P=0.2)	-2.91	-3.02	3.81	-1.03	-2.05 (P=0.4)	-2.89	-3.04	3.88	-1.19	-2.13	-3.00	-3.12
	# Subjects with Undetectable	2 (10.5%)	3 (15.8%)	8 (42.1%) (P=0.7)	15 (78.9%)	17 (+1 NA) (94.4%)	0 (0%)	3 (9.7%)	11 (35.5%) (P=0.9)	23 (74.2%)	30 (+1 NA) (100%)	3 (10.7%)	4 (14.3%)	10 (35.7%)	23 (82.1%)	27 (96.4%)

Overall, for studies BCX1812-211/311, there was a dose/response significant reduction in time to alleviation of symptoms for A/H1N1 virus and a trend for A/H3N2 virus. There was a dose/response significant reduction in viral shedding for A/H3N2 virus and a significant reduction at the lower dose for A/H1N1 virus. The number of subjects not shedding virus at day 3 for influenza A/H3N2 virus was statistically significant between peramivir 300 mg vs. placebo. There was no apparent activity in subjects infected with influenza B virus.

3.4. Study BCX1812-212

This study was a Phase 2 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of a single dose of peramivir (600 mg) administered as 2 bilateral IM injections of 300 mg versus placebo in adults with acute uncomplicated influenza. This study was conducted in a year when the circulating virus expressed H275Y and therefore efficacy was not demonstrated. This study enrolled 45 subjects at 69 study sites in the US, South Africa, Australia, and New Zealand. The primary efficacy endpoint was time to alleviation of clinical symptoms of influenza in subjects with confirmed influenza A virus infection (ITTI-A population), calculated as the number of hours from initiation of study drug until the start of the time period in which all 7 symptoms of influenza were either absent or present at a level no greater than mild for at least 21.5 hours. This study was conducted between July 2008 and April 2009, during which time the predominant circulating influenza virus strain was an influenza A/H1N1 virus that contained a H275Y amino acid substitution in the NA. This amino acid substitution results in reduced susceptibility to peramivir as compared to wild type (Abed et al., 2004; Okomo-Adhiambo et al., 2010; Whitley et al., 2013).

This study did not meet its primary endpoint of time to alleviation of symptoms. Among the 279 subjects with confirmed influenza A virus infection included in the primary efficacy analysis for whom data for time to symptom alleviation over the study period were available, the median time to alleviation of symptoms was 91.1

hours in subjects receiving peramivir and 106.9 hours in subjects receiving placebo (please see the review of the statistical reviewer). The time to alleviation of symptoms was analyzed based on influenza virus type/subtype (Table 14). The median time to alleviation of symptoms in subjects infected with influenza A/H1N1 H275Y virus was 92.13 hours (n=105) and 108.91 hours (n=62) in the 600 mg group and placebo group, respectively. The differences between the treatment arm and placebo arm were not statistically significant. The median time to alleviation of symptoms in subjects infected with influenza A/H1N1 virus was 69.77 hours (n=11) and 136.52 hours (n=4) in the 600 mg group and placebo group, respectively. The differences between the treatment arm and placebo arm were not statistically significant. The median time to alleviation of symptoms in subjects infected with influenza A/H3N2 virus was 81.36 hours (n=16) and 95.72 hours (n=20) in the 600 mg group and placebo group, respectively. The differences between the treatment arm and placebo arm were not statistically significant. The median time to alleviation of symptoms in subjects infected with influenza B virus was 138.32 hours (n=27) and 136.52 hours (n=25) in the 600 mg group and placebo group, respectively. The differences between the treatment arm and placebo arm were not statistically significant. There was no clear trend that was observed with respect to initiation of treatment relative to the time to onset of symptoms and the time to alleviation of symptoms for any of the influenza virus type/subtype. Of note, the median baseline IC_{50} value of peramivir using the neuraminidase inhibition assay were similar between the isolates from subjects infected with H1N1 H275Y and the H1N1 WT viruses which raises doubt about the sensitivity of the sponsor's genotypic assay. Additionally, the median baseline IC_{50} value of peramivir against isolates from subjects infected with influenza A/H3N2 or B viruses were similar to previous studies.

Table 14: Time to Alleviation of Symptoms and Baseline NA Activity

Time to Onset of Symptoms		Peramivir 600 mg				Placebo			
		Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)
H1N1 H275Y									
0 to 24 hours		N=54				N=62			
	Median	88.12	56 53	406.16	1.02	113.58	49.86	514.23	0.77
	Average	111.75	88 56	1209 23	1.62	126.74	68.53	1095 03	1.05
24+ to 36 hours		N=51				N=63			
	Median	100.62	40 09	425.94	0.83	104.37	56.45	521.72	1.31
	Average	126.79	53 64	822.87	1.37	118.15	68.04	884.24	1.46
Total		N=105				N=125			
	Median	92.13	44 34	417.23	0.96	108.91	52.34	516.83	0.95
	Average	119.13 (P=0 38)	71 51	1020 54	1.49	122.38	68.26	981.09	1.27
H1N1									
0 to 24 hours		N=5				N=3			
		181.42	22 36	425.41	0.98	105.62	55.07	1603.43	1.40
		131.45	22 36	425.41	0.98	150.85	55.07	1603.43	1.40
24+ to 36 hours		N=6				N=1			
	Median	62.43	130.00	728.55	1.22	167.42	2.47	0 93	0.92
	Average	91 35	130.00	728.55	1.22	167.42	2.47	0 93	0.92
Total		N=11				N=4			
	Median	69.77	56 58	425.41	1.10	136.52	51.18	571.00	0.92
	Average	109 58 (P=0.2)	94.12	627.50	1.14	154.99	37.53	1069 26	1.24
H3N2									
0 to 24 hours		N=5				N=13			
	Median	74 58	1.97	1 56	1.16	76 95	0.81	0 91	0.63

	Average	79 59	1.97	1 56	1.16	126.06	0.77	0.77	0.56
24+ to 36 hours		N=11				N=7			
	Median	88.13	28.47	311.49	0.20	150.00			
	Average	119.83	28.47	311.49	0.20	173.88			
Total		N=16				N=20			
	Median	81 36	3.75	2 84	0.68	95.72	0.81	0 91	0.63
	Average	107 25 (P=0.1)	10 80	104.87	0.84	141.16	0.77	0.77	0.56
A									
12+ to 24 hours		N=1							
	Median	106.08	76 57	860.65	0.99				
	Average	106.08	76 57	860.65	0.99				
Total		N=1							
	Median	106.08	76 57	860.65	0.99				
	Average	106.08	76 57	860.65	0.99				
B									
0 to 24 hours		N=14				N=13			
	Median	103.42	4.50	21.57	3.64	54 00	6.49	32.35	5.02
	Average	121.67	6.13	27.31	4.14	102.45	6.14	38.49	5.13
24+ to 36 hours		N=13				N=12			
	Median	161.30	5.97	24.58	5.49	115.04	6.48	39.19	6.35
	Average	170.73	5.89	30.68	5.37	130.27	6.90	44.22	6.63
Total		N=27				N=25			
	Median	138.32	4.50	21.57	4.12	112.92	6.49	37.36	6.19
	Average	145 29 (P=0.2)	6.03	28.72	4.65	115.80	6.47	40.98	5.78

The median change in virus titer ($\log_{10}$ $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 H275Y virus was -1.51 $\log_{10}$ $TCID_{50}$/mL and -1.75 $\log_{10}$ $TCID_{50}$/mL in the 600 mg group and placebo group, respectively (Table 15). The differences between the treatment arm and placebo arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was similar in the 600 mg (69/105, 65.7%) and in the placebo group (86/125, 68.8%). The differences between the treatment arm and placebo arm were not statistically significant. The median change in virus titer ($\log_{10}$ $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -0.05 $\log_{10}$ $TCID_{50}$/mL and -1.01 $\log_{10}$ $TCID_{50}$/mL in the 600 mg group and placebo group, respectively. The differences between the treatment arm and placebo arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was similar in the 600 mg (9/11, 81.8%) and in the placebo group (3/4, 75%). The differences between the treatment arm and placebo arm were not statistically significant. The median change in virus titer ($\log_{10}$ $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -0.19 $\log_{10}$ $TCID_{50}$/mL and -0.19 $\log_{10}$ $TCID_{50}$/mL in the 600 mg group and placebo group, respectively. The differences between the treatment arm and placebo arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was similar in the 600 mg (16/16, 100%) and in the placebo group (18/20, 90%). The differences between the treatment arm and placebo arm were not statistically significant. The median change in virus titer ($\log_{10}$ $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was -2.38 $\log_{10}$ $TCID_{50}$/mL and -2.25 $\log_{10}$ $TCID_{50}$/mL in the 600 mg group and placebo group, respectively. The differences between the treatment arm and placebo arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was lower in the 600 mg (14/27, 81.8%) than in the placebo group (17/25, 68%). The differences between the treatment arm and placebo arm were not statistically significant.

Table 15: Viral Load ($TCID_{50}$)

Time to Onset of Symptoms		Peramivir 600 mg				Placebo			
		$TCID_{50}$ (log_{10}/ml)							
		BL	Δ from BL Day 3	Δ from BL Day 4	Δ from BL Day 9	BL	Δ from BL Day 3	Δ from BL Day 4	Δ from BL Day 9
H1N1 H275Y									
0 to 24 hours		N=54				N=62			
	Median	2.63	-1.50	-1.76	-2.01	3.00	-1.75	-2.01	-2.26
	Average	2.77	-1.47	-1.78	-2.07	2.93	-1.72	-1.91	-2.19
	# Subjects with Undetectable	9 (16.7%)	34 (63%)	44 (81.5%)	50 (+3 NA) (98%)	9 (14.5%)	43 (69.4%)	47 (75 8%)	60 (+2 NA) (100%)
24+ to 36 hours		N=51				N=63			
	Median	2.75	-1.76	-1.89	-2.01	3.25	-1.76	-2.01	-2.26
	Average	2.93	-1.87	-2.11	-2.13	2.98	-1.91	-2.04	-2.23
	# Subjects with Undetectable	7 (13.7%)	35 (68.6%)	45 (88.2%)	49 (+1 NA) (98%)	2 (3 2%)	44 (69 8%)	50 (79.4%)	60 (95 2%)
Total		N=105				N=125			
	Median	2.75	-1.51	-1.76	-2.01	3.00	-1.75	-2.01	-2.26
	Average	2.85	-1 66 (P=0.2)	-1.94	-2.10	2.95	-1.82	-1.97	-2.21
	# Subjects with Undetectable	16 (15.2%)	69 (65.7%) (P=0.1)	89 (84.8%)	99 (+3 NA) (97.1%)	11 (8.8%)	86 (68 8%)	97 (77 6%)	120 (+1 NA) (96 8%)
H1N1									
0 to 24 hours		N=5				N=3			
	Median	0.74	0.00	0.00	0.00	1.75	-1.01	-1.01	-1.01
	Average	0.94	-0.10	-0.20	-0.20	2.00	-1.25	-1.26	-1.26
	# Subjects with Undetectable	4 (80%)	4 (80%)	5 (100%)	5 (100%)	1 (33%)	2 (66%)	3 (100%)	3 (100%)
24+ to 36 hours		N=6				N=1			
	Median	0.87	0.00	-0.13	-0.13	1.00	-0.26	-0.26	-0.26
	Average	1.08	0.00	-0.34	-0.34	1.00	-0.26	-0.26	-0.26
	# Subjects with Undetectable	3 (50%)	5 (83.3%)	6 (100%)	6 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)
Total		N=11				N=4			
	Median	0.74	0.00	0.00	0.00	1.38	-0.64	-0.64	-0.64
	Average	1.02	-0.05 (P=0 08)	-0.28	-0.28	1.75	-1.01	-1.01	-1.01
	# Subjects with Undetectable	7 (63.6%)	9 (81.8%) (P=0 2)	11 (100%)	11 (100%)	1 (25%)	3 (75%)	4 (100%)	4 (100%)
H3N2									
0 to 24 hours		N=5				N=13			
	Median	0.74	0.00	0.00	-0.18	0.74	0.00	0.00	0.00
	Average	0.94	-0.20	-0.20	-0.18	1.01	-0.29	-0.33	-0.27
	# Subjects with Undetectable	3 (60%)	5 (100%)	5 (100%)	4 (80%)	9 (69%)	12 (92 3%)	13 (100%)	13 (100%)
24+ to 36 hours		N=11				N=7			
	Median	0.74	0.00	0.00	-0.19	0.74	0.74	0.74	0.74
	Average	0.92	-0.18	-0.18	-0.19	0.74	0.74	0.74	0.74
	# Subjects with Undetectable	10 (90.9%)	11 (100%)	11 (100%)	11 (100%)	7 (100%)	7 (100%)	7 (100%)	7 (100%)
Total		N=16				N=20			
	Median	0.74	0.00	0.00	-0.19	0.74	0	0	0
	Average	0.93	-0.19 (P=0.5)	-0.19	-0.19	0.92	-0.19	-0.21	-0.25
	# Subjects with Undetectable	13 (81.3%)	16 (100%) (P=0 8)	16 (100%)	16 (100%)	17 (85%)	18 (90%)	20 (100%)	20 (100%)
A									

12+ to 24 hours		N=1							
	Median	3.50	-2.25	-2.76	-2.76				
	Average	3.50	-2.25	-2.76	-2.76				
	# Subjects with Undetectable	0 (0%)	0 (0%)	1 (100%)	1 (100%)				
Total		N=1							
	Median	3.50	-2.25	-2.76	-2.76				
	Average	3.50	-2.25	-2.76	-2.76				
	# Subjects with Undetectable	0 (0%)	0 (0%)	1 (100%)	1 (100%)				
B									
0 to 24 hours		N=14				N=13			
	Median	4.25	-2.75	-3.39	-3.51	3.50	-2.26	-2.26	-2.26
	Average	4.05	-2.27	-3.08	-3.31	3.88	-2.20	-3.03	-2.62
	# Subjects with Undetectable	0 (0%)	4 (28.6%)	10 (71.4%)	14 (100%)	0 (0%)	7 (53.8%)	12 (92 3%)	11 (+2 NA) (100%)
24+ to 36 hours		N=13				N=12			
	Median	3.25	-1.89	-2.50	-2.26	3.25	-1.26	-2.51	-2.51
	Average	2.88	-1.76	-2.01	-2.01	2.81	-1.74	-2.07	-2.07
	# Subjects with Undetectable	3 (23.1%)	10 (76.9%)	12 (92.3%)	12 (+1 NA) (100%)	1 (8 3%)	10 (83 3%)	12 (100%)	12 (100%)
Total		N=27				N=25			
	Median	3.75	-2.38	-2.76	-2.89	3.50	-2.25	-2.26	-2.26
	Average	3.49	-2 03 (P=0.4)	-2.56	-2.71	3.37	-1.98	-2.57	-2.34
	# Subjects with Undetectable	3 (11.1%)	14 (51.9%) (P=0 3)	22 (81.5%)	26 (+1 NA) (100%)	1 (4%)	17 (68%)	24 (96%)	23 (+2 NA) (100%)

Overall, for study BCX1812-212, the differences between the treatment arm and placebo arm were not statistically significant in any of the endpoints for all influenza types/subtypes.

3.5. Study 0815T0631

This study was a Phase 3, multinational, multicenter, oseltamivir controlled, double-blind, and parallel-design study with dynamic allocation using the minimization method in subjects with acute uncomplicated influenza. This study was conducted in a year when the circulating virus expressed H275Y and therefore efficacy was not demonstrated. This study enrolled 1099 subjects at 146 study sites in Japan, South Korea, and Taiwan. The objective of this study was to assess the non-inferiority of peramivir at the doses studied compared to oseltamivir. Subjects were enrolled within 48 hours of onset of influenza symptoms and randomized to 1 of 3 treatment groups (364 randomized to single IV dose of peramivir 300 mg; 362 randomized to single IV dose of peramivir 600 mg; 365 randomized to oral oseltamivir phosphate 75mg twice daily for 5 days). Clinical efficacy evaluations included subject self-assessments of influenza symptoms and body temperature from Screening to Day 14. Virology assessments included virus subtypes, viral titer ($TCID_{50}$ value), and NA-inhibitory activity (IC_{50} value).

This study was conducted between November 2008 – May 2009, during which time the predominant circulating influenza virus strain was an influenza A/H1N1 virus that contained an H275Y amino acid substitution in the NA (Kohno et al., 2011). This amino acid substitution results in reduced susceptibility to oseltamivir and peramivir compared to wild type (Abed et al., 2004; Okomo-Adhiambo et al., 2010; Whitley et al., 2013). The primary endpoint of this study was time to alleviation of symptoms (time to recovery of influenza symptoms), defined as the time from the start of study drug treatment to the time of recovery from influenza symptoms. The time of recovery from influenza symptoms was calculated as the time point where all of the 7 influenza symptoms (cough, sore throat, headache, nasal congestion, feeling feverishness or having chills, aches and

pains of the muscle or joints, and fatigue) recorded in the Subject Diary became "0: none" or "1: mild" and when this condition persisted for at least 21.5 hours.

The time to alleviation of symptoms was analyzed based on influenza virus type/subtype (Table 16). The median time to alleviation of symptoms in subjects infected with influenza A/H1N1 virus was 74.25 hours (n=203), 82.10 hours (n=205), and 86.43 hours (n=208) in the peramivir 300 mg group, peramivir 600 mg group, and oseltamivir group, respectively. The differences in the means between the treatment arms were not statistically significant. The median time to alleviation of symptoms in subjects infected with influenza A/H3N2 virus was 69.50 hours (n=115), 70.58 hours (n=112), and 75.07 hours (n=111) in the peramivir 300 mg group, peramivir 600 mg group, and oseltamivir group, respectively. The differences in the means between the treatment arms arm were not statistically significant. The median time to alleviation of symptoms in subjects infected with influenza B virus was 55.33 hours (n=21), 86.19 hours (n=28), and 92.65 hours (n=22) in the peramivir 300 mg group, peramivir 600 mg group, and oseltamivir group, respectively. The difference in the means between the 300 mg group and oseltamivir group was statistically significant (P <0.01). However, the relevance is unclear given that peramivir is not expected to better antiviral activity compared to oseltamivir based on cell culture studies. There was no clear trend that was observed with respect to initiation of treatment relative to the time to onset of symptoms and the time to alleviation of symptoms for any of the influenza virus type/subtype. The median baseline IC_{50} value of peramivir and oseltamivir (but not zanamivir) was reduced in subjects infected with influenza A/H1N1 virus, consistent with the predominant circulating influenza virus strain during this study was an influenza A/H1N1 virus that contained an H275Y amino acid substitution in the NA. The median baseline IC_{50} value of peramivir and oseltamivir was not reduced in subjects infected with influenza A/H3N2 and B viruses.

Table 16: Time to Alleviation of Symptoms and Baseline NA Activity

		Peramivir 300 mg				Peramivir 600 mg				Oseltamivir 75 mg BID			
Time to Onset of Symptoms		Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)	Time to Alleviation of Symptoms (hr)	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)
H1N1													
0 to 24 hours		N=57				N=67				N=60			
	Median	70 05	21 35	97.58	1 35	85.13	21.80	91.35	1.33	79.90	21.51	100 00	1.32
	Average	98 80	22 01	86.59	1 34	109.62	22.78	85.58	1.36	112.13	22.13	87.15	1.34
24+ to 48 hours		N=51				N=46				N=49			
	Median	80 92	21 66	100.00	1 34	80.37	21.51	100.00	1.34	93.10	21.42	100 00	1.35
	Average	105.55	22 05	88.63	1 35	101.54	22.61	89.16	1.38	120.29	21.93	88.38	1.36
Total		N=203				N=205				N=208			
	Median	74 25	21 56	100.00	1 34	82.10	21.66	100.00	1.33	86.43	21.46	100 00	1.34
	Average	102 39 (P=0.1)	22 03	87.68	1 34	105.16 (P=0.1)	22.68	87.54	1.37	116.41	22.03	87.79	1.35
H3N2													
0 to 24 hours		N=25				N=31				N=38			
	Median	66.79	0.83	0 60	1 90	72.25	0.76	0 60	1.86	91.43	0.79	0.61	1.84
	Average	103.00	0.82	0 62	1 90	108.46	0.79	0 60	1.90	94.70	0.83	0.63	2.06
24+ to 48 hours		N=38				N=43				N=37			
	Median	78.47	0.79	0 61	1 94	61.38	0 83	0 65	1.93	69.93	0.84	0.64	1.91
	Average	112.46	0.84	0 64	1 96	99.86	0 81	0 62	1.96	103.10	0.86	0.66	2.03
Total		N=115				N=112				N=111			
	Median	69 50	0.80	0 61	1 92	70.58	0 82	0 63	1.90	75.07	0.82	0.62	1.91
	Average	108.19 (P=0.6)	0.83	0 63	1 93	102.78 (P=0.4)	0 80	0 61	1.94	100.38	0.85	0.65	2.04

A													
0 to 24 hours		N=8				N=5				N=2			
	Median	144.03	19 03	89.11	1.42	93.38	1 07	0 86	1.98	92.17	19.21	67.63	1.45
	Average	145.58	16.47	61.76	1.46	101.52	8 88	37.11	1.90	96.53	13.06	51.38	1.53
24+ to 48 hours		N=6				N=5				N=4			
	Median	50.17	19 90	82.65	1.41	109.27	18.88	60.86	1.34	42.57	0.80	0.61	1.91
	Average	90 55	20.16	78.30	1 37	115.74	11.55	51.51	1.60	40.67	0.79	0.62	1.85
Total		N=22				N=18				N=16			
	Median	58 87	19.79	84.02	1.41	93.38	1 07	0 88	1.78	83.03	1.33	0.88	1.55
	Average	110.56	19.14	73.70	1 39	109.42	10.30	44.73	1.74	77.91	9.99	38.69	1.61
B													
0 to 24 hours		N=3				N=12				N=5			
	Median	44 33	3.66	16.98	9 93	126.08	3 62	16.18	10.01	97.57	3.55	16.50	9.55
	Average	55 39	3.73	17.39	10.07	129.78	3 53	15.92	10.12	112.15	3.44	16.32	9.70
24+ to 48 hours		N=12				N=8				N=9			
	Median	76 08	3.63	16.49	9 86	70.28	3 58	16.82	10.02	88.68	3.49	16.55	9.61
	Average	73.58 (P<0.01)	3.68	16.68	9 81	76 37 (P=0.1)	3 51	16.79	9.73	111.15	3.31	16.20	9.39
Total		N=21				N=28				N=22			
	Median	55 33	3.65	16.94	9 86	86.19	3 59	16.60	10.02	92.65	3.50	16.55	9.61
	Average	68 38	3.70	16.88	9 88	91.63	3 52	16.54	9.84	111.49	3.36	16.24	9.50

The median change in virus titer ($\log_{10}$ $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -3.06 $\log_{10}$ $TCID_{50}$/mL, -3.00 $\log_{10}$ $TCID_{50}$/mL, and -3.00 $\log_{10}$ $TCID_{50}$/mL in the peramivir 300 mg group, peramivir 600 mg group, and oseltamivir group, respectively (Table 17). The differences in the means between the treatment arms were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was similar in the peramivir 300 mg (74/203; 36.5%), 600 mg (78/205, 38%) and in the oseltamivir group (88/208, 42.3%). The differences between the treatment arms were not statistically significant. The median change in virus titer ($\log_{10}$ $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -2.76 $\log_{10}$ $TCID_{50}$/mL, -2.76 $\log_{10}$ $TCID_{50}$/mL, and -2.46 $\log_{10}$ $TCID_{50}$/mL in the peramivir 300 mg group, peramivir 600 mg group, and oseltamivir group, respectively. The differences in the means between the treatment arms were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was higher in the peramivir 300 mg (94/115; 81.7%) and 600 mg (97/112, 86.6%) than in the oseltamivir group (72/111, 64.9%). The difference between the 300 mg arm (P <0.004) and 600 mg arm (P <0.0002), and oseltamivir arm was statistically significant. The median change in virus titer ($\log_{10}$ $TCID_{50}$) from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was -3.46 $\log_{10}$ $TCID_{50}$/mL, -3.46 $\log_{10}$ $TCID_{50}$/mL, and -4.00 $\log_{10}$ $TCID_{50}$/mL in the peramivir 300 mg group, peramivir 600 mg group, and oseltamivir group, respectively. The differences in the means between the treatment arms arm were not statistically significant. The proportion of subjects who were not shedding virus at Visit 3 (Day 3) was higher in the peramivir 300 mg (8/21; 38.1%) and 600 mg (11/28, 39.3%) than in the oseltamivir group (6/23, 26.1%). The differences between the treatment arms were not statistically significant.

Table 17: Viral Load ($TCID_{50}$)

Time to Onset of Symptoms		Peramivir 300 mg				Peramivir 600 mg				Oseltamivir 75 mg BID			
		$TCID_{50}$ ($\log_{10}$/ml)											
		BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8
H1N1													

Time to Onset of Symptoms		Peramivir 300 mg				Peramivir 600 mg				Oseltamivir 75 mg BID			
		$TCID_{50}$ (log_{10}/ml)											
		BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8
0 to 24 hours		N=95				N=92				N=99			
	Median	4.80	-2.06	-3 30	-4 06	4.80	-2.40	-3.00	-4 06	4.80	-2.00	-3.18	-4.06
	Average	5.00	-2.27	-3.11	-4 33	4.87	-2.13	-2.95	-4.15	4.91	-1.93	-3.01	-4.20
	# Subjects with Undetectable	1 (1.1%	12 (12 6%)	35 (36.8%)	86 (+9 NA) (100%)	0 (0%)	8 (8.7%)	30 (32.6%)	87 (94 6%)	2 (2%)	9 (9.1%)	33 (33.3%)	96 (97%)
24+ to 48 hours		N=108				N=113				N=109			
	Median	4.65	-2.00	-3 06	-4 06	4.50	-2 30	-3.00	-3 91	4.50	-2.30	-3.00	-3.76
	Average	4.68	-2.32	-2 91	-4 00	4.59	-2 31	-3.09	-3 85	4.36	-2.24	-2.82	-3.57
	# Subjects with Undetectable	2 (1 9%)	13 (12%)	38 (35.2%)	108 (100%)	2 (1 8%)	23 (20.4%)	48 (42.5%)	109 (96 5%)	1 (0.9%)	9 (8.3%)	55 (50.5%)	104 (95.4%)
Total		N=203				N=205				N=208			
	Median	4.80	-2.06	-3 06	-4 06	4.80	-2 30	-3.00	-4 06	4.50	-2.30	-3.00	-3.76
	Average	4.83	-2.30	-3 00 (P=0.4)	-4.14	4.72	-2 23	-3.03 (P=0 3)	-3 98	4.62	-2.09	-2.91	-3.88
	# Subjects with Undetectable	3 (1 5%)	25 (12 3%)	74 (36.5%) (P=0.2)	194 (9 NA) (100%)	2 (1%)	31 (15.1%)	78 (38%) (P=0.4)	196 (95 6%)	3 (1.4%)	18 (8.7%)	88 (42.3%)	200 (96.2%)
H3N2													
0 to 24 hours		N=52				N=38				N=36			
	Median	4.20	-2.06	-3 26	-3.46	3.65	-2 30	-2.76	-2.76	4.20	-2.40	-2.91	-3.46
	Average	3.93	-2.33	-3 09	-3 23	3.60	-2 21	-2.71	-2 86	4.04	-2.25	-2.95	-3.30
	# Subjects with Undetectable	2 (3 8%)	13 (25%)	45 (86.5%)	49 (+2 NA)	1 (2 6%)	9 (23.7%)	32 (84.2%)	37 (97.4%)	0 (0%)	7 (19.4%)	21 (58.3%)	36 (100%)
24+ to 48 hours		N=63				N=74				N=75			
	Median	3.50	-2.40	-2.76	-2.76	3.35	-1.76	-2.61	-2.76	3.50	-1.60	-2.46	-2.46
	Average	3.45	-2.30	-2 61	-2 86	3.39	-2 02	-2.62	-2 67	3.41	-1.84	-2.41	-2.50
	# Subjects with Undetectable	4 (6 3%)	13 (20 6%)	49 (77.8%)	62 (98.4%)	1 (1.4%)	20 (27%)	65 (87.8%)	74 (100%)	0 (0%)	9 (12%)	51 (68%)	71 (+1 NA) (95.9%)
Total		N=115				N=112				N=111			
	Median	3.50	-2.35	-2.76	-3 06	3.50	-2 06	-2.76	-2.76	3.50	-2.00	-2.46	-2.76
	Average	3.66	-2.31	-2 83 (P=0.1)	-3 03	3.46	-2 09	-2.65 (P=0.4)	-2.73	3.61	-1.99	-2.59	-2.78
	# Subjects with Undetectable	6 (5 2%)	26 (22 6%)	94 (81.7%) (P<0 004)	111 (+1 NA) (97.4%)	2 (1 8%)	29 (25.9%)	97 (86.6%) (P<0 0002)	111 (99.1%)	0 (0%)	16 (14.4%)	72 (64.9%)	107 (+1 NA) (97.3%)
A													
0 to 24 hours		N=8				N=8				N=12			
	Median	2.50	-0.15	0 00	-1.76	1.50	0 00	-0.76	-1 56	2.50	-0.45	-1.15	-1.76
	Average	2.44	-0.13	-0.12	-1.70	1.86	-0.12	-0.60	-1.49	2.32	-0.76	-1.09	-1.56
	# Subjects with Undetectable	2 (25%)	2 (25%)	5 (62.5%)	8 (100%)	0 (0%)	1 (12.5%)	6 (75%)	8 (100%)	2 (16.7%)	0 (0%)	6 (50%)	12 (100%)
24+ to 48 hours		N=14				N=10				N=6			
	Median	2.00	-1.00	-0 58	-0.46	1.80	0 00	-0.91	-1 06	1.20	-1.18	-0.46	-0.46
	Average	2.03	-0.82	-0 82	-0 89	2.02	-0 37	-0.67	-1 28	1.50	-1.18	-0.68	-0.76
	# Subjects with Undetectable	3 (21.4%)	3 (21.4%)	8 (57.1%)	12 (85.7%)	2 (20%)	3 (30%)	9 (90%)	10 (100%)	0 (0%)	NA	5 (83.3%)	6 (100%)
Total		N=22				N=18				N=18			
	Median	2.20	-0.46	-0.46	-1 06	1.65	0 00	-0.76	-1 06	1.65	-0.45	-0.76	-0.76
	Average	2.18	-0.54	-0 54	-1 27	1.95	-0 28	-0.64	-1 34	2.04	-0.84	-0.95	-1.28
	# Subjects with Undetectable	5 (22.7%)	5 (22.7%)	13 (59.1%)	20 (90.9%)	2 (11.1%)	4 (22.2%)	15 (83.3%)	18 (100%)	2 (11.1%)	0 (0%)	11 (61.1%)	18 (100%)
B													
0 to 24		N=6				N=8				N=8			

Time to Onset of Symptoms hours		Peramivir 300 mg				Peramivir 600 mg				Oseltamivir 75 mg BID			
		$TCID_{50}$ (log_{10}/ml)											
		BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 8
	Median	6.15	1 00	-3 08	-5.41	4.50	-0 35	-2.40	-3.76	7.15	-2.70	-3.85	-6.41
	Average	5.72	-1.00	-2 66	-4 97	5.64	-0 35	-3.05	-4.79	6.63	-2.36	-3.93	-5.88
	# Subjects with Undetectable	0 (0%)	0 (0%)	3 (50%)	5 (83.3%)	0 (0%)	0 (0%)	2 (25%)	5 (62 5%)	0 (0%)	0 (0%0	1 (12.5%)	8 (100%)
24+ to 48 hours		N=15				N=20				N=15			
	Median	5.50	-2.05	-4 00	-4.73	5.65	-2.70	-3.58	-5 06	6.80	-3.00	-4.00	-6.06
	Average	6.09	-1.77	-3 37	-5 24	5.89	-2 88	-4.08	-5.42	6.23	-2.44	-3.84	-5.49
	# Subjects with Undetectable	0 (0%0	2 (13 3%)	5 (33.3%)	13 (+1 NA) (92.9%)	0 (0%)	0 (0%)	9 (45%)	20 (100%)	0 (0%)	0 (0%0	5 (33%)	15 (100%)
Total		N=21				N=28				N=23			
	Median	5.80	-1.70	-3.46	-4 91	5.50	-2.40	-3.46	-5 06	6.80	-2.85	-4.00	-6.06
	Average	5.98	-1.59	-3.17 (P=0.1)	-5.16	5.82	-2.42	-3.81 (P=0 5)	-5 24	6.37	-2.41	-3.87	-5.63
	# Subjects with Undetectable	0 (0%)	2 (9.5%)	8 (38.1%) (P=0.4)	18 (+1 NA) (90%)	0 (0%)	0 (0%)	11 (39.3%) (P=0 3)	28 (100%)	0 (0%)	0 (0%)	6 (26.1%)	23 (100%)

The median change in CRP levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -0.16 mg/L, -0.15 mg/L, and -0.14 mg/L in the 300 mg group, 600 mg group, and oseltamivir group, respectively (Table 18). The differences in the means between the treatment arms arm were not statistically significant. The median change in CRP levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -0.34 mg/L, -0.47 mg/L, and -0.07 mg/L in the 300 mg group, 600 mg group, and oseltamivir group, respectively. The differences in the means between the treatment arms arm were not statistically significant. The median change in CRP levels from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was -0.06 mg/L, -0.15 mg/L, and -0.07 mg/L in the 300 mg group, 600 mg group, and oseltamivir group, respectively. The differences in the means between the treatment arms arm were not statistically significant.

Table 18: CRP

		Peramivir 300 mg			Peramivir 600 mg			Oseltamivir 75 mg BID		
		CRP (mg/L)								
ONSET	SUBTYPE	BL	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 3	Δ from BL Day 8	BL	Δ from BL Day 3	Δ from BL Day 8
H1N1										
0 to 24 hours		N=57			N=67			N=60		
	Median	0.96	-0.04	-0.71	0.94	-0 09	-0 69	0.87	0.07	-0.56
	Average	1.60	-0.02	-1.12	1.47	0.14	-1.11	1.28	0.55	-0.90
24+ to 48 hours		N=51			N=46			N=49		
	Median	0.97	-0.22	-0.85	1.46	-0 31	-1.11	0.94	-0.36	-0.86
	Average	1.52	-0.48	-1.32	2.15	-0 56	-1.76	1.80	-0.68	-1.57
Total		N=203			N=205			N=208		
	Median	0.96	-0.16	-0.81	1.29	-0.15	-0 88	0.93	-0.14	-0.81
	Average	1.56	-0.27	-1.23	1.85	-0 24	-1.47	1.55	-0.09	-1.25
H3N2										
0 to 24 hours		N=25			N=31			N=38		
	Median	1.51	-0.10	-1.19	1.52	-0.12	-0 91	1.06	0.07	-0.89
	Average	2.28	-0.10	-1.93	1.77	-0.13	-1.19	1.74	0.25	-1.53
24+ to 48 hours		N=38			N=43			N=37		

	Median	1.93	-0.48	-1.59	2.38	-0.77	-1 95	2.48	-0.19	-1.98
	Average	2.41	-0.57	-1.90	2.52	-0 83	-1 98	2.72	-0.51	-2.43
Total		N=115			N=112			N=111		
	Median	1.66	-0.34	-1.46	1.82	-0.47	-1.44	1.79	-0.07	-1.54
	Average	2.35	-0.36	-1.92	2.27	-0 59	-1.72	2.40	-0.27	-2.13
A										
0 to 24 hours		N=8			N=5			N=2		
	Median	0.86	-0.05	-0.52	10.42	-0 57	-10.14	0.87	-0.03	-0.55
	Average	0.74	0.07	-0.52	9.80	2.13	-9 54	1.11	1.65	-0.56
24+ to 48 hours		N=6			N=5			N=4		
	Median	2.62	-1.21	-2.05	2.10	-0 60	-1 63	2.75	-1.92	-2.57
	Average	3.10	-1.45	-3.12	2.65	-1 04	-2.44	2.66	-1.56	-2.44
Total		N=22			N=18			N=16		
	Median	1.47	-0.75	-0.96	2.45	-0 57	-2.19	0.97	-0.25	-0.81
	Average	2.24	-0.87	-1.97	5.83	0.26	-5 59	1.63	0.58	-1.19
B										
0 to 24 hours		N=3			N=12			N=5		
	Median	0.44	0.05	-0.36	0.36	-0 08	-0 20	0.48	-0.18	-0.36
	Average	0.50	0.36	-0.39	0.49	0.37	-0 22	0.50	0.20	-0.16
24+ to 48 hours		N=12			N=8			N=9		
	Median	0.49	-0.10	-0.34	0.63	-0.17	-0 50	0.57	-0.06	-0.45
	Average	0.45	-0.09	-0.36	1.11	-0 28	-0 60	1.40	0.07	-1.21
Total		N=21			N=28			N=22		
	Median	0.49	-0.06	-0.34	0.55	-0.15	-0 35	0.57	-0.07	-0.43
	Average	0.47	0.04	-0.37	0.93	-0.11	-0.49	1.09	0.11	-0.85

Overall, for study 0815T0631, there was no significant reduction in time to alleviation of symptoms for A/H1N1 and A/H3N2 viruses despite a significant reduction in shedding for A/H3N2 virus. However, given the similar mechanism of action of peramivir and oseltamivir, there is no expectation that there would be a difference between the treatment arms. The number of subjects not shedding virus at day 3 was statistically significant between peramivir 300 mg or 600 mg vs. oseltamivir 75 mg BID for subjects infected with influenza A/H3N2 virus. The time to alleviation of symptoms was statistically significant between peramivir 300 mg vs. oseltamivir 75 mg BID for subjects infected with influenza B virus. As stated above, the relevance is unclear given that peramivir is not expected to better antiviral activity compared to oseltamivir based on cell culture studies.

3.6. Study 0816T0632

This study was a Phase 3, double-blind, non-controlled, multicenter study of IV peramivir in subjects with acute uncomplicated influenza and high-risk factors, such as poorly controlled diabetes, chronic respiratory disease, or use of drugs that might suppress immune function. This study enrolled 42 subjects at 37 study sites in Japan. Subjects were administered peramivir intravenously at a dose of 300 mg (n = 21 randomized) or 600 mg (n = 21 randomized) once daily for 1 to 5 days. Study drug was administered immediately after enrollment on Day 1. On Day 2 and later, study drug was continued at the discretion of the investigator based on the body temperature of ≥ 37.5°C or clinical manifestations. Within the safety analysis set, 88.1% of the subjects received either 1 or 2 doses of study drug. Study drug was administered by IV drip infusion over 15 to 60 minutes. Clinical efficacy evaluations included subject self-assessments of body temperature, severity of influenza symptoms, and assessment of ability to perform daily activities, assessed from Screening to Day 14 and recorded in a Subject Diary. An ITTI set and a Per Protocol Set (PPS) were established for the

effectiveness analysis. The PPS was the primary analysis set used for effectiveness, and it was confirmed that the results of this analysis were consistent with the results of the analysis of the ITTI. The primary endpoint for the study was the time to alleviation of symptoms (time to disappearance of influenza symptoms/duration of influenza), defined as the time from the start of investigational product dosing to the time point at which all of the 7 influenza symptoms (cough, sore throat, headache, nasal congestion, feverishness or chills, aches or pains of the muscle or joints, and fatigue) showed a score of “0: none” or “1: mild,” and when this condition persisted for at least 21.5 hours.

The median time to alleviation of symptoms in subjects infected with influenza A/H1N1 virus was 40.22 hours and 70.83 hours for the 300 mg and 600 mg group, respectively (Table 19). The median time to alleviation of symptoms in subjects infected with influenza A/H3N2 virus was 89.72 hours and 30.02 hours for the 300 mg and 600 mg group, respectively. The median time to alleviation of symptoms in subjects infected with influenza B virus was 79.75 hours and 291.91 hours for the 300 mg and 600 mg group, respectively. The median change in virus titer (log_{10} $TCID_{50}$) from the time of Screening up to Day 2 and Day 6 of dosing in subjects infected with influenza A/H1N1 virus was -2.30 log_{10} $TCID_{50}$/mL and -3.06 log_{10} $TCID_{50}$/mL, respectively (Table 19). The proportion of subjects who were not shedding virus at Day 2 and Day 6 were 24/111 (21.6%) and 101/111 (91%), respectively. The median change in CRP values from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H1N1 virus was -1.73 mg/L and -0.23 mg/L for the 300 mg and 600 mg group, respectively. The median change in CRP values from the time of Screening up to Day 3 of dosing in subjects infected with influenza A/H3N2 virus was -0.15 mg/L and 0.8 mg/L for the 300 mg and 600 mg group, respectively. The median change in CRP values from the time of Screening up to Day 3 of dosing in subjects infected with influenza B virus was 0 mg/L and 27.9 mg/L for the 300 mg and 600 mg group, respectively.

Table 19: Summary of Study 0816T0632

	$TCID_{50}$ (log_{10}/ml)				CRP (mg/L)						
	BL	Δ from BL Day 2	Δ from BL Day 3	Δ from BL Day 6	BL	Δ from BL Day 3	Δ from BL Day 6	Time to Alleviation of Symptoms (hr	BL Per IC_{50} value (nM)	BL Ose IC_{50} value (nM)	BL Zan IC_{50} value (nM)
Peramivir 300 mg (N=18)											
H1N1 (N=9)											
Median	3.5	-2 03	-2.41	-2.61	4.12	-1.73	-3.86	40 22	21 94	100	1.4
Average	3.64	-1.46	-1 51	-2.59	4 38	-1.69	-4.08	80 08	21 99	87.18	1.4
# Subjects with Undetectable	0 (0%)	4 (44%)	5 (55.6%)	8 (+1 NA) (100%)							
H3N2 (N=4)											
Median	4.5	-3.7	-3.76	-3.76	2.72	-0.15	-2.52	89.72	0.82	0.63	2.13
Average	4.4	-3 62	-3 66	-3.66	2 64	-0.06	-2.48	126.62	0.83	0.66	2.12
# Subjects with Undetectable	0 (0%)	1 (25%)	4 (100%)	4 (100%)							
A (N=1)											
Median	0.8	0.4	0	-0.06	1 21	0.95	-0.61	123.08	23 21	68.41	1.46
Average	0.8	0.4	0	-0.06	1 21	0.95	-0.61	123.08	23 21	68.41	1.46
# Subjects with Undetectable	0 (0%)	0 (0%)	0 (0%)	1 (100%)							
B (N=4)											
Median	7.85	-2 85	-2 55	-7.11	0.79	0	-0.36	79.75	3.46	17.79	9.42
Average	7.6	-2.75	-3.45	-6.86	1 25	0.03	-0.49	102.29	3.46	17.61	9.42
# Subjects with Undetectable	0 (0%)	0 (0%)	0 (0%)	4 (100%)							
Peramivir 600 mg (N=20)											
H1N1 (N=10)											
Median	4.85	-2.73	-3 88	-4.11	1 36	-0.23	-1.28	70 83	22 05	100	1.42
Average	5.14	-3.16	-4.15	-4.4	2 51	-0.6	-2.26	125.86	22.49	90.92	1.44

# Subjects with Undetectable	0 (0%)	2 (20%)	8 (80%)	10 (100%)							
H3N2 (N=9)											
Median	3.5	-2	-2.76	-2.76	1 53	0.8	-1.14	30 02	0.83	0.73	2.07
Average	3.48	-2 36	-2.73	-2.74	1 93	0.73	-1	58.9	0.86	0.73	2.11
# Subjects with Undetectable	2 (22.2%)	4 (44.4%)	8 (88.9%)	9 (100%)							
B (N=1)											
Median	0.74	0	0	0	2 55	27 9	-0.14	291.95	3.76	14.5	9.88
Average	0.74	0	0	0	2 55	27 9	-0.14	291.95	3.76	14.5	9.88
# Subjects with Undetectable	1 (100%)	1 (100%)	1 (100%)	1 (100%)							

3.7. Study 0918T0633

This study was a Phase 3, multicenter, non-controlled open trial study of IV administration of peramivir in pediatric subjects with influenza virus infection. This study enrolled 117 subjects at 35 study sites in Japan. Peramivir was administered intravenously at a dose of 10 mg/kg (600 mg for subjects weighing ≥60 kg) once daily in pediatric subjects with type A or type B influenza virus infection. Clinical efficacy evaluations included subject self-assessments (or assessments by the proxy consenter) of body temperature, severity of influenza symptoms, and evaluation of usual activities, recorded daily in a Study Diary from Screening until Day 14. Body temperature was taken 4 times a day from Screening until Day 3, and twice a day from Days 4 to 14. Severity of influenza symptoms was assessed twice daily and activities of daily living once daily. Other efficacy assessments included viral titer ($TCID_{50}$ value), NA-inhibitory activity (IC_{50} value), CRP determination, and assessment of influenza-related complications. The primary endpoint for this study was the time to disappearance of symptoms of influenza (duration of influenza), defined as the time from the start of investigational product dosing to the time point at which the symptoms of cough and nasal discharge/obstruction (as recorded in the Subject Diary) showed a score of “0: none” or “1: mild,” and the subject’s body temperature was <37.5°; this condition must have persisted for at least 21.5 hours.

The median time to alleviation of symptoms in subjects infected with influenza A/H1N1 virus was 29.10 hours. There were no subjects who were infected with influenza A/H3N2 or B viruses. The median change in virus titer (log_{10} $TCID_{50}$) from the time of Screening up to Day 2 and Day 6 of dosing in subjects infected with influenza A/H1N1 virus was -2.30 log_{10} $TCID_{50}$/mL and -3.06 log_{10} $TCID_{50}$/mL, respectively (Table 20). The proportion of subjects who were not shedding virus at Day 2 and Day 6 were 24/111 (21.6%) and 101/111 (91%), respectively. The median change in CRP values from the time of Screening up to Day 2 and Day 6 of dosing in subjects infected with influenza A/H1N1 virus was 0.05 mg/L and -0.25 mg/L, respectively.

Table 20: Summary of Study 0918T0633

	Peramivir 10 mg/kg									
	$TCID_{50}$ (log_{10}/mL			**CRP (mg/L)**						
	BL	**Δ from BL Day 2**	**Δ from BL Day 6**	**BL**	**Δ from BL Day 2**	**Δ from BL Day 6**	**Time to Alleviation of Symptoms (hr)**	**BL Per IC_{50} value (nM)**	**BL Ose IC_{50} value (nM)**	**BL Zan IC_{50} value (nM)**
H1N1 (N=111)										
Median	4.00	-2.30	-3 06	0.37	0.05	-0 25	29.10	0.35	0.52	1 04
Average	4.21	-2.19	-3 23	0.72	0.20	-0 54	49.76	0.36	0.54	1 06
# Subjects with Undetectable	4 (3.6%)	24 (21.6%)	101 (91%)							

3.8. Studies in Hospitalized Patients

The sponsor has conducted 3 studies in hospitalized patients. These studies are described in more detail in the Appendix. Briefly, subjects who were treated with peramivir did not demonstrate a statistically significant improved compared to subjects who received standard of care that did not include a neuraminidase inhibitor

with respect to time to clinical resolution or decline in viral titers. Interestingly, there was also no statistically significant difference between subjects who received standard of care with or without a neuraminidase inhibitor. These results indicate that peramivir did not provide a clinical benefit compared to those who did not receive any antiviral drug.

3.9. Clinical Resistance

Nasopharyngeal specimens collected from subjects were analyzed by RT-PCR-based assays and cultured in Madin-Darby canine kidney cell line (MDCK) cells. Real-time RT-PCR was used to determine the presence of influenza virus A or B type and to assess the hemagglutinin (HA) subtype (for influenza A virus strains). The susceptibility of viruses to inhibition by peramivir, oseltamivir, and zanamivir was determined by the 2-(4-methlyumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) technique.

The resistance analyses that the sponsor conducted for the studies by Shionogi were limited. For study 0722T0621, the NA gene of the influenza viruses in specimens from subjects demonstrating pretreatment or post-treatment IC_{50} values for peramivir that were greater than a threshold of a mean IC_{50} value + 3 standard deviations (SDs) for NA inhibition activity of peramivir defined for the pretreatment isolates and for the post-treatment (the last isolated virus on Day 2 to 5) isolates, respectively, was characterized by sequencing. The utility of the phenotypic analyses as a screen for resistance is suspect given the selection bias of the assay for wildtype virus when viruses in subject samples are amplified in cell culture prior to testing and the assay itself which can show a bias in mixtures of wild-type and resistant virus (Wetherall et al., 2003; Deyde et al., 2009; Yen et al., 2013). Additionally, NAI resistance appears to arise relatively quickly following treatment with oseltamivir (e.g. 2 days after start of treatment; Hu et al., 2013). Another source of bias is the inability of the neuraminidase assay to detect resistance developing in the viral hemagglutinin (Tai et al., 1998; Ison et al., 2006). Additionally, the type of substrate used affects the sensitivity of the assay (Wetherall et al., 2003). Lastly, the use of the mean IC_{50} value + 3 SDs is arbitrary since a clinical cutoff for the NA assay has not been established. For these reasons, the Division recommends that phenotypic assays not be used to identify which samples to conduct further resistance analyses and that subject samples be evaluated by genotypic assay directly without an intervening culture step. Site-directed mutant virus or plaque purified resistant virus should be used to assess phenotypic resistance to peramivir and cross-resistance to other NAIs. Additionally, the sponsor has not genotyped the HA in their pivotal Phase 2 study and has only provided limited HA genotypic data from their supportive Phase 2 studies. Amino acid substitutions in the HA conferring reduced susceptibility to peramivir have been selected in cell culture studies (see above). Of note, these substitutions were selected in the absence of any resistance-associated amino acid substitutions in the NA.

For the studies conducted by BioCryst, the sponsor genotyped samples based on the following criteria:

- Subjects who have detectable influenza virus by cell culture or RT-PCR at Day 5 or later.
- Subjects with virus isolates with peramivir IC_{50} values 3× the baseline median using the NAI assay.
- Subjects with isolates with a peramivir IC_{50} value <3× median baseline IC_{50} value with a last detectable RT-PCR at the last assessment that were not included in either of the other 2 subsets (for studies BCX1812-301 and BCX1812-303).

This nested RT-PCR-based assay has been validated for full length sequencing of NA and HA for influenza A/pdH1N1 2009, A/H3N2 and B viruses. (b) (4) previous sequencing methodology was often not sensitive enough to analyze post-baseline samples with lower viral loads unless material was amplified in culture. The sequence of the H1 subtypes circulating in 2007-2008 differs significantly from the 2009 H1 for which the assay was developed. As a result, the primers developed to sequence currently circulating H1 viruses with the nested RT-PCR-based assay are not suitable to sequence earlier H1 strains. (b) (4) has indicated that it will take up to 3 months to develop and validate new primers to assay earlier H1 viruses. Additionally, the list of

samples that the sponsor was originally proposing to sequence was inadequate because of the reasons described above. The sponsor noted during the pre-NDA meeting that due to the number of additional samples to be sequenced and the availability of the company that would be performing the sequencing, the NDA submission would be delayed by several months. The Division and the sponsor reached a compromise to submit the genotypic data based off of an agreed upon priority list and that the rest would be submitted as a PMR. Due to the reasons described above, the resistance data described below are limited. The number of subjects with paired sequences by influenza virus type/subtype and study number is summarized in Table 21. Most of the sequences were obtained from the studies in hospitalized subjects (BCX1812-201, BCX1812-301, and BCX1812-303). As mentioned above, the HA was not genotyped for samples from study 0722T0621. Additionally, none of the other samples from the studies conducted by Shionogi was genotyped.

Table 21: Number of Subjects with Paired Sequence

		Number of Subjects		
	Study ID	**Peramivir**	**Oseltamivir**	**Placebo**
H1N1	0722T0621	7	0	1
	BCX1812-201	0	1	0
	BCX1812-211	8	0	2
	BCX1812-301	29	0	8
	BCX1812-303	11	0	0
	Total	**55**	**1**	**11**
H3N2	BCX1812-201	3	2	0
	BCX1812-211	20	0	9
	BCX1812-301	21	0	14
	BCX1812-311	4	0	3
	Total	**48**	**2**	**26**
B	BCX1812-201	4	2	0
	BCX1812-211	6	0	4
	BCX1812-301	6	0	2
	Total	**16**	**2**	**6**

Influenza H1N1 Virus

Amino acid substitutions that developed in the NA and HA for subjects infected with influenza A/H1N1 virus are listed in Table 22 & Table 23. Only the NA H275Y amino acid substitution developed in more than one subject (see Appendix 2 Table B1 for individual resistance data). There were 9 subjects who developed the NA H275Y amino acid substitution on-treatment (0722T0621.AL1.080-1, 0722T0621.BI1.135-6, 0722T0621.BQ1.148-4, 0722T0621.CH1.163-5, 0722T0621.AP1.086-5, BCX1812-211.662.007, BCX1812-211.671.004, BCX1812-301.575.002, and BCX1812-303.143.003). Subjects BCX1812-301.575.002 and BCX1812-303.143.003 were from the study in hospitalized subjects. Additionally, there were 3 subjects who had the NA H275Y amino acid substitution at baseline (0722T0621.AF1.005-1, BCX1812-211.069.028, and BCX1812-301.202.039) and one subject who had the NA H275Y substitution at Day 3 but the baseline sample was not genotyped (0722T0621.CM1.172-4). As expected, all of the isolates with the NA H275Y substitution had reduced susceptibility to peramivir and oseltamivir but not to zanamivir.

Subject 0722T0621.AL1.080-1 had a baseline viral load of 4.4 $\log_{10}$ $TCID_{50}$/mL at baseline, 2.8 $\log_{10}$ $TCID_{50}$/mL at Day 3 but 3.1 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.97 nM at baseline and 14.5 nM at Day 5. (A pure virus population was not used to assess neuraminidase phenotype so these shifts in susceptibility may underrepresent the impact of resistant substitutions.) Subject 0722T0621.BI1.135-6 had a baseline viral

load of 4.1 $\log_{10}$ $TCID_{50}$/mL at baseline and continued to drop to 1.4 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 1.35 nM at baseline and 27.6 nM at Day 5. Subject 0722T0621.BQ1.148-4 had a baseline viral load of 5.4 $\log_{10}$ $TCID_{50}$/mL at baseline and continued to drop to 1.1 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 1.12 nM at baseline and 27.8 nM at Day 5. Subject 0722T0621.CH1.163-5 had a baseline viral load of 5.4 $\log_{10}$ $TCID_{50}$/mL at baseline, 2.8 $\log_{10}$ $TCID_{50}$/mL at Day 3, but 4.1 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 1.47 nM at baseline and 30.3 nM at Day 9 (Day 5 data not available). Subject 0722T0621.AP1.086-5 had a baseline viral load of 2.1 $\log_{10}$ $TCID_{50}$/mL at baseline, 1.1 $\log_{10}$ $TCID_{50}$/mL at Day 3, but 2.4 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 1.72 nM at baseline and 31.5 nM at Day 5. Subject BCX1812-211.662.007 had a baseline viral load of 5.3 $\log_{10}$ $TCID_{50}$/mL at baseline, 1.5 $\log_{10}$ $TCID_{50}$/mL at Day 3, but 3.5 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.12 nM at baseline and 8.34 nM at Day 5. Subject BCX1812-211.671.004 had a baseline viral load of 5.3 $\log_{10}$ $TCID_{50}$/mL at baseline, 0.75 $\log_{10}$ $TCID_{50}$/mL at Day 3, but 2.5 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 3. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 5.52 nM at baseline (Day 3 data not available). Subject BCX1812-301.575.002 had a baseline viral load of 4 $\log_{10}$ $TCID_{50}$/mL at baseline, 3 $\log_{10}$ $TCID_{50}$/mL at Day 3, but 4 $\log_{10}$ $TCID_{50}$/mL at Day 5 and 1.75 $\log_{10}$ $TCID_{50}$/mL at Day 6. The NA H275Y amino acid substitution was observed on Day 6. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.37 nM at baseline and 38.56 nM at Day 6. This subject received amantadine as part of the standard-of-care regimen. Subject BCX1812-303.143.003 had a baseline viral load of 3 $\log_{10}$ $TCID_{50}$/mL at baseline, <LLOQ at Day3, but was 4 $\log_{10}$ $TCID_{50}$/mL at Day 4. The NA H275Y amino acid substitution was observed on Day 4. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.01 nM at baseline and 31.02 nM at Day 4. This subject did not receive any antiviral as part of the standard-of-care regimen.

Subject 0722T0621.AF1.005-1 had a baseline viral load of 5.1 $\log_{10}$ $TCID_{50}$/mL at baseline and continued to drop to 1.1 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed at baseline. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 9.43 nM at baseline and 20.8 nM at Day 3. Subject BCX1812-211.069.028 had a baseline viral load of 3.5 $\log_{10}$ $TCID_{50}$/mL at baseline, 4.5 $\log_{10}$ $TCID_{50}$/mL at Day 3 and 1.5 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed at baseline. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 5.01 nM at baseline and 6.89 nM at Day 5. Subject BCX1812-301.202.039 had a baseline viral load of 3.5 $\log_{10}$ $TCID_{50}$/mL at baseline and continued to drop to <LLOQ at Day 5. The NA H275Y amino acid substitution was observed at baseline. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 43.92 nM at baseline (Day 3 data not available, though virus was still detectable). Subject 0722T0621.CM1.172-4 had a baseline viral load of 4.8 $\log_{10}$ $TCID_{50}$/mL at baseline and continued to drop to 1.1 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA H275Y amino acid substitution was observed on Day 3 but the baseline sample was not genotyped so it is unclear if this was a treatment emergent substitution. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 27.1 nM at Day 3.

Table 22: Amino Acid Substitutions in the NA (H1N1)

AA Substitution	Peramivir (n=55)	Oseltamivir (n=1)	Placebo (n=11)	Reference Sequence (Mississippi/03/2001)
G27R	1	0	0	G
S/R35S	1	0	0	S
N45H	0	1	0	H
H/N45H	1	0	0	H

AA Substitution	Peramivir (n=55)	Oseltamivir (n=1)	Placebo (n=11)	Reference Sequence (Mississippi/03/2001)
I48V	0	1	0	V
A76V	1	0	0	A
E78K	0	1	0	K
K78K/E	1	0	0	K
S/P82P	1	0	0	S
V106I	1	0	0	I
R/K130K	1	0	0	R
M/I188I	1	0	0	M
A204T/A	1	0	0	A
G214E	0	1	0	E
Q222R	0	1	0	Q
L224L/S	1	0	0	L
M234V	0	1	0	V
S237L	1	0	0	S
I/V241I	1	0	0	I
K249G	0	1	0	G
H275Y	9	0	0	H
T/I287T	1	0	0	T
I287T	0	1	0	T
Q/H308H	1	0	0	Q
E329K	0	1	0	K
K/E329K	1	0	0	K
N344D	0	1	0	N
G348E/G	1	0	1	G
D354G	0	1	0	G
G/D354G	1	0	0	G
S/I366S	1	0	0	R
L/I367I	1	0	0	L
S367S/L	1	0	0	L
K/N369K	1	0	0	K
N382D	0	1	0	D
K/I389I	1	0	1	V
K390N	1	0	0	K
V/I393I	1	0	0	V
G404E/G	1	0	1	G
C421C/W	1	0	0	C
W423L	1	0	0	W
R432K	0	0	0	K
S442G	1	0	0	S
L464C	0	1	0	L
P465R	0	1	0	P
F466H	0	1	0	F

Yellow: Known resistance-associated substitution.

Table 23: Amino Acid Substitutions in the HA (H1N1)

AA Substitution	Peramivir (n=41)	Oseltamivir (n=0)	Placebo (n=11)	Reference Sequence (A/California/07/2009)
V41I	1	0	0	V
L49I	1	0	0	L
L49L/P	1	0	1	L
S/L102S	1	0	0	S
N/D114D	1	0	0	D
V125V/M	1	0	0	V
A151T	1	0	0	A
T/A151T	1	0	0	A
G157R	1	0	0	G
G187R/G	1	0	0	G
P199L	1	0	0	P
P/S199P	1	0	0	P
P/S200P	1	0	0	S
T/S202S	1	0	0	S
T/A203A	1	0	0	A
A203T	1	0	0	A
P229P/S	1	0	0	P
R238R/G	1	0	0	R
P/Q253Q	1	0	0	P
A261T/A	1	0	0	A
P288S	1	0	0	P
N304K	1	0	0	N
L307L/H	1	0	0	L
K/E391E	1	0	0	E
T/I405T	1	0	0	T
E418G	1	0	0	E
D453E	1	0	0	D
K/N458K	1	0	0	N
Y463Y/H	1	0	1	Y
E464E/G	1	0	0	E
T491T/M	1	0	0	T
V/I496V	1	0	0	V
V/I530V	1	0	0	I

Influenza H3N2 Virus

Amino acid substitutions that developed in the NA and HA for subjects infected with influenza A/H3N2 virus are listed in Table 24 & Table 25. There were no amino acid substitutions that developed in more than one subject. The NA R292K amino acid substitution conferring reduced susceptibility to peramivir, oseltamivir, and zanamivir developed in one subject (see Appendix 2 Table B2 for individual resistance data). Subject BCX1812-211.461.052 had a baseline viral load of 4.3 $\log_{10}$ $TCID_{50}$/mL at baseline, 2.3 $\log_{10}$ $TCID_{50}$/mL at Day 3 and <LLOQ at Day 5. The NA R292K amino acid substitution was observed on Day 3. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.19 nM at baseline and 1.13 nM at Day 5. Subject BCX1812-211.004.012 had a viral load of 5.75 $\log_{10}$ $TCID_{50}$/mL at baseline, 1.5 $\log_{10}$ $TCID_{50}$/mL at Day 3 and 1.5 $\log_{10}$ $TCID_{50}$/mL at Day 5. The NA N294S conferring reduced susceptibility to peramivir, oseltamivir, and zanamivir was observed on Day 5. The IC_{50} value for peramivir in the neuraminidase inhibition assay was 0.05 nM at baseline and 1.43 nM at Day 5.

Table 24: Amino Acid Substitutions in the NA (H3N2)

AA Substitution	Peramivir (n=48)	Oseltamivir (n=2)	Placebo (n=26)	Reference Sequence (A/California/07/2009)
I26T	1	0	0	I
S45P	1	0	0	P
N/K47N	0	0	1	N
N/H48N	0	0	1	N
I62T	0	0	1	I
L68L/M	1	0	0	L
P90P/S	1	0	0	P
N147D	1	0	0	D
V149A	1	0	0	V
R156R/Q	1	0	0	R
L223R/S	0	0	1	L
N225T	1	0	0	T
F233F	1	0	0	I
R237C	1	0	0	C
P238T	1	0	0	T
N242T	1	0	0	T
N257I	1	0	0	I
A259E	1	0	0	E
V290V/A	1	0	0	V
R292K/R	1	0	0	R
D293N/D	1	0	0	D
N294N/S	1	0	0	N
I389K	0	1	0	S
F390L	0	0	1	L
V398V/I	1	0	0	V
G405G/S	0	0	1	G

Yellow: Known resistance-associated substitution.

Table 25: Amino Acid Substitutions in the HA (H3N2)

AA Substitution	Peramivir (n=44)	Oseltamivir (n=0)	Placebo (n=22)	Reference Sequence A/Brisbane/10/2007
T28K	0	0	1	T
V59I	1	0	0	V
Q60H	0	0	1	Q
T147A	1	0	0	T
A151T	1	0	0	T
R158S	1	0	0	R
H172H/Y	1	0	0	H
I/L173L	0	0	2	L
L173S	0	0	1	L
N189K	0	0	1	K
G202D	1	0	0	G
I339I/V	1	0	0	V
D391N	1	0	0	D
L444L/F	0	0	1	L

AA Substitution	Peramivir (n=44)	Oseltamivir (n=0)	Placebo (n=22)	Reference Sequence A/Brisbane/10/2007
K468K/R	1	0	0	K

Influenza B Virus
Amino acid substitutions that developed in the NA and HA for subjects infected with influenza B virus are listed in Table 26 & Table 27. There were no amino acid substitutions that developed in more than one subject. None of these amino acid substitutions conferred reduced susceptibility to peramivir, oseltamivir, or zanamivir.

Table 26: Amino Acid Substitutions in the NA (B)

AA Substitution	Peramivir (n=16)	Oseltamivir (n=2)	Placebo (n=6)	Reference Sequence Perth/211/2001
P51Q/P	1	0	0	P
P354H	0	1	0	H
Q453H	0	1	0	L

Table 27: Amino Acid Substitutions in the HA (B)

AA Substitution	Peramivir (n=16)	Oseltamivir (n=1)	Placebo (n=6)	Reference Sequence B/Hubei-Wujiagang/158/2009
	None	None	None	

4.0. Conclusions
Approval of this original NDA for RAPIVAB is recommended with respect to Clinical Virology. RAPIVAB is recommended as a single IV regimen for the treatment of acute uncomplicated influenza in patients 18 (b) (4) years infected with influenza A virus.

Microbiology Package Insert
The words with strikethroughs are the text the sponsor was requested to delete and the words in blue are recommended insertions.

INDICATIONS AND USAGE
RAPIVAB is indicated for the treatment of acute uncomplicated influenza in patients 18 years and older.
(b) (4) Limitations of Use:

- (b) (4)
- (b) (4)
- (b) (4)
- (b) (4)
- Influenza viruses change over time. Emergence of resistance substitutions (b) (4) could decrease drug effectiveness. Other factors (for example, changes in viral virulence) might also diminish clinical benefit of antiviral drugs. Prescribers should consider available information on influenza drug susceptibility patterns and treatment effects when deciding whether to use RAPIVAB [see Microbiology (12.4)].
- ~~Efficacy of RAPIVAB has not been established in patients with serious influenza requiring hospitalization. *[See Clinical Studies (14.2)]*~~

12.1 Mechanism of Action
Peramivir is an (b) (4) antiviral drug with activity against influenza virus *[See* (b) (4) *Microbiology (12.4)].*

12.4 Microbiology

Mechanism of Action

Peramivir is an inhibitor of influenza virus neuraminidase, an enzyme that releases viral particles from the plasma membrane of infected cells. The median neuraminidase inhibitory activity (IC_{50} values) of peramivir in biochemical assays against influenza A/H1N1 virus, influenza A/H3N2 virus, and influenza B virus clinical isolates were 0.16 nM (n=44; range 0.01- 1.77 nM), 0.13 nM (n=32; range 0.05-11 nM), and 0.99 nM (n=39; range 0.04-54.2 nM), respectively, in a neuraminidase assay with a fluorescently labeled MUNANA substrate.

Antiviral Activity

The antiviral activity [redacted] (b) (4) of peramivir against laboratory strains and clinical isolates of influenza virus was determined in cell culture [redacted] (b) (4) The concentrations of peramivir required for inhibition of influenza virus in cell culture varied depending on the assay method used and the virus tested. The median 50% effective concentrations (EC_{50} values) of peramivir in cell culture assays were [redacted] (b) (4) 2.6 nM (n=13; range (b) (4) 0.09-21 nM), 0.08 nM (n=17; range <0.01-1.9 nM) and 4.8 nM (n=11; range 0.06-120 nM) [redacted] (b) (4) for influenza A/H1N1, A/H3N2 and B virus [redacted] (b) (4) strains, respectively. [redacted] (b) (4) he relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, and the inhibition of influenza virus replication in humans has not been established.

Resistance

[redacted] (b) (4)

[redacted] (b) (4)

Cell culture: Influenza A and B virus isolates with reduced susceptibility to peramivir [redacted] (b) (4) recovered by serial passage of virus in cell culture in the presence of increasing concentrations of peramivir. Reduced

susceptibility of influenza virus to inhibition by peramivir may be conferred by amino acid substitutions in the viral neuraminidase and/or hemagglutinin proteins (Table X).

Table X: Amino Acid Substitutions Selected by Peramivir in Cell Culture Studies

	Type/Subtype		
Protein	**A/H1N1**	**A/H3N2**	**B**
HA	D129S*, R208K*	N63K*, G78D*, N145D*, K189E*	T139N, G141E*, R162M, D195N*, T197N*, Y319H
NA	N58D, I211T, H275Y		H275Y

(b) (4)

In vivo: (b) (4)

Table Y: Neuraminidase Amino Acid Substitutions Associated with Reduced Susceptibility to Peramivir

	Type/ Subtype		
Protein	**Influenza A/H1N1 (N1 numbering)**	**Influenza A/H3N2**	**Influenza B (B numbering in brackets)**

(b) (4)

(b) (4)

Circulating seasonal influenza strains expressing neuraminidase resistance-associated substitutions have been observed in individuals who have not received (b) (4) RAPIVAB. Prescribers should consider available information from the CDC on influenza virus drug susceptibility patterns and treatment effects when deciding whether to use RAPIVAB.

Cross Resistance

Cross-resistance between peramivir, oseltamivir and zanamivir (b) (4) observed in neuraminidase biochemical assays and cell culture assays. (b) (4)

(b) (4) The amino acid substitutions that resulted in reduced susceptibility to peramivir and either oseltamivir (b) (4) or zanamivir are summarized in Table Z.

Table Z: Summary of Amino Acid Substitutions with Cross-Resistance between Peramivir, Oseltamivir, and/or Zanamivir

		Type/ Subtype		
	Protein	A/H1N1	A/H3N2	B
Oseltamivir	HA	(b) (4)	N63K, N145D	-
	NA	E119V, D151G/N, Y155H, D198G, (b) (4) S246N, H275Y, N294S	E119 (b) (4) I/V, (b) (4) (b) (4) R292K, N294S	(b) (4) P139S, G140R, (b) (4) H275Y, R292K, R371K
Zanamivir	HA	(b) (4)	N63K, N145D	-
	NA	(b) (4) Q136K, (b) (4) Y155H, D198G, (b) (4) S246N, N294S	E119G (b) (4) V, T148I, D151A/G/N/V, R292K, N294S	(b) (4) E116A/D/G (b) (4) P139S, (b) (4) R149K, D198E/N, R292K, R371K

(b) (4)

No single amino acid substitution has been identified that could confer cross-resistance between the neuraminidase inhibitor class (peramivir, oseltamivir, zanamivir) and the M2 ion channel inhibitor class (amantadine, rimantadine). However, a virus may carry a neuraminidase inhibitor resistance-associated substitution in neuraminidase and an M2 ion channel inhibitor resistance-associated substitution in M2 and may therefore be resistant to both classes of inhibitors. The clinical relevance of phenotypic cross-resistance evaluations has not been established.

Immune Response
No influenza vaccine/peramivir interaction study has been conducted.

Clean Version
INDICATIONS AND USAGE
RAPIVAB is indicated for the treatment of acute uncomplicated influenza in patients 18 years and older.
(b) (4) Limitations of Use:

(b) (4)

- Influenza viruses change over time. Emergence of resistance substitutions could decrease drug effectiveness. Other factors (for example, changes in viral virulence) might also diminish clinical benefit of antiviral drugs. Prescribers should consider available information on influenza drug susceptibility patterns and treatment effects when deciding whether to use RAPIVAB [see Microbiology (12.4)].

12.1 Mechanism of Action
Peramivir is an antiviral drug with activity against influenza virus *[See Microbiology (12.4)]*.

12.4 Microbiology

Mechanism of Action

Peramivir is an inhibitor of influenza virus neuraminidase, an enzyme that releases viral particles from the plasma membrane of infected cells. The median neuraminidase inhibitory activity (IC_{50} values) of peramivir in biochemical assays against influenza A/H1N1 virus, influenza A/H3N2 virus, and influenza B virus clinical isolates were 0.16 nM (n=44; range 0.01- 1.77 nM), 0.13 nM (n=32; range 0.05-11 nM), and 0.99 nM (n=39; range 0.04-54.2 nM), respectively, in a neuraminidase assay with a fluorescently labeled MUNANA substrate.

Antiviral Activity

The antiviral activity of peramivir against laboratory strains and clinical isolates of influenza virus was determined in cell culture. The concentrations of peramivir required for inhibition of influenza virus in cell culture varied depending on the assay method used and the virus tested. The median 50% effective concentrations (EC_{50} values) of peramivir in cell culture assays were 2.6 nM (n=13; range ~~of~~ 0.09-21 nM), 0.08 nM (n=17; range <0.01-1.9 nM) and 4.8 nM (n=11; range 0.06-120 nM) [redacted] (b) (4) for influenza A/H1N1, A/H3N2 and B virus strains, respectively. The relationship between the antiviral activity in cell culture, inhibitory activity in the neuraminidase assay, and the inhibition of influenza virus replication in humans has not been established.

Resistance

Cell culture: Influenza A and B virus isolates with reduced susceptibility to peramivir [redacted] (b) (4) recovered by serial passage of virus in cell culture in the presence of increasing concentrations of peramivir. Reduced susceptibility of influenza virus to inhibition by peramivir may be conferred by amino acid substitutions in the viral neuraminidase and/or hemagglutinin proteins (Table X).

Table X: Amino Acid Substitutions Selected by Peramivir in Cell Culture Studies

	Type/Subtype		
Protein	**A/H1N1**	**A/H3N2**	**B**
HA	D129S*, R208K*	N63K*, G78D*, N145D*, K189E*	T139N, G141E*, R162M, D195N*, T197N*, Y319H
NA	N58D, I211T, H275Y		H275Y

[redacted] (b) (4)

In vivo: [redacted] (b) (4)

Table Y: Neuraminidase Amino Acid Substitutions Associated with Reduced Susceptibility to Peramivir

	Type/ Subtype		
Protein	**Influenza A/H1N1 (N1 numbering)**	**Influenza A/H3N2**	**Influenza B (B numbering in brackets)**
[redacted] (b) (4)			

[redacted] (b) (4)

Circulating seasonal influenza strains expressing neuraminidase resistance-associated substitutions have been observed in individuals who have not received RAPIVAB. Prescribers should consider available information from the CDC on influenza virus drug susceptibility patterns and treatment effects when deciding whether to use RAPIVAB.

Cross Resistance

Cross-resistance between peramivir, oseltamivir and zanamivir [redacted] (b) (4) observed in neuraminidase biochemical assays and cell culture assays. The amino acid substitutions that resulted in reduced susceptibility to peramivir and either oseltamivir [redacted] (b) (4) or zanamivir are summarized in Table Z.

Table Z: Summary of Amino Acid Substitutions with Cross-Resistance between Peramivir, Oseltamivir, and/or Zanamivir

		Type/ Subtype		
	Protein	**A/H1N1**	**A/H3N2**	**B**
Oseltamivir	**HA**	[redacted] (b) (4)	N63K, N145D	-
	NA	E119V, D151G/N, Y155H, D198G, [redacted] (b) (4) S246N, H275Y, N294S	E119 (b) (4) I/V, [redacted] (b) (4) (b) (4) R292K, N294S	[redacted] (b) (4) P139S, G140R, [redacted] (b) (4) H275Y, R292K, R371K
Zanamivir	**HA**	[redacted] (b) (4)	N63K, N145D	-
	NA	[redacted] (b) (4) Q136K, [redacted] (b) (4) Y155H, D198G, [redacted] (b) (4) S246N, N294S	E119G (b) (4) V, T148I, D151A/G N/V, R292K, N294S	[redacted] (b) (4) E116A/D/G, [redacted] (b) (4) P139S, [redacted] (b) (4) R149K, D198E/N, R292K, R371K

No single amino acid substitution has been identified that could confer cross-resistance between the neuraminidase inhibitor class (peramivir, oseltamivir, zanamivir) and the M2 ion channel inhibitor class (amantadine, rimantadine). However, a virus may carry a neuraminidase inhibitor resistance-associated substitution in neuraminidase and an M2 ion channel inhibitor resistance-associated substitution in M2 and may therefore be resistant to both classes of inhibitors. The clinical relevance of phenotypic cross-resistance evaluations has not been established.

Immune Response
No influenza vaccine/peramivir interaction study has been conducted.

APPENDICES

Appendix 1: Tables for Nonclinical Virology Studies

Table A1: Activity of Oral Peramivir in Mice – Once-Daily or BID Treatment for 5 days beginning 4 hours before Viral Inoculation

Study Number	Influenza Virus	Drug	Dose (mg/kg/day)[a]	Survival	P Value vs Vehicle Control	Findings for Other Endpoints of Activity Studied
DD99057	A/Shangdong/09/93 (H3N2)	Vehicle control (saline)	0	1/20	NA	NA
		Peramivir	25	10/10	P<0.001	Increased mean days to death, arterial oxygen saturation; lower lung scores and lung virus titers
EDMS-USRA-2705266	B/Hong Kong/5/72	Vehicle control (saline)	0	0/19	NA	NA
		Peramivir	1 10 100	4/10 6/10 10/10	<0.01 <0.001 <0.001	Increased mean days to death (except at lowest dose), arterial oxygen saturation; lower lung scores and lung virus titers
		RBV	75	9/10	<0.001	Comparable to peramivir
Bantia et al., 2001	A/Turkey/Mass/76X A/Beijing/32/92 (H6N2)	Vehicle control (saline)	0	1/9	NA	NA
		Peramivir	0.1 1	7/9 9/9	<0.015 <0.001	No effect on mean days to dea h; decreased body weight loss
	A/NWS/33 (H1N1)	Vehicle control (saline)	0	0/10	NA	NA
		Peramivir	1 10	4/7 8/8	<0.015 <0.001	Increased mean days to dea h; decreased body weight loss
EDMS-USRA-3251731	A/Shangdong/09/93 (H3N2)	Vehicle control (saline)	0	8/20	NA	NA
		Peramivir	10	10/10	<0.01	Increased mean days to dea h; improved arterial oxygen satura ion and lung scores. No effect on lung viral titers
EDMS-USRA-3251543	A/Turkey/Mass/76X A/Beijing/32/92 (H6N2)	Vehicle control (0.5 % CMC in saline)	0	0/10	NA	NA
		Peramivir	1[c] 10[c]	7/10 9/9	<0.05 <0.001	No effect on body weight loss
EDMS-USRA-3251811	A/Shangdong/09/93 (H3N2)	Vehicle control (saline)	0	0/20	NA	NA

Study Number	Influenza Virus	Drug	Dose (mg/kg/day)[a]	Survival	P Value vs Vehicle Control	Findings for Other Endpoints of Activity Studied
		Peramivir	1 10 100	5/10 9/10 10/10	<0.001 <0.001 <0.001	Increased mean days to death at 1 and 100 mg/kg/day; improved arterial oxygen saturation, lung scores; lower lung virus titers
		Oseltamivir	1 10	0/10 5/10	NS <0.001	Improvements in other endpoints comparable to peramivir
		RBV	75	10/10	<0.001	Comparable to peramivir
EDMS-USRA-3251808	A/Shangdong/09/93 (H3N2)	Vehicle control (saline)	0	2/20	NA	NA
		Peramivir	1 10 100	4/10 5/10 10/10	NS <0.05 <0.001	Increased mean days to death at 100 mg/kg/day; improved arterial oxygen saturation, lung weight, lung scores; lower lung virus titers
		Oseltamivir	1 10	0/10 8/9	NS <0.001	Improved oxygen saturation at 10 mg/kg/day decrease; no effects on lung scores, lung weight, or lung viral titers at either dose
		RBV	75	9/9	<0.001	Comparable to peramivir
DD00012	A/Victoria/3/75 (H3N2)	Vehicle control (saline)	0	2/16	NA	NA
		Peramivir	1 10 100	0/10 7/10 10/10	NS <0.01 <0.001	Increased mean days to death at 100 mg/kg/day, improved arterial oxygen saturation at 10 and 100 mg/kg/day, and lower lung virus titers at 100 mg/kg/day
		Oseltamivir	10	1/9	NS	No statistically significant effect on these endpoints
DD99046	A/Bayern/57/93 (H1N1)	Vehicle control (saline)	0	1/20	NA	NA

Study Number	Influenza Virus	Drug	Dose (mg/kg/day)[a]	Survival	P Value vs Vehicle Control	Findings for Other Endpoints of Activity Studied
		Peramivir	1 10 100	4/10 8/10 6/9	<0.05 <0.001 <0.001	Improved arterial oxygen saturation and lung scores; lower virus titers in lung
		RBV	75	9/9	<0.001	Comparable to peramivir
P04-1812-001	A/NWS/33 (H1N1)	Vehicle control (saline)	0[b]	0/5	NA	NA
		Peramivir	1[b] 10[b]	2/5 5/5	<0.003 <0.003	Increased mean days to dea h; decreased body weight loss
		Oseltamivir	2[b] 10[b]	5/5 5/5	<0.003 <0.003	Comparable to peramivir
EDMS-USRA-3252053	A/Turkey/Mass/76X A/Beijing 32/92 [R] (H6N2)	Vehicle control (0.5% CMC in saline)	0	1/9	NA	NA
		Peramivir zwitterion	0.1 1	7/9 9/9	<0.015 <0.001	Decreased body weight loss
		Peramivir trifluoroacetate salt	0.1 1	3/9 9/9	NS <0.001	None
Sidwell et al., 2001	A/NWS/33 (H1N1)	Vehicle control (saline)	0	3/20	NA	NA
		Peramivir	1 10	10/10 10/10	<0.001 <0.001	Increased mean days to death; improved oxygen saturation, lung score, lung weight; lower virus titer from lung
	A/Shangdong/09/93 (H3N2)[c]	Vehicle control (saline)	0	0/20	NA	NA
		Peramivir	1 10 100	5/10 9/10 10/10	<0.001 <0.001 <0.001	Increased mean days to death (at 1 and 100 mg/kg/day only); improved oxygen saturation, lung score, lung weight; lower lung virus titer (at 1 and 100 mg/kg/day)
	A/Victoria/3/75 (H3N2)	Vehicle control (saline)	0	2/16	NA	NA

Study Number	Influenza Virus	Drug	Dose (mg/kg/day)[a]	Survival	P Value vs Vehicle Control	Findings for Other Endpoints of Activity Studied
		Peramivir	1 10 100	0/10 7/10 10/10	NS <0.01 <0.001	Increased mean days to death (at 100 mg/kg/day only); improved oxygen saturation (at 10 and 100 mg/kg/day), lung score, lung weight (at 10 and 100 mg/kg/day); lower lung virus titer (at 10 and 100 mg/kg/day)
	B/Hong Kong/05/72	Vehicle control (saline)	0	0/19	NA	NA
		Peramivir	1 10 100	4/10 6/10 10/10	<0.01 <0.001 <0.001	Increased mean days to death; improved oxygen saturation, lung score, lung weight (at 10 and 100 mg/kg/day); lower lung virus titer (at 10 and 100 mg/kg/day)
	A/NWS/33 (H1N1)	Oseltamivir	1 10	7/10 10/10	<0.001 <0.001	Comparable to peramivir
	A/Shangdong/09/93 (H3N2)[c]	Oseltamivir	1 10	0/10 5/10	NS <0.001	Improvements in other endpoints comparable to peramivir
	A/Victoria/3/75 (H3N2)	Oseltamivir	10	1/9	NS	Improvement only in lung score
	B/Hong Kong/05/72	Oseltamivir	1 10	1/10 1/10	NS NS	Improvements in other endpoints comparable to peramivir
	A/Shangdong/09/93 (H3N2)[c]	RBV	75	10/10	<0.001	Comparable to peramivir
	B/Hong Kong/05/72	RBV	75	9/10	<0.001	Comparable to peramivir
DD00013	B/Lee/40	Vehicle control (saline)	0	0/20	NA	NA
		Peramivir	1 10 100	5/10 3/10 8/10	<0.001 <0.05 <0.001	Improved oxygen saturation (at 100 mg/kg/day only), lung score, and lung weight on at least one sampling day

Study Number	Influenza Virus	Drug	Dose (mg/kg/day)[a]	Survival	P Value vs Vehicle Control	Findings for Other Endpoints of Activity Studied
		RBV	75	10/10	<0.001	Improved oxygen saturation, lung score on at least one sampling day
DD01044	Avian A/HK/156/97 (H5N1)	Vehicle control (saline)	0	0/11	NA	NA
		Peramivir	0 01 0.1 1 10	3/10 7/10 9/10 9/9	<0.015 <0.001 <0.001 <0.001	Improved brain and lung virus titers
		Oseltamivir	0 01 0.1 1	2/6 6/6 5/5	<0.01 <0.001 <0.001	Improved brain virus titers only
	Avian A/quail/HK/G1/97 (H9N2)	Vehicle control (saline)	0	0/10	NA	NA
		Peramivir	0 01 0.1 1 10	3/12 4/11 12/12 11/11	NS <0.05 <0.001 <0.001	Improved brain and lung virus titers
		Oseltamivir	0 01 0.1 1 10	5/11 4/11 11/11 12/12	<0.05 <0.01 <0.001 <0.001	Improved brain virus titers only

a. Given BID unless otherwise noted
b. Dosed once daily
c. Data presented are from one of 2 similar experiments in this study

Table A2: Activity of Oral Peramivir in Mice – Treatment for 3 or 5 days After Viral Inoculation

Study Number	Influenza Virus	Drug	Dosing Regimen	Dose (mg/kg/day)	Dosing Initiated (Hours After Viral Inoculation)[a]	Survival	P Value vs Vehicle Control
EDMS-USRA-3252035	A/Turkey/Mass/ 76X A/Beijing 32/92[R] (H6N2)	Vehicle Control (0.5% methyl cellulose in saline)	BID for 5 days	0	+24	0/8	NA
		Peramivir	BID for 5 days	10 10	+24 +48	9/10 0/10	<0.001 Not Significant

Study Number	Influenza Virus	Drug	Dosing Regimen	Dose (mg/kg/day)	Dosing Initiated (Hours After Viral Inoculation)[a]	Survival	P Value vs Vehicle Control
		Oseltamivir	BID for 5 days	0 10 10	+24 +24 +48	0/8 10/10 1/10	NA <0.001 Not Significant
Mishin et al., 2006	A/PR/8/34 (H1N1)	Vehicle Control (phosphate buffered saline)	BID for 5 days	0	+1	4/24	NA
		Peramivir	QD for 5 days	10	+1	12/12	<0.05
DD99004	A/NWS/33 (H1N1)	Vehicle Control (saline)	BID for 5 days	0	+4	6/20	NA
		Peramivir	BID for 5 days	10 10 10 10 10	+4 +24 +36 +48 +60	10/10 9/9 10/10 10/10 10/10	<0.001 <0.001 <0.001 <0.001 <0.001
		RBV	QD for 1 Day	75 75	+4 +24	10/10 10/10	<0.001 <0.001
EDMS-USRA-3425608	A/Shangdong/09/93 (H3N2)	Vehicle Control (saline)	BID for 5 days	0	+24	1/20	NA
		Peramivir	BID for 5 days	10	+24	0/10	Not Significant[c]
DD99045	A/Shangdong/09/93 (H3N2)	Vehicle Control (saline)	TID	0	+4	0/19	NA
		Peramivir	QD for 5 days	1 3 6	+12	1/10 1/10 7/10	Not Significant Not Significant <0.001
			BID for 5 days	1 3 6	+12	2/10 2/10 3/10	<0.05 <0.05 <0.05
			TID for 5 days	1 3 6	+12	0/10 1/10 6/10	Not Significant Not Significant <0.001
DD01044	A/Hong Kong/156/97 (H5N1) avian	Vehicle Control (phosphate buffered saline)	BID for 5 days	0	Not stated	0/10	NA

Study Number	Influenza Virus	Drug	Dosing Regimen	Dose (mg/kg/day)	Dosing Initiated (Hours After Viral Inoculation)[a]	Survival	P Value vs Vehicle Control
		Peramivir	BID for 5 days	10	+24 +36 +48 +60	8/10 6/10 5/10 4/10	NR NR NR NR
		Oseltamivir	BID for 5 days	10	+24 +36 +48 +60	9/10 7/10 7/10 7/10	NR NR NR NR
DD01029[b]	A/Shangdong/0 9/93 (H3N2)	Vehicle Control (saline)	TID	0	+12	3/20	NA
		Peramivir	QD	5 10	+12 +12	1/10 3/10	Not Significant Not Significant
			BID	5 10	+12 +12	7/10 6/10	<0.01 <0.05
			TID	5 10	+12 +12	6/10 6/10	<0.05 <0.05
DD00010	A/NWS/33 (H1N1)	Vehicle Control (saline)	BID for 3 days	0	+24	1/20	NA
		Peramivir	BID for 3 days	1 10 100 1 10 100 1 10 100	+24 +24 +24 +42 +42 +42 +60 +60 +60	7/10 8/10 10/10 9/10 10/10 10/10 9/10 9/10 8/8	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
		Vehicle Control (saline)	BID for 5 days	0	+24	0/18	NA

Study Number	Influenza Virus	Drug	Dosing Regimen	Dose (mg/kg/day)	Dosing Initiated (Hours After Viral Inoculation)[a]	Survival	P Value vs Vehicle Control
		Peramivir	BID for 5 days	1 10 100 1 10 100 1 10 100	+24 +24 +24 +42 +42 +42 +60 +60 +60	10/10 10/10 9/9 10/10 10/10 10/10 9/9 10/10 10/10	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001

a. In all studies, inoculation of mice with virus occurred prior to initial dosing of drug, after which the mice were dosed with drug one or more times daily for 5 days; in study DD00010, animals were dosed for 3 days and 5 days. Activity endpoints comprised a number of parameters including mortality, mean arterial oxygen saturation, lung consolidation, and/or virus titer in lung and/or brain.
b. These data are from Experiment NIA-174.
c. Peramivir was active when dosed 4 h prior to infection in this study.

Table A3: Activity of Intravenous Peramivir in Mice – Initiation of Treatment before or Immediately After Viral Inoculation

Study Number	Influenza Virus	Drug	Dosing Regimen	Dosing Initiated (Hours Relative to Viral Inoculation)	Dose (mg/kg/day)	Survival	P Value vs Vehicle Control[a]
S-021812-EB-103-N	A/PR/8/34 (H1N1)	Vehicle Control (0.5% Methyl cellulose)	BID oral for 5 days	Immediately after	0	0/20	NA
		Peramivir	Single IV dose	Immediately after	0.1 0.3 1 3 10	0/10 4/10 8/10 10/10 10/10	Not Significant <0.05 <0 05[b] <0 05[b] <0.05[c]
		Oseltamivir	BID oral for 5 days	Immediately after	0.3 1 3 10 30	0/9 2/10 3/10 6/10 8/10	Not Significant Not Significant <0.05 <0.05 <0.05
		Oseltamivir	Single oral dose	Immediately after	10 30	1/10 1/10	Not Significant Not Significant

Study Number	Influenza Virus	Drug	Dosing Regimen	Dosing Initiated (Hours Relative to Viral Inoculation)	Dose (mg/kg/day)	Survival	P Value vs Vehicle Control[a]
S-021812-EB-104-N	A/Kumamoto/Y5 /67 (H2N2)	Vehicle Control (0.5% Methyl cellulose)	BID oral for 5 days	Immediately after	0	0/20	NA
		Peramivir	Single IV dose	Immediately after	0.1 0.3 1	0/10 0/10 6/10	Not Significant Not Significant <0 05[b]
					3 10	10/10 10/10	<0 05[b] <0.05[c]
		Oseltamivir	BID oral for 5 days	Immediately after	0.3 1 3 10 30	0/10 0/10 4/10 10/10 10/10	Not Significant Not Significant <0.05 <0.05 <0.05
		Oseltamivir	Single oral dose	Immediately after	10 30	0/10 1/10	Not Significant Not Significant
S-021812-EB-105-N	A/Victoria/3/75 (H3N2)	Vehicle Control (0.5% Methyl cellulose)	BID oral for 5 days	Immediately after	0	0/20	NA
		Peramivir	Single IV dose	Immediately after	0.1 0.3 1 3 10	0/10 2/10 8/10 9/10 10/10	Not Significant Not Significant <0 05[b] <0.05 <0.05[c]
		Oseltamivir	BID oral for 5 days	Immediately after	0.3 1 3 10 30	0/10 0/10 5/10 7/10 10/10	Not Significant Not Significant <0.05 <0.05 <0.05
		Oseltamivir	Single oral dose	Immediately after	10 30	0/10 3/10	Not Significant <0.05
S-021812-EB-106-N	B/Lee/40	Vehicle Control (0.5% Methyl cellulose)	BID oral for 5 days	Immediately after	0	0/20	NA

Study Number	Influenza Virus	Drug	Dosing Regimen	Dosing Initiated (Hours Relative to Viral Inoculation)	Dose (mg/kg/day)	Survival	P Value vs Vehicle Control[a]
		Peramivir	Single IV dose	Immediately after	0.1 0.3 1 3 10	0/10 0/10 5/10 10/10 10/10	Not Significant Not Significant <0 05[b] <0 05[b] <0.05[c]
		Oseltamivir	BID oral for 5 days	Immediately after	0.3 1 3 10 30	0/10 0/10 2/10 5/10 10/10	Not Significant Not Significant Not Significant <0.05 <0.05
		Oseltamivir	Single oral dose	Immediately after	10 30	0/10 0/10	Not Significant Not Significant
PMV-EB-039-N	B/Maryland/1/59	Vehicle Control (0.5% Methyl cellulose)	BID oral for 5 days	Immediately after	0	0/20	NA
		Peramivir	Single IV dose	Immediately after	0.03 0.1 0.3 1 3 10	1/10 2/10 9/10 10/10 10/10 10/10	Not Significant Not Significant <0 05[b] <0 05[b] <0.05 <0.05[c]
		Oseltamivir	BID oral for 5 days	Immediately after	0.3 1 3 10	0/10 4/10 6/10 10/10	NS <0.05 <0.05 <0.05
		Oseltamivir	Single oral dose	Immediately after	10	2/10	Not Significant
S-021812-EB-123-N	A/Victoria/3/75 (H3N2)	Vehicle Control (saline)	Single IV dose	Immediately after	0	0/20	NA
		Peramivir	Single IV dose	Immediately after	1 3 10 30	6/10 9/10 10/10 10/10	<0.01[c] <0.01[c] <0.01[c] <0.01[c]

Reference ID: 3613983

Study Number	Influenza Virus	Drug	Dosing Regimen	Dosing Initiated (Hours Relative to Viral Inoculation)	Dose (mg/kg/day)	Survival	P Value vs Vehicle Control[a]
		Oseltamivir	Single IV dose	Immediately after	1 3 10 30	0/10 0/10 0/10 1/10	Not Significant Not Significant Not Significant Not Significant
S-021812-EB-082-N	A/Hong Kong/483/97) (H5N1) highly pathogenic virus	Vehicle Control (0.5% Methyl cellulose)	BID oral for 5 days	Immediately after	0	0/20	NA
		Peramivir	Single IV dose	Immediately after	10 30	6/10 7/10	<0.01 <0.01
		Peramivir	Once daily IV for 5 days	Immediately after	10 30	9/10 10/10	<0.01[bd] <0 01[d]
		Oseltamivir	BID oral for 5 days	Immediately after	10 30	3/10 7/10	<0.05 <0.01
		Zanamivir	BID IV for 5 days	Immediately after	10 30	1/10 5/10	Not Significant <0.01
NIA-527	A/Duck/MN/152 5/81 (H5N1) Avian virus	Vehicle Control (saline)	Single IV dose	1 h before	0	9/20	NA
		Peramivir	Single IV dose	1 h before	3 10 20	5/10 10/10 10/10	Not Significant <0.01 <0.01
		Oseltamivir	Single IV dose	1 h before	20	6/10	Not Significant
		Oseltamivir	BID oral for 5 days	4 h before	10	7/10	Not Significant

a. P values for other comparisons are given as footnotes
b. P <0.05 compared with oseltamivir (oral, BID for 5 days) at the same dose (and at same time for relevant studies)
c. P <0.05 compared with oseltamivir (single dose) at the same dose
d. P <0.05 compared with zanamivir (IV, BID for 5 days)

Table A4: Intravenous Activity of Peramivir in Mice with Comparison to Oseltamivir when Administered at 24 hours or Later After Viral Inoculation

Study Number	Viral Strain Influenza Virus	Drug	Dosing Regimen	Time Dosing Initiated (Hours After Viral Inoculation)	Total Daily Dose (mg/kg/day)	Survival	P Value vs Vehicle Control
PMV-EB-038-N	A/WS/33 (H1N1)	Vehicle Control (0.5% Methyl Cellulose)	BID oral for 5 days	48	0	0/20	NA

Study Number	Viral Strain Influenza Virus	Drug	Dosing Regimen	Time Dosing Initiated (Hours After Viral Inoculation)	Total Daily Dose (mg/kg/day)	Survival	P Value vs Vehicle Control
		Peramivir	Single IV dose	48	0.3	0/10	Not Significant
					1	3/10	Not Significant
					3	8/10	<0.001
					10	10/10	<0.001[c]
		Oseltamivir	BID oral for 5 days	48	0.3	0/10	Not Significant
					1	0/10	Not Significant
					3	5/10	Not Significant
					10	10/10	Not Significant
		Oseltamivir	Single oral dose	48	10	2/10	Not Significant
S-021812-EB-059-N	A/WS/33 (H1N1)	Vehicle Control (0.5% Methyl Cellulose)	BID oral for 5 days	48	0	0/20	NA
		Peramivir	Single IV dose	48	0.1	0/10	Not Significant
					0.3	0/10	Not Significant
					1	1/10	Not Significant
					3	3/10	<0.05
					10	8/10	<0.05
		Peramivir	IV once daily for 5 days	48	0.1	0/10	Not Significant
					0.3	0/10	Not Significant
					1	10/10	<0.05[ab]
					3	9/10	<0 05[a]
					10	10/10	<0.05
		Oseltamivir	BID oral for 5 days	48	0.3	0/10	Not Significant
					1	1/10	Not Significant
					3	3/10	<0.05
					10	10/10	<0.05
S-021812-EB-052-N	A/WS/33 (H1N1)	Vehicle Control (Saline)	4 IV doses: one each at 48, 51, 54, and 57 h after viral inoculation	48	0	0/20	NA
		Peramivir	Single IV dose	48	0.25	0/10	ND

Study Number	Viral Strain Influenza Virus	Drug	Dosing Regimen	Time Dosing Initiated (Hours After Viral Inoculation)	Total Daily Dose (mg/kg/day)	Survival	P Value vs Vehicle Control
					0.5	1/10	ND
					1	4/10	ND
					2	9/10	ND
					4	10/10	ND
					8	10/10	ND
		Peramivir	2 IV doses: one at 48 and one at 54 h after viral inoculation	48	0.25	3/10	ND
					0.5	2/10	ND
					1	6/10	ND
					2	10/10	ND
					4	10/10	ND
					8	10/10	ND
		Peramivir	4 IV doses: one each at 48, 51, 54, and 57 h after viral inoculation	48	0.25	1/10	ND
					0.5	2/10	ND
					1	5/10	ND
					2	10/10	ND
					4	10/10	ND
					8	10/10	ND
S-021812-EB-058-N	A/WS/33 (H1N1)	Vehicle Control (0.5% Methyl Cellulose)	BID oral for 5 days	24	0	0/10	NA
		Peramivir	Single IV dose	24	10	10/10	<0.05
				48	10	10/10	<0.05
				60	10	10/10	<0.05
				72	10	6/10	<0 05[a]
				84	10	0/10	Not Significant
				96	10	1/10	Not Significant
		Oseltamivir	BID oral for 5 days	24	10	10/10	<0.05
				48	10	9/10	<0.05
				60	10	9/10	<0.05
				72	10	0/10	Not Significant

Study Number	Viral Strain Influenza Virus	Drug	Dosing Regimen	Time Dosing Initiated (Hours After Viral Inoculation)	Total Daily Dose (mg/kg/day)	Survival	P Value vs Vehicle Control
				84	10	0/10	Not Significant
				96	10	0/10	Not Significant

a. P <0.05 compared to oseltamivir (oral, BID for 5 days) at the same dose (and at the same time for relevant studies).
b. P <0.05 compared to peramivir (IV, single) at the same dose.
c. P <0.001 compared to oseltamivir (single dose, oral) at the same time and same dose.

Table A5: Activity of Peramivir Administered at 100 mg/kg/day for 5 days in Immunocompromised Mice

Study No.	Immune Status[a]	Route of Drug Administration	Survival		Virus Strain	P value vs Control
			Peramivir	Vehicle		
DD00016	Immunosuppressed by treatment with CP at 100 mg/kg IP on Days -1, 3, and 7	Oral[b]	10/10	2/20	A/NWS/33 (H1N1)	<0.001
DD00043	Immunosuppressed by treatment with CP at 100 mg/kg IP on Days -1, +3, +7, +11, +15, +19, +23, +27, +31, and +35)	Oral[c]	10/10	0/20	A/NWS/33 (H1N1)	<0.001
DD01034	Immunosuppressed SCID mice	Oral[d]	7/8	13/18	A/NWS/33 (H1N1)	NR
S-021812-EB-131-N	Immunosuppressed by treatment with CP at 50 mg/kg IP on Days -1, +3, +7	IV[e]	10/10	0/20	A/NWS/33 (H1N1)	<0.05
Kitano et al.., 2013	Immunosuppressed by treatment with CP at 100 mg/kg IP on Days -1, +3, +7, +11, +15, and +19	IV[f]	78%[g] 90%[h]	0%	A/H1N1pdm	<0.01 <0.001

a. Virus inoculation on Day 0.
b. Dosed beginning 24 h after virus inoculation.
c. Dosed beginning 2.5 or 8 days after virus inoculation.
d. Dosed beginning 8 days after virus inoculation.
e. Dosed beginning 21 days after virus inoculation.
f. Peramivir 40 mg/kg once daily for 1, 5, 10, or 20 days, beginning at 1, 24, 48, or 72 h after virus inoculation.
g. Survival in animals dosed with peramivir 40 mg/kg once daily for 10 days.
h. Survival in animals dosed with peramivir 40 mg/kg once daily for 20 days.

Table A6: Activity of Peramivir against Mutant Virus in NA Inhibition Assay

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
Baz et al., 2007	Cell culture passaging with peramivir or zanamivir; recombinant[c]	H1N1	1 WT	-	0.11	1.1	1.4
		H1N1	1	H275Y	36.5	679.5	1 8
Gubareva et al., 2002[d]	Cell culture passaging with oseltamivir or zanamivir	H1N1	1 WT	NA	0.6	2.0	2 0
		H1N1	1	H275Y	525	>1000	2 5
Mishin et al., 2005	Cell culture passaging with oseltamivir or zanamivir	H1N1	2 WT	-	0.3–0.4	1.4–2.0	0.8–0.9
		H1N1	2	H275Y	2.4–40	220–>1000	0.9–1.0
Gubareva et al., 2001	In vitro passaging with oseltamivir or zanamivir, plus oseltamivir insusceptible clinical isolate	H1N1	1 WT	-	1.1	0.4	2 5
		H1N1	1	H275Y	40	400	1 5
Gubareva et al., 2010[e]	Clinical	2009 H1N1	1440 WT	-	0.03–0.50	0.05–2.41	0.04–1.24
		2009 H1N1	19–23	H275Y	0.45–15.65	6.24–155.00	0.18–0.53
Hurt et al., 2009	Clinical[f] (passaging in MDCK cells)	H1N1	372 WT	-	0 2	0.5	0.4
		H1N1	11	Q136K[g]	1.3–35.2	0.2–0.6	6.0–238.8
Hurt et al., 2009	Clinical	H1N1	96 WT	-	0 2	0.9	0.77
		H1N1	168	H275Y	111.6	1363.4	0 96
S–021812–EB–117–N	Clinical	H1N1	>1 WT	-	0.54–0.61	Not tested	Not tested
		H1N1	10	H275Y	19.9–88.9	112–378	1.25–2.63
Nguyen et al., 2010	Clinical	H1N1	2 WT	-	0.1	NR	NR
		H1N1	2	H275Y	13.8–15.0	NR	NR
		2009 H1N1	3 WT	-	0.1	NR	NR
		2009 H1N1	4	H275Y	9.2–13.1	NR	NR
S–021812–EB–133–N	Recombinant NA[h]	N1	1 WT	-	0.28	1.3	1 0
		N1	1	H275Y	17	180	0.76
		N1	1	N294S	1.6	36	1.6
S–021812–EB–135–N	Recombinant	H1N1	2 WT	-	0.55–0.62	1.13–1.35	1.17–1.39
Abed et al., 2004	Recombinant[c]	H1N1	1 WT	-	0.25	1.0	0.75
		H1N1	1	H275Y	12.2	440.6	0 95
		H1N1	1	E119Q	0.4	9.0	3.1
Abed et al., 2006	Recombinant NA[i]	N1	1 WT	NA	0.1	0.8	0.6

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
		N1	1	E119V	606.0	1295.7	1201.0
		N1	1	H275Y	31.3	565.5	0.6
		N1	1	N294S	1 5	147.5	3 2
Okomo-Adhiambo et al., 2010	Clinical samples passaged in cellculture	H1N1	1 WT	-	0.17	0.52	0 51
		H1N1	1	H275Y	22.6	189.3	0 95
		H1N1	2	D151G	0.05	0.41	0 83
		H1N1	8	D151G + H275Y	197.3	618.3	6 92
		H1N1	20	D151N	0.07	0.5	0 54
		H1N1	15	D151N + H275Y	122.1	415.5	1 28
Pizzorno et al., 2011	Recombinant	2009 H1N1	1 WT	-	0.1	0.5	0 2
		2009 H1N1	1	E119G	4.6	1.3	124.9
		2009 H1N1	1	E119V	2 3	27.7	85.7
		2009 H1N1	1	D198G	0 2	7.7	0 9
		2009 H1N1	1	I222V	0 2	2.6	0.4
		2009 H1N1	1	H275Y	26.6	451.9	0.1
		2009 H1N1	1	N294S	1 0	95.8	0 5
		2009 H1N1	1	I222V + H275Y	119.8	797.4	0 3
		2009 H1N1	1	S334N + H275Y	16.0	302.8	0.1
		2009 H1N1	1	S334N	0.1	0.4	0 2
Hurt et al., 2012	Recombinant	2009 H1N1	1 WT	-	0.09	0.3	0 24
		2009 H1N1	1	I117V	0.08	1.42	0 54
		2009 H1N1	1	I117V + H275Y	47.08	568.84	0.44
		2009 H1N1	1	H275Y	19.72	195.02	0 26
Hurt et al., 2011	Recombinant	2009 H1N1	1 WT	-	0.09	0.30	0 24
		2009 H1N1	1	S246N	0.18	0.96	0 57
		2009 H1N1	1	H275Y	19.72	195.02	0 26
		2009 H1N1	1	S246N + H275Y	60.91	2149.96	1 25

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
Pizzorno et al., 2012	Recombinant	2009 H1N1	1 WT	-	0.06	0.46	0.15
		2009 H1N1	1	I222V	0.15	2.63	0 35
		2009 H1N1	1	I222R	0.60	24.48	1.10
		2009 H1N1	1	H275Y	39.66	451.9	0.14
		2009 H1N1	1	H275Y + I222V	162.39	797.4	0 32
		2009 H1N1	1	H275Y + I222R	1040.89	757.39	2 32
Eshaghi et al., 2011	Clinical	2009 H1N1	1 WT	-	NT	0.34	0 20
		2009 H1N1	1	I222R	NT	9.49	2.46
Nguyen et al., 2010	Clinical	2009 H1N1	1 WT	-	0.24	1.03	0 39
		2009 H1N1	1	I222K	1.06	40.06	2 32
Hurt et al., 2009	Recombinant[j]	H5N1	1 WT	-	0 3	0.8	0 5
		H5N1	1	H275Y	142.4	695.6	0.6
		H5N1	1	I222M + H275Y[m]	891.4	6126.2	1.4
		H5N1	1	I222V + H275Y[n]	562.1	1469.7	1.1
		H5N1	1	E119G	43.6	3.1	677.3
	Recombinant[k]	H5N1	1 WT	-	0 3	0.2	0.6
		H5N1	1	H275Y	27.0	498.9	1 0
	Recombinant[l]	H5N1	1 WT	-	0 2	1.5	0.7
		H5N1	1	H275Y	82.8	1178.3	0 8
		H5N1	1	I222M + H275Y[o]	95.5	3009.1	0 5
		H5N1	1	I222V + H275Y[p]	213.0	1502.8	1 5
Ilyushina et al., 2012	Cell culture with or without oseltamivir, zanamivir, or peramivir	H5N1	1 WT	-	1.7	12.2	4.4
		H5N1	1	R156K	59.1	26.5	1049.9
		H5N1	1 WT	-	2 8	39.0	8 3
		H5N1	1	E119A	188.8	380.6	2116.3
Earhart et al., 2010	Clinical	H5N1	1 WT	-	0.46	1.5	0 5
		H5N1	1	N294S	1.7	138.9	1 5
Gubareva et al., 2002[d]	Cell culture passaging with oseltamivir or zanamivir	H1N2	1 WT	-	1.7	0.9	3 0
		H1N2	4	E119D	2.0–95	1.0–>1000	1000–10,000

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
Gubareva et al., 2001	Cell culture passaging with oseltamivir or zanamivir, plus oseltamivir insusceptible clinical isolate	H1N2	3	E119G	1 8	0.5	100
				E119A	1.6	1.1	50
				E119D	9 5	0.5	150
		H1N2	1 WT	-	1.1	0.4	2 5
		H1N2	1	R292K	30	3750	20
S–021812–EB–135–N	Recombinant	H1N2	2	H274Y	18.2–21.5	150–152	1.60–1.65
P98–1812– 001[q]	Cell culture passaging with peramivir	H2N2	1 WT	-	0.4	1.5	2.1
		H2N2	1	R292K	4	4500	210
Mishin et al., 2005	Cell culture passaging with oseltamivir or zanamivir	H2N2	1 WT	-	0.4	0.6	3 0
		H2N2	1	R292K	28	>1000	15
Mishin et al., 2005	Cell culture passaging with oseltamivir or zanamivir	H3N2	2 WT	-	0.4–1.3	0.3–0.8	1.0–2.0
		H3N2	2	E119V	0.5–1.5	2.8–105	2.5–83
Baz M et al., 2006	Clinical	H3N2	1	E119V + I222V	1.71	2012	5 29
		H3N2	WT	-	0.23	2.0	3.6
Okomo–Adhiambo et al., 2010	Clinical with subsequent passaging in MDCK cells	H3N2	1[r]	E119I	214.1	430.6	18.6
DD99005	Cell culture passaging with zanamivir	H4N2	1 WT	-	1.3–1.4	0.2–0.4	3
		H4N2	3	E119D	8 5	2.7	350
				E119G	2 0	0.25	210
				E119A	1 3	1.0	150
		H4N2	1	R292K	27	5000	24
Mishin et al., 2005	Cell culture passaging with oseltamivir or zanamivir	H4N2	1 WT	-	0.6	0.4	2 0
		H4N2	4	R292K	26	3000	15
				E119A	0 8	3.5	200
				E119D	19	1.8	645
				E119G	1 5	0.6	400
S–021812–EB–133–	Recombinant[h]	N2	1 WT	-	0.82	0.50	2 8

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
N		N2	7	D151A	81	31	>1000
				D151N	120	100	840
				D151E	2.4	1.5	7 2
				D151G	73	34	960
				R292K	140	>1000	86
				N294S	2 5	78	16
				D151V	>1000	340	>1000
			4	E119V	2 9	37	11
				E119G	>1000	>1000	>1000
				E119A	>1000	>1000	>1000
				E119D	>1000	>1000	>1000
Abed et al., 2006	Recombinant[h,s]	N2	1 WT	-	0.4	0.4	0 3
		N2	1	E119V	1 2	442.0	2.6
		N2	1	H274Y	0.4	3.3	1.7
		N2	1	R292K	294.7	>5000	45.0
		N2	1	N294S	0.4	808.0	2 8
DD00048	Cell culture passaging with peramivir	B	1 WT	-	8	50	29
		B	1	H274Y	127	120	14
DD99005	Cell culture passaging with zanamivir	B	1 WT	-	1.5–2.1	5–10	3
		B	1	R152K	≥ 1000	600	100
Sleeman et al, 2011	Clinical	B	39 WT	-	0.61	8.33	3 31
		B	2	I222V/I	2.43-2.77	18.98-20.39	8.13-8 58
Fujisaki et al, 2012	Clinical with 5 passages in MDCK cells	B	WT	-	0.62	3.17	1 37
		B	1	E105K	66.05	8.28	10.37
Fujisaki et al, 2013	Clinical with passages in MDCK cells	B	WT	-	0.32	1.58	0.75
		B	1	Q138R	18.31	3.18	9 27
		B		P139S	8.32	5.87	6.65
		B		G140R	12.74	7.07	6.18
Gubareva et al.,	Cell culture passaging with	B	1 WT	-	2 0	40	3 2

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
2002[d]	oseltamivir or zanamivir	B	1	R152K	>1000	>1000	10,000
Mishin et al., 2005	Cell culture passaging with oseltamivir or zanamivir	B	2 WT	-	1–1.2	4.0–33	1.6–3.2
		B	2	R152K	400	400	220
				D198N	5 8	304	15
Gubareva et al., 2001	Cell culture passaging with oseltamivir or zanamivir, plus oseltamivir insusceptible clinical isolate	B	1 WT	-	1.4	4	3 5
		B	1	R152K	570	750	100
Hurt et al., 2006	Clinical	B	1 WT[t]	-	0 9	17.8	2 8
		B	1	D198E	16.3	209.3	18.0
Ison et al., 2006	Clinical	B	1	D198N	NT	86.0	2 2
			1 WT	-	NT	37.0	1.4
McSharry et al., 2004	Clinical	B	1 WT	-	0.1	3.4	0 5
		B	1	R152K	81.4	320.3	36.9
S–021812–EB–133–N	Recombinant[h]	B	1 WT	-	3 5	21	12
		B	10	R152K	>1000	>1000	460
				D198N	9 3	69	34
				D198Y	110	430	200
				D198E	30	91	56
				N221K	9 9	31	16
				I222T	36	100	22
				S250G	3 2	27	200
				H274Y	82	52	6 0
				R371K	310	>1000	230
Jackson et al., 2005	Recombinant[u]	B	1 WT	-	0 3	1.8	1 0
		B	1	E116D	>550	>550	>550
		B	1	E116A	>550	>550	>550
		B	1	E116V	191	>550	1 9
		B	1	E116G	>550	57.0	>550
		B	1	R149K	73.8	462.2	4.6

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
		B	1	R292K	172	>550	27.9
Higgins et al., 2012	Clinical with 1 passage in cell culture	B	1 WT	-	0.32	1.32	1 35
		B	1	H275Y	11.12	19.4	0 93
McKimm–Breschkin et al., 2012	Cell culture passaging with oseltamivir or zanamivir	H1N1/09[v]	1 WT	-	0 3	7.7	1.4
		H1N1/09	1	D198G	2.7	85.5	9.6
		H1N1/09	1	D198G H275Y	1700	14000	12.6
		H1N1/09	1	N146S + G158E[w]	0.35	14.8	2 8
McKimm–Breschkin et al., 2013	Cell culture passaging	H5N1[x]	1 WT	-	0.7	0.6	2.1
			1	P154S	0.4	1.8	4.7
		H5N1[y]	1 WT	-	0.6	19.6	1 3
			1	I222T	1 3	86.1	2 2
			1	I222M	2 0	310.3	2.6
			1	I222V	1 9	70.1	3.1
McKimm–Breschkin et al., 2013	Cell culture passaging	H1N1[z]	1 WT	-	0 5	2.6	1 3
			2	Y155H	18.5	78.6	172.2
				V114I	2	17.9	4 5
Okomo–Adhiambo et al., 2013	Clinical[aa]	H1N1/09	326	-	0.03–0.13	0.10–0.56	0.08–0.40
		H3N2	407	-	0.04–0.28	0.03–0.27	0.14–0.89
		B	346	-	0.15–2.47	2.13–33.22	0.46–3.24
	Clinical, passaged in MDCKs	H1N1 2009	1	N325K	0.16	4.27	1.74
		H1N1 2009	1	G460S	0.26	0.8	1 06
		H1N1 2009	2	S246N	0.15-0.17	0.56-0.73	0.36-0 39
		H1N1 2009	1	D198N	0.21	0.65	0.49
		B	1	G140R + N144K	210.68	68.70	11.73
		B	1	I120V	4.36	21.10	0 98
		B	1	A136S	4.77	33.22	0.6
		B	1	M400V	4.36	21.10	0 98
Tamura et al., 2013	Cell culture passaging P2[bb]	H3N2	1777	E119V	0.20	65.36	1.19
	Cell culture passaging P3[bb]	H3N2	1777	E119V	10.42	720.45	195.00

Report Number	Source of Variant Virus	Influenza Virus Subtype	No. of Virus Strain Tested	NA Amino Acid Substitution	Range of IC_{50} Value (nM)		
					Peramivir	Oseltamivir	Zanamivir
	Recombinant	H3N2	1	None	0.08	0.16	0 21
				E119V	0.12	44.93	0 54
				T148I	0.06	0.04	1 33
				E119V, T148I	9.54	984.62	220.48

a. Where the data range was not provided in the report, the mean IC_{50} value is presented.
b. Oseltamivir.
c. Recombinant influenza A/WSN/33 (H1N1) virus.
d. Data presented here were taken from Assay 1 results in this publication.
e. This publication used clinical isolates, without one–to–one comparisons with WT strains. Overall, 3335 clinical isolates were tested for susceptibility to oseltamivir and zanamivir, and a subset of 1440 of these isolates was tested for susceptibility to peramivir.
f. A number of strains were isolated in the years 2006–2008 with variable IC_{50} values for peramivir and zanamivir. The Q136K amino acid substitution was not detected in the primary clinical isolates. It was present only in virus grown in MDCK cells.
g. Includes recombinant virus with 7 segments from A/PuertoRico/8/1934 and the NA segment from A/Philippines/1279/2006 virus.
h. This report provided information only regarding N1 or N2 subtype.
i. Recombinant NA proteins from influenza A/WSN/33 (H1N1) and A/Sydney/5/97-like (H3N2) viruses.
j. Recombinant virus with NA gene from influenza A/Chicken/Laos/26/2006 (H5N1) virus and other genes from A/PR/8/34 (H1N1) virus.
k. Recombinant virus with HA and NA genes from A/Vietnam/1203/2004 and internal genes from A/PR/8/34 (H1N1) virus.
l. Recombinant from influenza A/New Caledonia/20/99 (H1N1).and A/PR/8/34 (H1N1) virus.
m. Values for variant I222M were 0.3, 27.8, and 0.5, for peramivir, oseltamivir and zanamivir, respectively.
n. Values for variant I222V were 0.3, 5.3, and 0.7, for peramivir, oseltamivir and zanamivir, respectively.
o. Values for variant I222M were 0.2, 11.9, and 0.4, for peramivir, oseltamivir and zanamivir, respectively.
p. Values for variant I222V were 0.3, 4.5, and 1.3 for peramivir, oseltamivir and zanamivir, respectively.
q. The data in this report were also included in DD00011. Data were only presented as fold–increases for viruses with reduced suscept bility in the report; actual values have been calculated and presented here.
r. Data are presented from a fluorescent NA assay using clone 2. Initial work in this paper used isolates with mixed E119V and E119I amino acid substitutions; those data are not presented in this table.
s. Recombinant from influenza A/Sydney/5/97 (H3N2) virus.
t. This publication used a clinical isolate that was composed of a mixed viral population; a comparison was therefore made between components of the mixed population that did or did not have the D198E mutation, rather than to WT.
u. Recombinant from influenza B/Beijing/1/87 virus.
v. H1N1/09 pandemic influenza virus (A/Swine/Shepparton/6/2009).
w. HA mutation; all other NA mutant viruses had WT HA.
x. Clade 1.1 avian viruses; IC_{50} values obtained from a reaction in which virus was preincubated with inh bitor for 30 min prior to substrate addition
y. Clade 2.1 avian viruses; IC_{50} values obtained from a reaction in which virus was preincubated with inh bitor for 30 min prior to substrate addition.
z. A/Hokkaido/15/02 H1N1 virus; IC50 values obtained from a reaction in which virus was preincubated with inh bitor for 30 min prior to substrate addition.
aa. This publication used clinical isolates obtained globally during 2011; no comparisons were made with WT strains. Mean IC_{50} values are presented, with exclusion of extreme outliers.
bb. This publication tested 1777 clinical isolates obtained during the 2010–2011, of which 1 virus, A/Mississippi/05/2011 was found to be oseltamivir–resistant and selected for further study. (This virus was collected from a patient treated with oseltamivir). P2 and P3 refer to virus isolates passaged 2 and 3 times, respectively, on MDCK cells.

Table A7: Activity of Peramivir against Virusus with Decreased Susceptibility to Peramivir in Mice

Report Number	Influenza Virus	Protein	Amino Acid Substitution	Drug	Dosing Regimen	Dose (mg/kg/day)	Survival	P Value vs Vehicle Control	Other information
DD00020	A/Shandon g/09/92 (H3N2)	HA	K189E	Vehicle control (Saline)	BID oral for 5 days beginning 4 h before inocula ion	0	1/19	NA	Viral titer used for inoculation not given
				Peramivir	BID oral for 5 days beginning 4 h before inocula ion	1	6/10	<0.01	Increase in mean oxygen saturation
				Peramivir	BID oral for 5 days beginning 4 h before inocula ion	10	7/10	<0.001	Increase in mean oxygen saturation
				Peramivir	BID oral for 5 days beginning 4 h before inocula ion	100	9/9	<0.001	Increase in mean days to death and mean oxygen saturation
DD00047	A/Shandon g/09/92 (H3N2)	WT NA	NA	Vehicle control (Saline)	BID oral for 3 5 days beginning 4 h prior to inocula ion	0	Not evaluated	NA	Viral titer used for inoculation not given Mean EC50 in cell culture of virus from lung = 0.002 µM
				Peramivir	BID oral for 3 5 days beginning 4 h prior to inocula ion	100	Not evaluated	NA	Mean EC50 in cell culture of virus from lung = 0.002 µM
	A/Shandon g/09/92 (H3N2) Variant	HA	K189E	Vehicle control (Saline)	BID oral for 3 5 days beginning 4 h prior to inocula ion	0	Not evaluated	NA	Mean EC50 in cell culture of virus from lung = 11.7 µM
				Peramivir	BID oral for 3 5 days beginning 4 h prior to inocula ion	100	Not evaluated	NA	Mean EC50 in cell culture of virus from lung = 1.8 µM. Decreased viral lung titer on D3 compared to
S-021812-E B-134-N	A/PR/8/34 (H1N1)	NA	H275Y	Vehicle control (0.5% MC)	BID oral for 5 days beginning immediately after	0	0/20	NA	Inoculated with 6.4 x 10-5 TCID50/mouse

Report Number	Influenza Virus	Protein	Amino Acid Substitution	Drug	Dosing Regimen	Dose (mg/kg/day)	Survival	P Value vs Vehicle Control	Other information
					inocula ion				
				Peramivir	Single IV dose immediately after inocula ion	1	2/10	<0.05	None
						3	7/10	<0.05	None
						10	8/10	<0.05	None
						30	9/10	<0.05	None
						100	10/10	<0.05	None
				OSE	BID oral for 5 days beginning immediately after inocula ion	1	0/10	NS	None
						3	0/10	NS	None
						10	0/10	NS	None
						30	5/9	<0.05	None
						100	9/9	<0.05	None
EPB09-1812-001	A/WSN/33 (H1N1) WT	WT	NA	Vehicle Control (Saline)	IM QD 5 days beginning 1 h before viral inoculation	0	0/8	NA	Inoculated with 8 x 10^3 PFU
				Peramivir	IM 1 h before viral inocula ion	45	8/8	ND	Less body weight loss vs. control mice infected with WT virus
						90	8/8	ND	Less body weight loss vs. control in mice infected with WT virus
					IM QD 5 days beginning 1 h before viral inoculation	45	8/8	ND	Less body weight loss and decreased viral lung titer vs. control mice infected with WT virus
	A/WSN/33 (H1N1) variant	NA	H275Y	Vehicle Control (Saline)	IM QD 5 days beginning 1 h before inocula ion	0	1/8	NA	NA
				Peramivir	IM once at 1 h before viral inoculation	45	7/8	ND	Decreased viral lung titer vs control mice infected with variant virus
						90	8/8	ND	Less body weight loss and decreased viral lung titer vs. control mice infected with variant virus
					IM QD 5 days beginning 1 h before viral inoculation	45	8/8	ND	Less body weight loss and decreased viral lung titer vs. control mice infected with variant virus

Reference ID: 3613983

Report Number	Influenza Virus	Protein	Amino Acid Substitution	Drug	Dosing Regimen	Dose (mg/kg/day)	Survival	P Value vs Vehicle Control	Other information
Abed et al., 2012[a]	A/WSN/33 (H1N1) variant	NA	H275Y	Untreated	NA	0	2/8	NA	Inoculated with 5.7 x 103 PFU
				Peramivir	IM QD 5 days beginning 24 h after inocula ion	45	8/8	<0.0001	Less body weight loss and decreased viral lung titer vs. untreated controls
					IM once at 24 h after viral inoculation	90	8/8	<0.0001	Less body weight loss and decreased viral lung titer vs. untreated controls
				OSE	Oral, QD 5 days beginning 24 h after viral inoculation	1	2/8	NS	None
					Oral, QD 5 days 5 days 24 h after viral inocula ion	10	8/8	<0.0001	Less body weight loss and decreased viral lung titer vs. untreated controls
				Untreated	NA	0	2/8	5.7	Inoculated with 8 x 103 PFU
				Peramivir	IM QD 5 days beginning 48 h after inocula ion	45	8/8	<0.0001	Less body weight loss and decreased viral lung titer vs. untreated controls
					IM once at 48 h after viral inoculation	90	8/8	<0.0001	Less body weight loss and decreased viral lung titer vs. untreated controls
				OSE	Oral, QD 5 days beginning 48 h after viral inoculation	1	0/8	NS	None
					Oral, QD 5 days beginning 48 h after viral inoculation	10	5/8	NS	None

a. In this study, animals infected with WT virus were also evaluated. In the mice infected with WT virus, peramivir and oseltamivir were equal in preventing mortality and weight loss, and reducing viral lung titers, even when dosed beginning 48 hours after infection.

Appendix 2: Resistance Data (Individual Subjects)

Table B1: Individual Resitance Data (Subjects Infected with A/H1N1)

Subject ID	Isolate Date	NA	HA	$TCID_{50}$ (log_{10} copies/mL)	Per IC_{50} value (nM)	Ose IC_{50} value (nM)	Zan IC_{50} value (nM)	Time to Alleviation of Symptoms
0722T0621.AF1.005-1	Day 1	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y^{BL}, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, V427I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	5.10	9.43	44.00	2.30	
0722T0621.AF1.005-1	Day 3	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y^{BL}, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, V427I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	3.40	20.80	94.00	2.48	61.83
0722T0621.AL1.080-1	Day 1	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	4.40	0.97	1.81	2.67	
0722T0621.AL1.080-1	Day 5	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	3.10	14.50	100.00	1.62	54.42
0722T0621.BI1.135-6	Day 1	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	4.10	1.35	2.48	3.53	
0722T0621.BI1.135-6	Day 5	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	1.40	27.60	100.00	1.76	44.97
0722T0621.BQ1.148-4	Day 1	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	5.40	1.12	1.98	2.85	
0722T0621.BQ1.148-4	Day 5	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	1.10	27.80	100.00	1.78	174.08
0722T0621.CH1.163-5	Day 1	H45N^{BL}, V48I^{BL}, I75V^{BL}, K78E^{BL}, E214G^{BL}, V234M^{BL}, G249K^{BL}, T287I^{BL}, K329E^{BL}, G354D^{BL}, D382N^{BL}, K432R^{BL}	N/A	5.40	1.47	2.65	3.94	
0722T0621.CH1.163-5	Day 5	H45N^{BL}, V48I^{BL}, I75V^{BL}, K78E^{BL}, E214G^{BL}, V234M^{BL}, G249K^{BL}, H275Y, T287I^{BL}, K329E^{BL}, G354D^{BL}, D382N^{BL}, K432R^{BL}	N/A	4.10				140.08
0722T0621.CH1.163-5	Day 9	H45N^{BL}, V48I^{BL}, I75V^{BL}, K78E^{BL}, E214G^{BL}, V234M^{BL}, G249K^{BL}, H275Y, T287I^{BL}, K329E^{BL}, G354D^{BL}, D382N^{BL}, K432R^{BL}	N/A	1.10	30.30	100.00	1.89	140.08
0722T0621.CM1.172-4	Day 1	N/A	N/A	4.80				
0722T0621.CM1.172-4	Day 3	H45S, V48I, I75V, K78E, E214G, V234M, G249K, H275Y, T287I, K329E, G354D, D382N, K432R, S451G, T453I	N/A	1.10	27.10	87.70	1.94	58.60
0722T0621.AP1.086-5	Day 1	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, I195V^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	2.10	1.72	2.13	4.21	
0722T0621.AP1.086-5	Day 5	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, I195V^{BL}, N209K^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y, G331R^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, K432R^{BL}, S451G^{BL}, T453I^{BL}	N/A	2.40	31.50	100.00	2.34	29.87
BCX1812-211.069.028	Day 1	N68S^{BL}, I75V^{BL}, Q222R^{BL}, H275Y^{BL}, E332K^{BL}, N344D^{BL}, K432R^{BL}, N450D^{BL}, W458G^{BL}, P459Q^{BL}, D460T^{BL}, G461V^{BL}, A462L^{BL}		3.50	5.01	64.42	0.25	

Subject ID	Isolate Date	NA	HA	$TCID_{50}$ (log_{10} copies/mL)	Per IC_{50} value (nM)	Ose IC_{50} value (nM)	Zan IC_{50} value (nM)	Time to Alleviation of Symptoms
BCX1812-211.069.028	Day 3	N68S^{BL}, I75V^{BL}, Q222R^{BL}, H275Y^{BL}, E332K^{BL}, N344D^{BL}, K432R^{BL}, N450D^{BL}, W458G^{BL}, P459Q^{BL}, D460T^{BL}, G461V^{BL}, A462L^{BL}		4.50	6.89		0.26	31.57
BCX1812-211.662.007	Day 1	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, L367I^{BL}, D382N^{BL}, V393I^{BL}, I396L^{BL}, K432R^{BL}		5.25	0.12	6.89	4.45	
BCX1812-211.662.007	Day 5	V48I^{BL}, I75V^{BL}, S82P^{BL}, A86V^{BL}, R130K^{BL}, M188I^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y, L367I^{BL}, D382N^{BL}, V393I^{BL}, I396L^{BL}, K432R^{BL}		3.50	8.34	95.45	2.12	31.93
BCX1812-211.671.004	Day 1	H45H/N^{BL}, V48I^{BL}, I75V^{BL}, K78K/E^{BL}, S82S/P^{BL}, R130R/K^{BL}, M188M/I^{BL}, E214G^{BL}, V234M^{BL}, I267M^{BL}, T287T/I^{BL}, K329K/E^{BL}, G354G/D^{BL}, L367L/I^{BL}, D382N^{BL}, V393V/I^{BL}, I396I/L^{BL}, K432R^{BL}		5.25	5.52	15.96	7.86	
BCX1812-211.671.004	Day 3	H/N45H, V48I^{BL}, I75V^{BL}, K/E78K, S/P82P, R/K130K, M/I188I, E214G^{BL}, V234M^{BL}, I267M^{BL}, H275Y, T/I287T, K/E329K, G/D354G, L/I367I, D382N^{BL}, V/I393I, I/L396L, 421C/W, K432R^{BL}		0.75				315.60
BCX1812-301.202.039	Day 1	I13V^{BL}, S14C^{BL}, I15M^{BL}, A16T^{BL}, I19M^{BL}, S21N^{BL}, M23I^{BL}, A34I^{BL}, T40L^{BL}, S42N^{BL}, N44S^{BL}, H45Q^{BL}, T46I^{BL}, G47E^{BL}, V48T^{BL}, R52S^{BL}, I53V^{BL}, S59N^{BL}, H64Q^{BL}, N70S^{BL}, V74F^{BL}, K78Q^{BL}, D79S^{BL}, K80V^{BL}, T81V^{BL}, T84K^{BL}, S93P^{BL}, I94V^{BL}, T101S^{BL}, H126P^{BL}, V149I^{BL}, A157T^{BL}, L163I^{BL}, A166V^{BL}, K173R^{BL}, M188I^{BL}, G189N^{BL}, E214D^{BL}, K220R^{BL}, K221N^{BL}, Q222N^{BL}, N248D^{BL}, A250Q^{BL}, K257R^{BL}, V263I^{BL}, T264V^{BL}, I267V^{BL}, L269M^{BL}, F274Y^{BL}, H275Y^{BL}, T285S^{BL}, G286S^{BL}, T287E^{BL}, V288I^{BL}, M289T^{BL}, D311E^{BL}, V321I^{BL}, K329N^{BL}, G331K^{BL}, E332T^{BL}, N336G^{BL}, T339S^{BL}, V340S^{BL}, D341N^{BL}, Y351F^{BL}, N365I^{BL}, R366S^{BL}, L367S^{BL}, D382G^{BL}, S385N^{BL}, D386N^{BL}, V389I^{BL}, V393I^{BL}, A395G^{BL}, T397N^{BL}, D398E^{BL}, V427I^{BL}, L430R^{BL}, T436I^{BL}, I437W^{BL}, W438T^{BL}, T439S^{BL}, S440G^{BL}, G441S^{BL}, S443I^{BL}, I444S^{BL}, S445F^{BL}, F446C^{BL}, C447G^{BL}, G448V^{BL}, V449N^{BL}, N450S^{BL}, S451D^{BL}, D452T^{BL}, T453V^{BL}, A454G^{BL}, N455W^{BL}, W456S^{BL}, S457W^{BL}, W458P^{BL}, P459D^{BL}, D460G^{BL}	P100S^{BL}, S160G^{BL}, S202T^{BL}, A214T^{BL}, S220T^{BL}, I338V^{BL}, E391K^{BL}, S468N^{BL}	3.50	43.92	499.70	0.38	

Subject ID	Isolate Date	NA	HA	$TCID_{50}$ (log_{10} copies/mL)	Per IC_{50} value (nM)	Ose IC_{50} value (nM)	Zan IC_{50} value (nM)	Time to Alleviation of Symptoms
BCX1812-301.202.039	Day 3	I13V^{BL}, S14C^{BL}, I15M^{BL}, A16T^{BL}, I19M^{BL}, S21N^{BL}, M23I^{BL}, A34I^{BL}, T40L^{BL}, S42N^{BL}, N44S^{BL}, H45Q^{BL}, T46I^{BL}, G47E^{BL}, V48T^{BL}, R52S^{BL}, I53V^{BL}, S59N^{BL}, H64Q^{BL}, N70S^{BL}, V74F^{BL}, K78Q^{BL}, D79S^{BL}, K80V^{BL}, T81V^{BL}, T84K^{BL}, S93P^{BL}, I94V^{BL}, T101S^{BL}, H126P^{BL}, V149I^{BL}, A157T^{BL}, L163I^{BL}, A166V^{BL}, K173R^{BL}, M188I^{BL}, G189N^{BL}, E214D^{BL}, K220R^{BL}, K221N^{BL}, Q222N^{BL}, N248D^{BL}, A250Q^{BL}, K257R^{BL}, V263I^{BL}, T264V^{BL}, I267V^{BL}, L269M^{BL}, F274Y^{BL}, H275Y^{BL}, T285S^{BL}, G286S^{BL}, T287E^{BL}, V288I^{BL}, M289T^{BL}, D311E^{BL}, V321I^{BL}, K329N^{BL}, G331K^{BL}, E332T^{BL}, N336G^{BL}, T339S^{BL}, V340S^{BL}, D341N^{BL}, Y351F^{BL}, N365I^{BL}, R366S^{BL}, L367S^{BL}, D382G^{BL}, S385N^{BL}, D386N^{BL}, V389I^{BL}, V393I^{BL}, A395G^{BL}, T397N^{BL}, D398E^{BL}, V427I^{BL}, L430R^{BL}, T436I^{BL}, I437W^{BL}, W438T^{BL}, T439S^{BL}, S440G^{BL}, G441S^{BL}, S443I^{BL}, I444S^{BL}, S445F^{BL}, F446C^{BL}, C447G^{BL}, G448V^{BL}, V449N^{BL}, N450S^{BL}, S451D^{BL}, D452T^{BL}, T453V^{BL}, A454G^{BL}, N455W^{BL}, W456S^{BL}, S457W^{BL}, W458P^{BL}, P459D^{BL}, D460G^{BL}	P100S^{BL}, S160G^{BL}, S202T^{BL}, A214T^{BL}, S220T^{BL}, I338V^{BL}, E391K^{BL}, S468N^{BL}	<LLOQ				123.00
BCX1812-301.575.002	Day 1	I13V^{BL}, S14C^{BL}, I15M^{BL}, A16T^{BL}, I19M^{BL}, I20A^{BL}, S21N^{BL}, M23I^{BL}, A34I^{BL}, T40L^{BL}, S42N^{BL}, N44S^{BL}, H45Q^{BL}, T46I^{BL}, G47E^{BL}, V48T^{BL}, R52S^{BL}, I53V^{BL}, S59N^{BL}, H64Q^{BL}, N70S^{BL}, V74F^{BL}, I75A^{BL}, K78Q^{BL}, D79S^{BL}, K80V^{BL}, T81V^{BL}, T84K^{BL}, S93P^{BL}, I94V^{BL}, T101S^{BL}, H126P^{BL}, V149I^{BL}, A157T^{BL}, L163I^{BL}, A166V^{BL}, K173R^{BL}, M188I^{BL}, G189N^{BL}, E214D^{BL}, K220R^{BL}, K221N^{BL}, Q222N^{BL}, V232A^{BL}, N248D^{BL}, A250Q^{BL}, K257R^{BL}, V263I^{BL}, T264V^{BL}, I267V^{BL}, L269M^{BL}, F274Y^{BL}, T285S^{BL}, G286S^{BL}, T287E^{BL}, V288I^{BL}, M289T^{BL}, D311E^{BL}, V321I^{BL}, K329N^{BL}, G331K^{BL}, E332T^{BL}, N336G^{BL}, T339S^{BL}, V340S^{BL}, D341N^{BL}, Y351F^{BL}, N365I^{BL}, R366S^{BL}, L367S^{BL}, D382G^{BL}, S385N^{BL}, D386N^{BL}, V389I^{BL}, V393I^{BL}, A395G^{BL}, T397N^{BL}, D398E^{BL}, V427I^{BL}, L430R^{BL}, T436I^{BL}, I437W^{BL}, W438T^{BL}, T439S^{BL}, S440G^{BL}, G441S^{BL}, S443I^{BL}, I444S^{BL}, S445F^{BL}, F446C^{BL}, C447G^{BL}, G448V^{BL}, V449N^{BL}, N450S^{BL}, S451D^{BL}, D452T^{BL}, T453V^{BL}, A454G^{BL}, N455W^{BL}, W456S^{BL}, S457W^{BL}, W458P^{BL}, P459D^{BL}, D460G^{BL}	P100S^{BL}, H155Q^{BL}, S160G^{BL}, S202T^{BL}, A214T^{BL}, S220T^{BL}, P288L^{BL}, I338V^{BL}, E391K^{BL}	4.00	0.37	1.20	0.58	
BCX1812-301.575.002	Day 6	I13V^{BL}, S14C^{BL}, I15M^{BL}, A16T^{BL}, I19M^{BL}, I20A^{BL}, S21N^{BL}, M23I^{BL}, A34I^{BL}, T40L^{BL}, S42N^{BL}, N44S^{BL}, H45Q^{BL}, T46I^{BL}, G47E^{BL}, V48T^{BL}, R52S^{BL}, I53V^{BL}, S59N^{BL}, H64Q^{BL}, N70S^{BL}, V74F^{BL}, I75A^{BL}, K78Q^{BL}, D79S^{BL}, K80V^{BL}, T81V^{BL}, T84K^{BL}, S93P^{BL}, I94V^{BL}, T101S^{BL}, H126P^{BL}, V149I^{BL}, A157T^{BL}, L163I^{BL}, A166V^{BL}, K173R^{BL}, M188I^{BL}, G189N^{BL}, E214D^{BL}, K220R^{BL}, K221N^{BL}, Q222N^{BL}, V232A^{BL}, N248D^{BL}, A250Q^{BL}, K257R^{BL}, V263I^{BL}, T264V^{BL}, I267V^{BL}, L269M^{BL}, F274Y^{BL}, H275Y, T285S^{BL}, G286S^{BL}, T287E^{BL}, V288I^{BL}, M289T^{BL}, D311E^{BL}, V321I^{BL}, K329N^{BL}, G331K^{BL}, E332T^{BL}, N336G^{BL}, T339S^{BL}, V340S^{BL}, D341N^{BL}, Y351F^{BL}, N365I^{BL}, R366S^{BL}, L367S^{BL}, D382G^{BL}, S385N^{BL}, D386N^{BL}, V389I^{BL}, V393I^{BL}, A395G^{BL}, T397N^{BL}, D398E^{BL}, V427I^{BL}, L430R^{BL}, T436I^{BL}, I437W^{BL}, W438T^{BL}, T439S^{BL}, S440G^{BL}, G441S^{BL},	P100S^{BL}, H155Q^{BL}, S160G^{BL}, S202T^{BL}, A214T^{BL}, S220T^{BL}, P288L^{BL}, I338V^{BL}, E391K^{BL}	1.75	38.56	387.80	0.29	48.00

Subject ID	Isolate Date	NA	HA	$TCID_{50}$ (log_{10} copies/mL)	Per IC_{50} value (nM)	Ose IC_{50} value (nM)	Zan IC_{50} value (nM)	Time to Alleviation of Symptoms
		S443I^{BL}, I444S^{BL}, S445F^{BL}, F446C^{BL}, C447G^{BL}, G448V^{BL}, V449N^{BL}, N450S^{BL}, S451D^{BL}, D452T^{BL}, T453V^{BL}, A454G^{BL}, N455W^{BL}, W456S^{BL}, S457W^{BL}, W458P^{BL}, P459D^{BL}, D460G^{BL}						
BCX1812-303.143.003	Day 1	I13V^{BL}, S14C^{BL}, I15M^{BL}, A16T^{BL}, I19M^{BL}, I20A^{BL}, S21N^{BL}, M23I^{BL}, A34I^{BL}, T40L^{BL}, S42N^{BL}, H45Q^{BL}, T46I^{BL}, G47E^{BL}, V48T^{BL}, R52S^{BL}, I53V^{BL}, S59N^{BL}, H64Q^{BL}, V67I^{BL}, N70S^{BL}, V74F^{BL}, I75A^{BL}, K78Q^{BL}, D79S^{BL}, K80V^{BL}, T81V^{BL}, T84K^{BL}, N88S^{BL}, S93P^{BL}, I94V^{BL}, T101S^{BL}, H126P^{BL}, A157T^{BL}, L163I^{BL}, A166V^{BL}, K173R^{BL}, M188I^{BL}, G189N^{BL}, E214D^{BL}, K220R^{BL}, K221N^{BL}, Q222N^{BL}, V232A^{BL}, I241V^{BL}, N248D^{BL}, A250Q^{BL}, K257R^{BL}, V263I^{BL}, T264V^{BL}, I267V^{BL}, L269M^{BL}, F274Y^{BL}, T285S^{BL}, G286S^{BL}, T287E^{BL}, V288I^{BL}, M289T^{BL}, D311E^{BL}, V321I^{BL}, K329N^{BL}, G331K^{BL}, E332K^{BL}, N336G^{BL}, T339S^{BL}, V340S^{BL}, D341N^{BL}, Y351F^{BL}, N365S^{BL}, R366S^{BL}, L367S^{BL}, K369N^{BL}, D382G^{BL}, S385N^{BL}, D386N^{BL}, V389I^{BL}, V393I^{BL}, A395G^{BL}, T397N^{BL}, D398E^{BL}, V427I^{BL}, L430R^{BL}, T436I^{BL}, I437W^{BL}, W438T^{BL}, T439S^{BL}, S440G^{BL}, G441S^{BL}, S443I^{BL}, I444S^{BL}, S445F^{BL}, F446C^{BL}, C447G^{BL}, G448V^{BL}, V449N^{BL}, N450S^{BL}, S451D^{BL}, D452T^{BL}, T453V^{BL}, A454G^{BL}, N455W^{BL}, W456S^{BL}, S457W^{BL}, W458P^{BL}, P459D^{BL}, D460G^{BL}	P100S^{BL}, Y215H^{BL}, S220T^{BL}, I338V^{BL}, E391K^{BL}	3.00	0.01	0.32	0.25	
BCX1812-303.143.003	Day 4	I13V^{BL}, S14C^{BL}, I15M^{BL}, A16T^{BL}, I19M^{BL}, I20A^{BL}, S21N^{BL}, M23I^{BL}, A34I^{BL}, T40L^{BL}, S42N^{BL}, H45Q^{BL}, T46I^{BL}, G47E^{BL}, V48T^{BL}, R52S^{BL}, I53V^{BL}, S59N^{BL}, H64Q^{BL}, V67I^{BL}, N70S^{BL}, V74F^{BL}, I75A^{BL}, K78Q^{BL}, D79S^{BL}, K80V^{BL}, T81V^{BL}, T84K^{BL}, N88S^{BL}, S93P^{BL}, I94V^{BL}, T101S^{BL}, H126P^{BL}, A157T^{BL}, L163I^{BL}, A166V^{BL}, K173R^{BL}, M188I^{BL}, G189N^{BL}, E214D^{BL}, K220R^{BL}, K221N^{BL}, Q222N^{BL}, V232A^{BL}, I241V^{BL}, N248D^{BL}, A250Q^{BL}, K257R^{BL}, V263I^{BL}, T264V^{BL}, I267V^{BL}, L269M^{BL}, F274Y^{BL}, H275Y, T285S^{BL}, G286S^{BL}, T287E^{BL}, V288I^{BL}, M289T^{BL}, D311E^{BL}, V321I^{BL}, K329N^{BL}, G331K^{BL}, E332K^{BL}, N336G^{BL}, T339S^{BL}, V340S^{BL}, D341N^{BL}, Y351F^{BL}, N365S^{BL}, R366S^{BL}, L367S^{BL}, K369N^{BL}, D382G^{BL}, S385N^{BL}, D386N^{BL}, V389I^{BL}, V393I^{BL}, A395G^{BL}, T397N^{BL}, D398E^{BL}, V427I^{BL}, L430R^{BL}, T436I^{BL}, I437W^{BL}, W438T^{BL}, T439S^{BL}, S440G^{BL}, G441S^{BL}, S443I^{BL}, I444S^{BL}, S445F^{BL}, F446C^{BL}, C447G^{BL}, G448V^{BL}, V449N^{BL}, N450S^{BL}, S451D^{BL}, D452T^{BL}, T453V^{BL}, A454G^{BL}, N455W^{BL}, W456S^{BL}, S457W^{BL}, W458P^{BL}, P459D^{BL}, D460G^{BL}	P100S^{BL}, Y215H^{BL}, S220T^{BL}, P288S, I338V^{BL}, E391K^{BL}	4.00	31.02	282.40	0.28	429.32

Yellow: Known resistance-associated substitution.

Table B2: Individual Resitance Data (Subjects Infected with A/H3N2)

Subject ID	Isolate Date	NA	HA	$TCID_{50}$ (log_{10} copies/mL)	Per IC_{50} value (nM)	Ose IC_{50} value (nM)	Zan IC_{50} value (nM)	Time to Alleviation of Symptoms

Subject ID	Isolate Date	NA	HA	$TCID_{50}$ (log_{10} copies/mL)	Per IC_{50} value (nM)	Ose IC_{50} value (nM)	Zan IC_{50} value (nM)	Time to Alleviation of Symptoms
BCX1812-211.461.052	Day 1	I26T^{BL}, H150R^{BL}, V194I^{BL}, K221E^{BL}, Y310H^{BL}, S372L^{BL}, N387K^{BL}		4.25	0.19	0.27	0.45	
BCX1812-211.461.052	Day 3	I26T^{BL}, H150R^{BL}, V194I^{BL}, K221E^{BL}, R292K/R, Y310H^{BL}, S372L^{BL}, N387K^{BL}		2.25	1.13	0.41	0.25	33.87
BCX1812-211.004.012	Day 1	H150R^{BL}, V194I^{BL}, K221E^{BL}, Y310H^{BL}, S335G^{BL}, L370S^{BL}, S372L^{BL}, N387K^{BL}	N/A	5.75	0.05	0.67	0.01	
BCX1812-211.004.012	Day 5	H150R^{BL}, V194I^{BL}, K221E^{BL}, N294N/S, Y310H^{BL}, S335G^{BL}, L370S^{BL}, S372L^{BL}, N387K^{BL}	N/A	1.5	1.43	1.63	1.41	47.13

Yellow: Known resistance-associated substitution.

Appendix 3: Studies in Hospitalized Patients

A3.1. Study BCX1812-201

This study was a Phase 2 multinational, randomized, double-mask, double-dummy study to compare the efficacy and safety of peramivir administered intravenously once daily for 5 days versus oseltamivir administered orally twice daily for 5 days in adults hospitalized with acute serious or potentially life-threatening influenza. A total of 137 subjects enrolled in this study. Subjects with signs and symptoms compatible with acute influenza virus infection (present for no more than 72 hours) and for whom a positive test for influenza virus infection using either the Rapid Antigen Test (RAT) or other test for influenza virus antigen using another methodology performed in a clinical laboratory were evaluated for participation. Enrollment was stratified according to duration of symptoms of influenza and oxygen saturation at screening and subjects were randomized to receive 1 of 3 treatments:

- Treatment Group 1: Peramivir 200 mg administered intravenously once daily for 5 days (5 doses).
- Treatment Group 2: Peramivir 400 mg administered intravenously once daily for 5 days (5 doses).
- Treatment Group 3: Oseltamivir 75mg oral suspension administered orally twice daily for 5 days (10 doses).

Subjects were evaluated at least twice daily while hospitalized to assess the criteria for clinical stability (measurements of temperature, respiration rate, systolic blood pressure, heart rate and transcutaneous oxygen saturation). Viral shedding was evaluated by determination of the $TCID_{50}$ value from anterior nose and posterior pharynx specimens (swabs) that were collected at screening and then twice daily (every 12 hours) for the first 48 hours after initiation of study drug treatment and again at 72 and 96 hours after initiation of treatment or at hospital discharge, if discharge occurred prior to Day 5 of treatment. At least once during each day, subjects were assessed by study staff for presence and severity of each of the 7 symptoms of influenza (sore throat, cough, nasal congestion, headache, myalgia, feverishness, malaise or fatigue). Subjects were asked to provide a daily assessment of ability to perform their usual daily activities. The duration of hospital confinement, indicators of possible clinical relapse, and incidence of mortality were also assessed.

Among all 122 subjects with confirmed influenza included in the primary efficacy analysis, the median time to clinical stability was 25.3 hours (95% CI 22.3, 37.0) (Table C1). The median time to clinical stability was 23.7 hours in the group treated with peramivir 200 mg, 37.0 hours in the group treated with peramivir 400 mg, and 28.1 hours in the group treated with oseltamivir. The median time to clinical stability was not significantly different among treatments or between any pair of treatments.

Table C1: Time to Clinical Stability: ITTI Population

Time to Clinical Resolution	Peramivir		Oseltamivir n=41	Total n=122
	200 mg n=41	400 mg n=40		
Mean (hr) (SD)	35.9 (5.55)	46.6 (6.33)	42.6 (7.55)	42.9 (4.12)
Median (hr)	23.7	37	28.1	25.3
Overall P-value				0.306
Pairwise comparison to oseltamivir	0.447	0.471		
Pairwise comparison to peramivir		0.190		

The virologic efficacy endpoint was the change (reduction) in influenza virus titer as measured by log_{10} $TCID_{50}$/mL. Nasopharyngeal specimens were collected at 12, 24, 36, 48, 72, and 96 hours after treatment initiation. Among the 122 subjects with confirmed influenza, a total of 112 subjects had positive virus cultures obtained from baseline nasopharyngeal specimens. Virologic data from these 112 subjects with culture-positive influenza virus were used in this analysis. A total of 80 subjects with positive virus cultures had influenza caused by either influenza A/H3N2 virus (n=60) or influenza A/H1N1 virus (n=20). Influenza B virus

was present at enrollment in 32 subjects with positive virus cultures. The protocol-specified analysis of the secondary virologic endpoint grouped influenza virus A and B subjects together. In post hoc analyses, the secondary virologic endpoint was analyzed separately for subjects with influenza A virus and influenza B virus. When all virus types and subtypes were combined (n=112), changes in virus titers were similar across the 3 treatment groups at all sampled time points.

A3.2. Study BCX1812-301

This study was a Phase 3 multicenter, randomized, double-blind, controlled study to evaluate the efficacy and safety of peramivir (i.v. 600 mg q.d. for 5 days) administered intravenously in addition to standard of care compared to standard of care alone in subjects six years of age and older who are hospitalized due to influenza. The primary endpoint is defined as time to clinical resolution as specified in the protocol. The secondary endpoint was the change in quantitative viral load over time and change from baseline measured from nasopharyngeal swab samples, as determined by quantitative virus culture (and retrospectively by real-time RT-PCR, if available). The data monitoring committee reviewed available data on 397 subjects enrolled in study BCX1812-301. In addition, a scheduled interim analysis was performed on 119 subjects in the Intent-To-Treat (ITT), non-neuraminidase inhibitor (NAI) sub-group, with focus on the “time to clinical resolution” endpoint, as specified by the protocol. Interim efficacy analysis demonstrated that the difference between drug and control groups for the endpoint of “time to clinical resolution” was small, resulting in a sample size re-calculation of >320 subjects. As determined by a decision tree that was discussed and agreed upon by the committee and the sponsor prior to the interim analysis, the committee recommended that the BCX1812-301 study be terminated for futility. This study was terminated on November 2, 2012. The final enrollment was approximately 70% of planned enrollment. A total of 1675 subjects were screened for participation in this study, with 405 subjects enrolling. A total of 360/405 subjects (89%) completed the study and 45/405 subjects (11%) discontinued prematurely (Table C2). The most frequent reason for premature discontinuation was withdrawal of informed consent (23/405 subjects, 6%). One subject (<1%) discontinued due to adverse events, and no subjects discontinued due to influenza-related complications. Three subjects (1%) died during the study. Study completion and reasons for withdrawal were similar between treatment groups. A total of 118/137 (86%) of subjects in the placebo group and 236/268 (88%) of subjects in the peramivir group had a confirmed influenza virus infection. Among the 354 subjects who had a confirmed influenza virus infection, most had the influenza A virus, H3N2 subtype (154/405 subjects, 44%), and the proportion of subjects with this subtype in each treatment group was slightly higher among subjects in the placebo treatment group (58/118 [49%] in the placebo group, 96/236 [41%] in the peramivir group). Most of the remaining confirmed influenza virus infections were by the 2009 H1N1 subtype of influenza A virus (35/118 [30%] in the placebo group, 72/236 [31%] in the peramivir group). A total of 65/354 (18%) of subjects (19/118 [16%] in the placebo group, 46/236 [19%] in the peramivir group) had influenza B virus, and 3/236 subjects (1%) in the peramivir group were positive for both influenza A virus and influenza B virus.

Table C2: Populations for Analysis

	Number of Subjects, n (%)		
	Placebo+SOC N = 137	**Peramivir+SOC N = 268**	**Total N = 405**
Subjects Enrolled	137 (100%)	268 (100%)	405 (100%)
Intent-to-Treat Population	137 (100%)	268 (100%)	405 (100%)
Randomized Not Treated	3 (2%)	4 (1%)	7 (2%)
Safety Population	134 (98%)	264 (99%)	398 (98%)

Subjects without a Confirmed Influenza Virus Infection	15 (11%)	28 (10%)	43 (11%)
Subjects from a Site with GCP Inconsistencies	2 (1%)	13 (5%)	15 (4%)
Subjects without a Confirmed Influenza Virus Infection and from a Site with GCP Inconsistencies	1 (1%)	1 (< 1%)	2 (< 1%)
Intent-to-Treat Infected Population	116 (85%)	222 (83%)	338 (83%)
Subjects Who Were Intended to Receive NAI-containing SOC as Recorded in the CRF	73 (53%)	144 (54%)	217 (54%)
Intent-to-Treat Infected Non-NAI Population	43 (31%)	78 (29%)	121 (30%)

Majority of the subjects (73/137 [53%] of placebo subjects and 144/268 [54%] of peramivir subjects) were intended to receive NAI (oseltamivir or zanamivir) as a part of their standard of care. The remaining subjects were not intended to receive NAI treatment, and were included in the ITTI Non-NAI population (43/137 placebo subjects [31%] and 78/268 peramivir subjects [29%]), which was the primary population for efficacy analyses.

A total of 121 subjects comprised the ITTI-Non-NAI population. For this population, the median time to clinical resolution was similar between treatment groups (49.5 hours for placebo, 42.5 hours for peramivir; P = 0.973; hazard ratio = 1.03) (Table C3). The range of times to clinical resolution was broad for these subjects, particularly for the peramivir treatment group (range: 2.0 to 207.2 hours [placebo] and 0.7 to 238.9 hours [peramivir]). Most of the subgroups were either too small to provide meaningful information or showed no differences between subgroups. However, differences in time to clinical resolution were notable for subgroups by region. The overall median time to clinical resolution was 19.0 hours for subjects in the US and Canada (n = 12), 36.0 hours for subjects in India (n = 56), and 70.9 hours for subjects in Eastern Europe (n = 48); the remaining regions each enrolled fewer than 10 subjects in the primary analysis population. In addition, among subjects who were admitted to the ICU at Baseline, there was a trend toward a shorter time to clinical resolution for subjects who received peramivir (median time to clinical resolution, 50.2 hours placebo [n = 8] versus 31.5 hours peramivir [n = 15]).

Table C3: Time to Clinical Resolution (Intent-to-Treat Infected Non-NAI Population)

Time to Clinical Resolution	Placebo + SOC N = 43	Peramivir + SOC N = 78
Mean (hr) (SD)	57.7 (6.82)	53.9 (5.04)
Median (hr) (SD)	49.5 (40.0, 61.9)	42.5 (34.0, 57.9)
P Value		0.973
Hazard Ratio		1.03 (0.69, 1.55)

The hazard ratio was calculated using a Cox regression model with stratification factors of duration of illness at randomization, ICU status at Baseline, use of supplemental oxygen at Baseline, influenza season, and influenza virus type. P value is based on the Wilcoxon-Gehan statistic stratified by duration of illness at randomization, ICU status at Baseline, use of supplemental oxygen at Baseline, influenza season, and influenza virus type.

Time to clinical resolution was also calculated for the ITTI population. As in the ITTI-Non-NAI population, time to clinical resolution was not significantly different between treatment groups (P = 0.794; hazard ratio = 0.92) (Table C4). The median values for time to clinical resolution (48.0 hours, placebo; 40.8 hours, peramivir) were similar to those seen in the ITTI-Non-NAI population. The range of times to clinical resolution was broad, ranging from 0.3 to 686.8 for placebo subjects, and 0.3 to 830.0 hours for peramivir subjects. Time to clinical resolution was calculated for the subgroups based on duration of illness (≤48 hours vs. >48 to ≤72 hours), age (adults vs. children and adolescents), gender, ICU admission (admitted vs. not admitted at Baseline), hemisphere and season of enrollment, need for supplemental oxygen at Baseline, viral subtype, country, region, site, and use of NAI. As with the ITTI-Non-NAI population, most of the subgroups were either too small to provide meaningful information or showed no differences between subgroups. Subgroups by region again showed differences in the overall time to clinical resolution: the overall median was 40.2 hours for subjects in the United States and Canada, 36.0 hours for subjects in India, and 62.0 hours for subjects in Eastern Europe.

Table C4: Time to Clinical Resolution (Intent-to-Treat Infected Population)

Time to Clinical Resolution	Placebo + SOC N = 43	Peramivir + SOC N = 78
Mean (hr) (SD)	65.7 (6.15)	84.4 (7.36)
Median (hr) (SD)	48.0 (37.0, 59.0)	40.8 (35.4, 48.9)
P Value		0.794
Hazard Ratio		0.92 (0.72, 1.18)

The hazard ratio was calculated using a Cox regression model with stratification factors of duration of illness at randomization, ICU status at Baseline, use of supplemental oxygen at Baseline, influenza season, and influenza virus type. P value is based on the Wilcoxon-Gehan statistic stratified by duration of illness at randomization, ICU status at Baseline, use of supplemental oxygen at Baseline, influenza season, and influenza virus type.

Most subjects in the ITTI-Non-NAI population had a positive Baseline viral titer as measured by RT-PCR (34/42 subjects in the placebo group and 61/78 subjects in the peramivir group) (Table). For those subjects, mean and median viral titers declined rapidly from Baseline through 60 hours post-dose, and declined more slowly through Day 5. The differences between treatment groups were not statistically significant. For the ITTI population, 146 subjects (47/115 placebo, 99/221 peramivir) had a positive viral titer at Baseline (as measured by $\log_{10}$ $TCID_{50}$/mL) and were included in the analysis of viral titer. As with the ITTI-Non-NAI population, viral titers decreased rapidly. For subjects in the peramivir treatment group, the median viral titer was undetectable (<0.50 $\log_{10}$ $TCID_{50}$/mL) as of 24 hours postdose; for subjects in the placebo group, median viral titer was undetectable at 36 hours. As of 84 hours postdose, the mean viral titer was almost undetectable for subjects in both treatment groups (0.53 for subjects who received placebo and 0.61 for subjects who received peramivir). A total of 201 subjects (66/115 subjects in the placebo group and 135/221 subjects in the peramivir group) had positive RT-PCR results at baseline. Similar to the results from the ITTI-Non-NAI population, mean and median viral titers decreased rapidly between Baseline and 60 hours postdose, and then declined slowly through Day 5. Decreases in influenza virus were similar between treatment groups. For the ITTI-Non-NAI and the full ITTI populations, change in viral titer and change from Baseline in RT-PCR were analyzed by the following subgroups: duration of illness (≤48 hours vs. >48 to ≤72 hours), age (adults vs. children and adolescents), gender, ICU admission (admitted vs. not admitted at Baseline), hemisphere and season of enrollment, need for supplemental oxygen at Baseline, viral subtype, country, region, site, and the use of an NAI-containing regimen. Most of the subgroups were too small to draw meaningful conclusions, including subgroups by age, hemisphere and season of enrollment, and viral subtype. For the larger subgroups, no differences were seen between subgroups.

A3.3. Study BCX1812-303

This study was a Phase 3 open-label, randomized, multi-center study of the antiviral activity, safety, and tolerability of intravenous peramivir in hospitalized subjects 6 years of age or older with confirmed or suspected influenza virus infection who did not have clinical or laboratory evidence of severe organ dysfunction. Enrollment continued until 234 subjects were enrolled, at which time the sponsor decided to end enrollment. Peramivir was administered intravenously according to one of the two following dose regimens (once daily or twice daily) for a total duration of 5 days, during which time clinical assessments were made and serial specimens were obtained for viral culture and virologic studies.

Treatment Group 1:

- Adults (≥18 years): Peramivir 600 mg, administered intravenously, once daily (every 24 hrs).
- Children and Adolescents (6 to 17 years of age): Peramivir 10 mg/kg (not to exceed a maximum dose of 600 mg) administered intravenously, once daily (every 24 hours).

Treatment Group 2:

- Adults (≥18 years): Peramivir 300 mg, administered intravenously, twice daily (every 12 hours).
- Children and Adolescents (6 to 17 years of age): Peramivir (BCX1812) 5mg/kg (not to exceed a maximum dose of 300 mg) administered intravenously, twice daily (every 12 hours).

Adults and adolescents ≥12 years old who did not meet the protocol-defined criteria of clinical resolution on Day 5, or who had quantifiable virus by real-time RT-PCR from a sample collected on Study Day 4 after dosing (where available), continued treatment for a further 5 days at the same total daily dose received on Day 5 of treatment administered once daily.

All 234 subjects who enrolled in the study were included in the ITT population. Four subjects (Subject 125.004 [300 mg BID group], Subject 312.001 [300 mg BID group], Subject 132.001 [600 mg QD group], and Subject 151.009 [600 mg QD group] were never dosed with study drug and were excluded from the Safety population (Table C5). Overall, 45% of subjects in the ITT population did not have a confirmed influenza virus infection; therefore, excluding those subjects who did not receive at least one dose of study drug, the ITTI population included only 54% of the ITT population. In the ITTI population, 49% of subjects in the 300 mg BID group and 60% of subjects in the 600 mg QD group had confirmed influenza (by primary viral culture, RT-PCR, or paired acute and convalescent serology specimens demonstrating at least a 4-fold increase in antibody titer against influenza virus A or B). Thirty percent of subjects in the 300 mg BID group and 40% of subjects in the 600 mg QD group had an available plasma concentration of peramivir and at least one post-baseline efficacy assessment and were included in the Exposure Response population.

Table C5: Populations for Analysis

	Number of Subjects, n (%)		
	300 mg BID (N=117)	**600 mg BID (N=117)**	**Total (N=234)**
Subjects enrolled	117 (100)	117 (100)	234 (100)
Intent-to-Treat Population	117 (100)	117 (100)	234 (100)
Subjects who did not receive ≥1 dose of study drug	2 (2)	2 (2)	4 (2)
Safety Population	115 (98)	115 (98)	230 (98)

Subjects without confirmed influenza virus infection	59 (50)	47 (40)	106 (45)
Intent-to-Treat Infected Population	57 (49)	70 (60)	127 (54)
Determined by (b) (4) RT-PCR	43	54	97
Determined by serology	4	3	7
Determined by positive viral titer	22	26	48
Determined by local lab	47	52	99
Subjects with no available plasma concentration of peramivir	9 (8)	7 (6)	16 (7)
Subjects with no post-baseline $TCID_{50}$ value	13 (11)	17 (15)	30 (13)
Exposure Response Population	35 (30)	47 (40)	82 (35)

The primary efficacy endpoint was the change (reduction) in influenza virus titer measured by $TCID_{50}$ at 48 hours. A total of 44 subjects had both a quantifiable Baseline and post-Baseline viral titer measured by $TCID_{50}$ and contributed data to the analysis of time-weighted changes from Baseline in log_{10} $TCID_{50}$/mL. The overall mean and median time-weighted changes from Baseline in log_{10} $TCID_{50}$/mL at 48 hours, i.e. for the combined arms, were identical with no significant differences in both the mean or median values in the 300 mg BID and 600 mg QD treatment groups.

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/s/

TAKASHI E KOMATSU
08/21/2014

ERIC F DONALDSON
08/21/2014

JULIAN J O REAR
08/21/2014

Product Quality Microbiology Review

29 JULY 2014

NDA: 206426

Drug Product Name
Proprietary: RAPIVAB®
Non-proprietary: Peramivir Solution

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
12/21/2013	12/21/2013	12/22/2013	12/23/2013
6/5/2014	6/5/2014	N/A	N/A

Applicant/Sponsor
Name: BioCryst Pharmaceuticals, Inc.
Address: 4505 Emperor Blvd, Suite 200
Durham, NC 2770

Representative: Elliot Berger
4505 Emperor Blvd, Suite 200
Durham, NC 2770

Telephone: 919-859-7919

Name of Reviewer: Neal J. Sweeney, Ph.D.

Conclusion: Recommended for Approval

Product Quality Microbiology Data Sheet

A. **1.** **TYPE OF SUBMISSION:** 505 (b) (1) Original NDA

2. **SUBMISSION PROVIDES FOR:** New drug product

3. **MANUFACTURING SITE:** [redacted] (b) (4)

4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** Peramivir 10 mg/mL solution (200 mg in 20 mL) in single-use vials, supplied in a carton containing 3 vials, for intravenous administration following dilution.

5. **METHOD(S) OF STERILIZATION:** [redacted] (b) (4)

6. **PHARMACOLOGICAL CATEGORY:** Antiviral indicated for the treatment of acute uncomplicated influenza in patients 18 years and older.

B. **SUPPORTING/RELATED DOCUMENTS:**
DMF [redacted] (b) (4)
DMF [redacted] (b) (4)
DMF [redacted] (b) (4)

C. **REMARKS:** The [redacted] (b) (4) manufacturing facility Warning Letter Follow-up Inspection [redacted] (b) (4) resulted in 18 observations related to the manufacturing of sterile products, and included deficiencies associated with the following: [redacted] (b) (4) However, none of the observations cited the manufacturing of Peramivir (the subject of this review), which is [redacted] (b) (4) The 483 was issued [redacted] (b) (4) Resolution of 483 issues (or identification of an alternate drug product manufacturer) prior to the December 23, 2014 PDUFA date is questionable.

File name: N206426R1.doc

Reference ID: 3604894

Executive Summary

I. Recommendations

A. Recommendation on Approvability - Recommended for Approval.

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – N/A

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology – (b) (4)

B. Brief Description of Microbiology Deficiencies – Based upon the information provided, no microbiology deficiencies were identified.

C. Contains Potential Precedent Decision(s)- ☐ Yes ☒ No

(If yes, provide a brief description and a reference to the page where the precedent is discussed in depth)

III. Product Quality Microbiology Risk Assessment

A. Initial Product Quality Microbiology Risk Assessment

CQA	Risk Factor	Prob. of Occ. (O)	Modifier for O[3, 4, 5]	Severity of Effect (S)	Detect. (D)	Risk Priority Number[6] (RPN)	Additional Review Emphasis based on Risk (in addition to normal review process)
(b) (4)							

(b) (4)

Reference ID: 3604894

B. Final Risk Assessment -

Although the antiviral peramivir drug product is (b) (4) the applicant provided data demonstrating that (b) (4) solution for the established maximum (b) (4) hold time (in-process control), results in bioburden levels (b) (4) over the (b) (4) hold period. Additionally the drug product is (b) (4) Container/closure integrity was demonstrated for product containers exposed to the (b) (4) The drug product is diluted prior to use, but labeling indicates that the diluted product must be either administered immediately or stored at 2ºC-8ºC for ≤ 24 hours prior to administration. Therefore, the applicant has mitigated the risk of drug product non-sterility.

IV. Administrative

A. Reviewer's Signature _____

Neal J. Sweeney, Ph.D. (Primary Reviewer)

B. Endorsement Block _____

John W. Metcalfe, Ph.D. (Secondary Reviewer)

C. CC Block

N/A

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/s/

NEAL J SWEENEY
08/05/2014

JOHN W METCALFE
08/05/2014
I concur.

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 206426 **Applicant: BioCryst Pharmaceuticals** **Stamp Date: 12/23/13**

Drug Name: Peramivir **NDA Type: Original**

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		
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?	X		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	X		
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		
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?			Not applicable
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?	X		
11	Have all the study reports, published articles, and other references been included and cross-referenced in the	X		

File name: Virology Filing Checklist for a NDA or Supplement 010908

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	annotated draft labeling or summary section of the submission?			
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 VIROLOGY SECTION OF THE APPLICATION FILEABLE? ___YES _____

If the NDA is not fileable from the virology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Komatsu, Takashi 1/29/14
Reviewing Virologist Date

O'Rear, Jules 1/29/14
Virology Team Leader Date

File name: Virology Filing Checklist for a NDA or Supplement 010908

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/s/

TAKASHI E KOMATSU
01/29/2014

JULIAN J O REAR
01/29/2014

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 206426 **Applicant:** Biocryst Pharmaceuticals Inc. **Letter Date:** 12/21/2013

Drug Name: RAPIVAB Injection (200 mg/20 mL) **NDA Type: 505(b)(2)** Standard **Stamp Date:** 12/23/2013

The following are necessary to initiate a review of the NDA application:

	Content Parameter	Yes	No	Comments
1	Is the product quality microbiology information described in the NDA and organized in a manner to allow substantive review to begin? Is it legible, indexed, and/or paginated adequately?	X		eCTD
2	Has the applicant submitted an overall description of the manufacturing processes and microbiological controls used in the manufacture of the drug product?	X		3.2.P.3.3 (b) (4) (b) (4) [redacted].
3	Has the applicant submitted protocols and results of validation studies concerning microbiological control processes used in the manufacture of the drug product?	X		3.2.P.3.5.: Validation studies provided for both (b) (4) [redacted]
4	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	
5	Has the applicant submitted preservative effectiveness studies (if applicable) and container-closure integrity studies?	X		3.2.P.3.5.17. C/C integrity AET: N/A (single-dose vials)
6	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?	X		3.2.P.5.1. (release and stability) sterility and endotoxin, 3.2.P.5.6.1.7 justification for sterility and endotoxin limit
7	Has the applicant submitted the results of analytical method verification studies?	X		3.2.P.5.3 (sterility and endotoxin testing)
8	Has the applicant submitted all special/critical studies/data requested during pre-submission meetings and/or discussions?		X	No Product Quality Micro studies requested during pre-submission meetings.
9	If sterile, are extended post-constitution and/or post-dilution hold times in the draft labeling supported by microbiological data?		X	Diluted with (b) (4) [redacted] 0.9% or 0.45% Sodium Chloride Inj, 5% Dextrose or Lactated Ringer's, and may be stored up to 24 hrs at 2-8°C. Discarded after 24 hours. (Study not necessary)
10	Is this NDA fileable? If not, then describe why.	X		

Additional Comments: (None)

27 January 2014

Neal J. Sweeney, Ph.D., Reviewing Microbiologist Date

Bryan S. Riley, Ph.D., Acting Team Leader Date

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/s/

NEAL J SWEENEY
01/28/2014

BRYAN S RILEY
01/28/2014
I concur.