

Antiviral Susceptibility of Influenza Viruses After Therapy (Rx) with Peramivir (PVR) Vs. Placebo (PBO) or Oseltamivir (OSE) in Influenza Subjects in Phase 2 and 3 Trials

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INTRODUCTION

- Early treatment with NAIs is recommended for subjects hospitalized with influenza¹
- Concerns about emergence of resistance were raised after most seasonal H1N1 influenza A isolates in 2008 were found to be resistant to oseltamivir (OSE)², most with the H275Y mutation
- There are few reports of H275Y isolates after peramivir (PVR) treatment
- Peramivir is an intravenous neuraminidase inhibitor (NAI) approved in Japan and South Korea for treatment of influenza and in Phase 3 global trials to evaluate treatment of influenza patients in the hospital setting
- Peramivir has in vitro activity superior to OSE and zanamivir (ZAN) against wild-type strains of influenza^{3,4}
- Resistance of seasonal H1N1 influenza A to PVR is only selected after multiple cycles of in vitro passage⁵
- In 3 completed randomized double-blind Phase 2 and 3 trials of IV and IM peramivir, subjects treated with peramivir demonstrated reductions in viral shedding and time to alleviation of influenza symptoms similar to those treated with oseltamivir⁶ and superior to placebo^{7,8}
- With viral susceptibility data from 4 peramivir trials now available, we have combined these data to assess the potential development of resistance to peramivir in patients with influenza treated with peramivir

METHODS

Study Design and Treatments

Peramivir Clinical Trials with Virology Susceptibility Data

Study:	BCX1812-211	0722T0621	BCX1812-201	BCX1812-303
Phase	2	2	2	3
Design	R, DB, PG	R, DB, PG	R, DB, DD, PG	R, PG
Years Conducted	2006-08	2007-08	2007-08	2009-10
Countries	7	1	7	5
Sites	151	75	84	59
Peramivir	150 mg IM 300 mg IM	300 mg IV 600 mg IV	200 mg IV 400 mg IV	300 mg IV BID 600 mg IV QD
Control	Placebo	Placebo	Oseltamivir 75 mg BID	None
Treatment Duration	Single dose	Single dose	5 days	5-10 days

IV=intravenous, IM=intramuscular, R=randomized, DB=double blind, PG=parallel group, DD=double dummy

- 4 completed randomized phase 2 and 3 studies conducted from December 2006 to October 2010
- Eligible patients were randomly assigned to treatment with peramivir (PVR) IV or IM, oral oseltamivir, or matched placebo

Study Subjects

- Adult or adolescent inpatients or outpatients
- Symptoms consistent with diagnosis of acute influenza infection
- Confirmation of influenza A or B infection by influenza A or B infection by Rapid Antigen Test prior to randomization except for study BCX1812-303

Study Assessments

- Virus was collected from nasopharyngeal swabs at baseline and post treatment
- Influenza A subtype and B infection at baseline was determined by RT-PCR, culture, or serology
- H275Y status was assessed by pyrosequencing in H1N1 specimens
- Seasonal A/H1N1 isolates without the H275Y mutation by pyrosequencing were considered wild type (WT)
- Neuraminidase enzyme inhibition (NAI) assay was performed on virus MDCK culture supernatants by MUNANA technique to assess the IC₅₀ of PVR, oseltamivir (OSE) and zanamivir (ZAN) at baseline and post treatment. IC₅₀ values could not be determined for all virus subtypes that were confirmed by RT-PCR, culture or serology
- Genotypic sequencing at baseline and post treatment was performed in 2 populations:
 - IC₅₀ subset: Paired isolates with change in NAI IC₅₀ from baseline to last timepoint > mean + 2 SD, or > 3 fold
 - Delayed clearance: Subjects with positive culture post-treatment at day 5 or day 9

RESULTS

Study Subjects

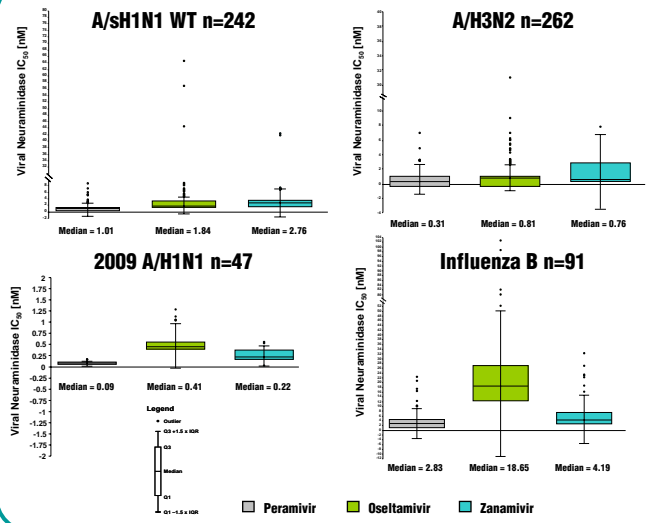
Subjects in Peramivir Clinical Trials by Dose and Influenza Strain at Baseline

Study:	BCX1812-211	0722T0621	BCX1812-201	BCX1812-303	Total
Enrolled Subjects	344 outpatients	300 outpatients	137 inpatients	234 inpatients	1015
Treated Subjects	PVR 150 IM SD = 113 PVR 300 IM SD = 115 Placebo IM SD = 114	PVR 300 IV SD = 99 PVR 600 IV SD = 99 Placebo IV SD = 100	PVR 200 QD IV SD = 45 PVR 400 QD IV SD = 46 OSE 75 BID PO = 46	PVR 300 BID IV 5-10d = 114 PVR 600 BID IV 5-10d = 116	1007
Subjects with A/H1N1 WT*	PVR 150 IM SD = 32 PVR 300 IM SD = 21 Placebo IM SD = 20	PVR 300 IV SD = 74 PVR 600 IV SD = 69 Placebo IV SD = 72	PVR 200 QD IV SD = 5 PVR 400 QD IV SD = 5 OSE 75 BID PO = 6	PVR 300 BID IV 5-10d = 3 PVR 600 BID IV 5-10d = 2	316
Subjects with A/H3N2*	PVR 150 IM SD = 50 PVR 300 IM SD = 57 Placebo IM SD = 53	PVR 300 IV SD = 21 PVR 600 IV SD = 25 Placebo IV SD = 24	PVR 200 QD IV SD = 20 PVR 400 QD IV SD = 26 OSE 75 BID PO = 22	PVR 300 BID IV 5-10d = 3 PVR 600 BID IV 5-10d = 10	298
Subjects with B*	PVR 150 IM SD = 19 PVR 300 IM SD = 25 Placebo IM SD = 24	PVR 300 IV SD = 2 PVR 600 IV SD = 1 Placebo IV SD = 0	PVR 200 QD IV SD = 15 PVR 400 QD IV SD = 6 OSE 75 BID PO = 11	PVR 300 BID IV 5-10d = 3 PVR 600 BID IV 5-10d = 2	108
Subjects with A/Indeterminate*	PVR 150 IM SD = 3 PVR 300 IM SD = 2 Placebo IM SD = 2	PVR 300 IV SD = 2 PVR 600 IV SD = 1 Placebo IV SD = 4	PVR 200 QD IV SD = 1 PVR 400 QD IV SD = 1 OSE 75 BID PO = 1	PVR 300 BID IV 5-10d = 10 PVR 600 BID IV 5-10d = 16	42
Subjects with A/H1N1H275Y**					3
Subjects with 2009 A/H1N1*				PVR 300 BID IV 5-10d = 43 PVR 600 BID IV 5-10d = 51	94
Subjects with Indeterminate*				PVR 300 BID IV 5-10d = 1 PVR 600 BID IV 5-10d = 1	2

IV=intravenous, IM=intramuscular, SD=single dose, PO=oral
 *Determined by RT PCR, culture or paired serology, either by a local or central lab
 **Determined by pyrosequencing

- 1007 subjects were treated once or over 5-10 days with PVR delivered IM or IV or OSE delivered PO
- Two studies were placebo controlled
- Influenza was confirmed at baseline and the strain identified by RT PCR, culture or paired serology in 863 subjects

Viral Susceptibility at Baseline



- Mean and median IC₅₀ values for all influenza A and B viruses isolated at baseline were lowest for peramivir compared to the two other NAIs
- 3 isolates in the 201 study had the H275Y mutation at baseline; they had high IC₅₀ values for oseltamivir and intermediate IC₅₀ values for peramivir

Change in Viral Susceptibility

Seasonal A/H1N1 WT

Treatment Group	NAI, median (min, max)			
	Peramivir	Oseltamivir	Zanamivir	
PBO, n=81	IC ₅₀ (nM) of last positive culture	0.90 (0.01, 2.98)	1.63 (0.23, 13.02)	2.55 (0.06, 32.64)
	Fold change from baseline*	0.90 (0.04, 14.00)	0.96 (0.19, 7.92)	0.95 (0.12, 40.00)
PVR 150 IM SD, n=20	IC ₅₀ (nM) of last positive culture	0.22 (0.01, 8.34)	0.95 (0.10, 95.45)	0.54 (0.08, 10.39)
	Fold change from baseline*	1.91 (0.21, 69.50)	1.15 (0.12, 28.81)	0.76 (0.08, 14.60)
PVR 300 IM SD, n=19	IC ₅₀ (nM) of last positive culture	0.14 (0.01, 9.68)	1.72 (0.04, 24.37)	1.04 (0.21, 5.44)
	Fold change from baseline*	1.08 (0.04, 29.33)	1.40 (0.34, 36.92)	1.15 (0.49, 23.54)
PVR 300 IV SD, n=57	IC ₅₀ (nM) of last positive culture	0.95 (0.58, 2.64)	0.95 (0.41, 2.64)	0.97 (0.31, 3.53)
	Fold change from baseline*	1.21 (0.79, 20.30)	1.86 (0.10, 94.00)	2.48 (1.62, 5.15)
PVR 600 IV SD, n=49	IC ₅₀ (nM) of last positive culture	1.14 (0.74, 24.82)	1.00 (0.04, 2.88)	0.99 (0.48, 1.85)
	Fold change from baseline*	0.05 (0.01, 0.31)	1.11 (0.68, 2.31)	0.82 (0.08, 2.49)
PVR 200 IV, n=5	IC ₅₀ (nM) of last positive culture	0.79 (0.16, 1.00)	0.60 (0.45, 1.31)	0.79 (0.28, 1.71)
	Fold change from baseline*	0.40 (0.01, 0.88)	2.24 (0.89, 2.65)	1.00 (0.42, 2.00)
PVR 400 IV, n=5	IC ₅₀ (nM) of last positive culture	0.88 (0.57, 1.31)	0.98 (0.33, 2.60)	0.68 (0.55, 1.42)
	Fold change from baseline*	2.23 (0.14, 4.20)	2.65 (0.91, 3.41)	1.72 (0.11, 2.53)
OSE, n=6	Fold change from baseline*	1.86 (0.26, 5.19)	1.27 (0.21, 2.38)	1.06 (0.08, 1.98)

*Values = 1 indicate no change, values > 1 indicate a fold increase, values < 1 indicate a fold decrease

Treatment Group	NAI, median (min, max)			
	Peramivir	Oseltamivir	Zanamivir	
PBO, n=72	IC ₅₀ (nM) of last positive culture	0.34 (0.01, 2.63)	0.70 (0.06, 20.19)	0.80 (0.03, 8.30)
	Fold change from baseline*	1.04 (0.16, 18.00)	0.99 (0.16, 23.60)	1.07 (0.14, 25.38)
PVR 150 IM SD, n=44	IC ₅₀ (nM) of last positive culture	0.17 (0.02, 1.78)	0.50 (0.02, 3.99)	0.71 (0.05, 3.56)
	Fold change from baseline*	1.21 (0.11, 53.00)	1.06 (0.07, 6.23)	1.40 (0.25, 141.00)
PVR 300 IM SD, n=44	IC ₅₀ (nM) of last positive culture	0.21 (0.04, 4.65)	0.51 (0.11, 13.09)	0.82 (0.04, 14.15)
	Fold change from baseline*	1.60 (0.28, 59.00)	1.22 (0.26, 36.36)	1.40 (0.08, 36.20)
PVR 300 IV SD, n=21	IC ₅₀ (nM) of last positive culture	1.21 (1.04, 1.67)	0.84 (0.75, 1.24)	2.97 (2.50, 4.22)
	Fold change from baseline*	0.80 (0.42, 1.28)	0.76 (0.42, 1.23)	0.86 (0.61, 1.27)
PVR 600 IV SD, n=24	IC ₅₀ (nM) of last positive culture	1.41 (0.88, 1.92)	1.06 (0.49, 1.86)	3.41 (2.61, 4.56)
	Fold change from baseline*	0.97 (0.47, 1.21)	0.96 (0.42, 1.40)	0.89 (0.70, 1.22)
PVR 200 IV SD, QD, n=16	IC ₅₀ (nM) of last positive culture	0.33 (0.06, 4.83)	1.36 (0.09, 34.93)	0.58 (0.17, 5.56)
	Fold change from baseline*	0.95 (0.33, 21.95)	1.23 (0.03, 36.39)	0.85 (0.09, 5.10)
PVR 400 IV SD, QD, n=21	IC ₅₀ (nM) of last positive culture	0.45 (0.04, 2.33)	1.76 (0.05, 10.21)	1.03 (0.13, 4.20)
	Fold change from baseline*	0.86 (0.04, 2.69)	0.99 (0.21, 7.59)	0.87 (0.03, 6.80)
OSE PO 5d BID, n=20	IC ₅₀ (nM) of last positive culture	0.60 (0.13, 7.10)	1.17 (0.26, 6.22)	1.02 (0.20, 4.17)
	Fold change from baseline*	0.90 (0.32, 177.50)	1.01 (0.10, 4.49)	0.65 (0.07, 25.80)

*Values = 1 indicate no change, values > 1 indicate a fold increase, values < 1 indicate a fold decrease

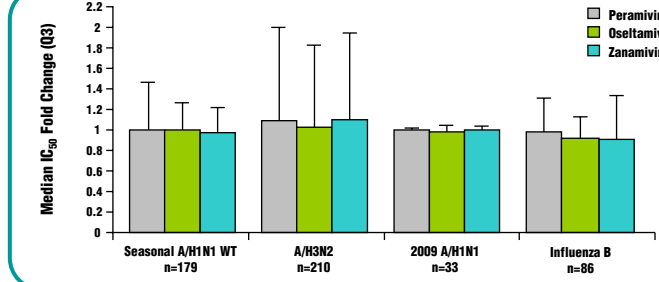
Treatment Group	NAI, median (min, max)			
	Peramivir	Oseltamivir	Zanamivir	
PVR 300 IV 5-10d BID, n=22	IC ₅₀ (nM) of last positive culture	0.09 (0.06, 31.02)	0.37 (0.23, 282.40)	0.23 (0.08, 0.39)
	Fold change from baseline*	1.00 (0.58, 3102.00)	1.00 (0.39, 882.50)	1.05 (0.29, 1.56)
PVR 600 IV 5-10d QD, n=25	IC ₅₀ (nM) of last positive culture	0.10 (0.07, 0.13)	0.38 (0.28, 0.93)	0.25 (0.11, 0.34)
	Fold change from baseline*	0.95 (0.67, 1.63)	0.92 (0.62, 1.69)	1.00 (0.28, 1.69)

*Values = 1 indicate no change, values > 1 indicate a fold increase, values < 1 indicate a fold decrease

Treatment Group	NAI, median (min, max)			
	Peramivir	Oseltamivir	Zanamivir	
PBO, n=19	IC ₅₀ (nM) of last positive culture	2.53 (0.02, 6.30)	17.63 (0.06, 54.63)	2.81 (0.02, 11.97)
	Fold change from baseline*	1.12 (0.50, 3.04)	1.15 (0.24, 2.52)	1.03 (0.30, 2.41)
PVR 150 IM SD, n=16	IC ₅₀ (nM) of last positive culture	2.75 (1.02, 11.69)	15.04 (3.98, 32.95)	5.24 (1.90, 14.91)
	Fold change from baseline*	1.03 (0.21, 2.08)	0.94 (0.24, 1.19)	1.06 (0.36, 2.71)
PVR 300 IM SD, n=21	IC ₅₀ (nM) of last positive culture	1.74 (0.29, 3.41)	13.12 (0.53, 32.57)	2.83 (0.52, 8.80)
	Fold change from baseline*	0.96 (0.03, 15.25)	0.94 (0.13, 4.08)	0.92 (0.37, 6.50)
PVR 300 IV SD, n=2	IC ₅₀ (nM) of last positive culture	2.89 (2.64, 3.14)	8.52 (7.28, 9.75)	9.98 (7.75, 10.20)
	Fold change from baseline*	1.03 (1.01, 1.05)	0.99 (0.88, 1.09)	0.97 (0.91, 1.03)
PVR 600 IV SD, n=1	IC ₅₀ (nM) of last positive culture	2.49	7.07	7.86
	Fold change from baseline*	0.89	0.97	0.91
PVR 200 IV SD, QD, n=15	IC ₅₀ (nM) of last positive culture	4.42 (0.41, 17.60)	21.02 (0.33, 61.25)	4.06 (0.50, 37.65)
	Fold change from baseline*	0.87 (0.04, 2.91)	0.81 (0.03, 1.23)	0.78 (0.11, 1.96)
PVR 400 IV SD, QD, n=6	IC ₅₀ (nM) of last positive culture	2.58 (0.06, 6.26)	30.52 (4.62, 71.51)	2.55 (1.67, 10.83)
	Fold change from baseline*	0.90 (0.02, 3.85)	0.70 (0.44, 1.97)	0.85 (0.50, 1.69)
OSE PO 5d BID, n=11	IC ₅₀ (nM) of last positive culture	6.89 (0.26, 12.91)	41.92 (8.17, 88.63)	5.90 (2.11, 21.21)
	Fold change from baseline*	0.78 (0.10, 5.43)	0.91 (0.41, 22.73)	0.82 (0.43, 8.66)

*Values = 1 indicate no change, values > 1 indicate a fold increase, values < 1 indicate a fold decrease

- In pairs of influenza isolates obtained at baseline and up to 9 days post therapy, there were no meaningful decreases in susceptibility in any treatment group



- When the subjects from all 4 studies and treatment groups are assessed, there were no meaningful changes in viral susceptibility for subjects with any viral influenza strain

Genotypic Analysis

Subjects in Peramivir Clinical Trials with Post-Baseline Treatment-Emergent H275Y Mutation

Subject	Study	Treatment	Sex	Age	Influenza Subtype	Peramivir IC ₅₀ (nM)		Fold Change from BL
						Baseline	Post-Rx*	
662-007	211	PVR 150 IM single dose	F	20	A/H1N1	0.12	8.34	70
671-004**	211	PVR 300 IM single dose	F	27	A/H1N1	5.52	1.09	0.2
080-1	621	PVR 600 IV single dose	F	38	A/H1N1	0.97	14.5	15
086-5	621	PVR 600 IV single dose	F	39	A/H1N1	1.72	31.5	18
135-6	621	PVR 600 IV single dose	F	21	A/H1N1	1.35	27.6	20
148-4	621	PVR 600 IV single dose	M	22	A/H1N1	1.12	27.8	25
163-5	621	PVR 600 IV single dose	F	25	A/H1N1	1.47	30.3	21
143-003	303	PVR 300 BID IV 5-10 d	F	67	2009 A/H1N1	0.01	31.02	3102

IV = intravenous, IM = intramuscular, BL = baseline
 *Last positive culture
 **Last positive culture for this subject (Day 5) did not yield a result for sequence analysis. Sequence performed on Day 3 specimen

- Genotypic analyses of the limited number of isolates from single dose studies (paired isolates with change in IC₅₀ > mean + 2 SD, n=31; subjects with viral shedding post baseline, n=17) found 7 subjects (1.6%) with treatment-emergent mutations, all of which were H275Y substitutions in the NA gene. No additional known resistance mutations were identified. In study 621, there was no difference in clinical outcomes when subjects with the H275Y mutation post therapy were compared to those without (data not shown).⁹
- In the 5-10 day studies, genotyping found only 1 subject (0.5%) with a treatment-emergent H275Y mutation out of those tested (paired isolates with change in IC₅₀ > mean + 2 SD, n=13; subjects with viral shedding post baseline, n=18); that subject had received oseltam