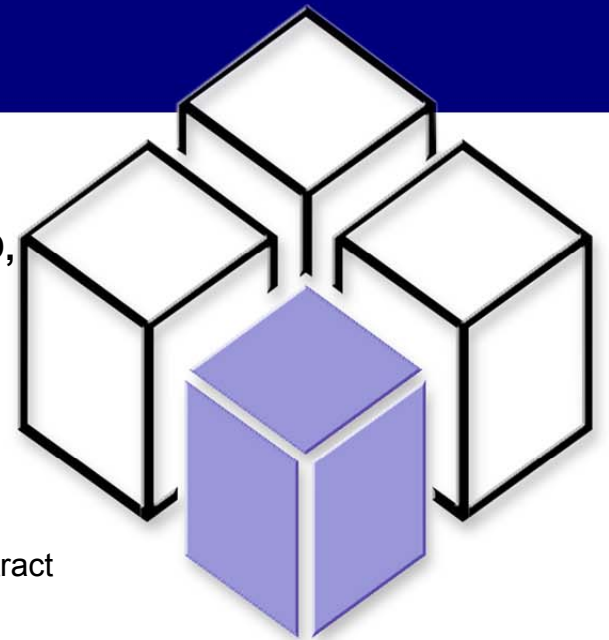


Antiviral Susceptibility of Influenza Viruses after Therapy with Peramivir Vs. Placebo or Oseltamivir in Influenza Subjects in Phase 2 and 3 Clinical Trials

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Background

- Early treatment with NAIs is recommended for subjects hospitalized with influenza and for subjects with risk factors for serious disease¹
- Concerns about emergence of NAI 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
- Peramivir is an intravenous neuraminidase inhibitor (NAI) approved in Japan and South Korea for treatment of influenza and in Phase 3 global trials for US approval
- Peramivir has in vitro activity similar or superior to OSE and zanamivir (ZAN) against wild-type strains of influenza^{3,4}
- There are few reports of H275Y isolates after peramivir (PVR) treatment⁵

1. Lee N, Choi KW, Chan PK, et al. Outcomes of adults hospitalized with severe influenza. *Thorax* 2010; 65:510-5

2. Hurt AC, Ernest J, Deng YM, et al. Emergence and spread of oseltamivir-resistant A(H1N1) influenza viruses in Oceania, South East Asia and South Africa. *Antiviral Res* 2009; 83:90-3

3. Boivin G, Goyette N. Susceptibility of recent Canadian influenza A and B virus isolates to different neuraminidase inhibitors. *Antiviral Res* 2002; 54:143-7

4. Gubareva LV, Trujillo A, Okomo-Adhiambo M, et al. Comprehensive assessment of 2009 pandemic influenza A (H1N1) virus drug susceptibility in vitro. *Antiviral Ther* 2010; 15:1151-9

5. Kohno S, Kida H, Mizuguchi M, Shimada J; S-021812 Clinical Study Group. Efficacy and safety of intravenous peramivir for treatment of seasonal influenza virus infection. *Antimicrob Agents Chemother*. 2010;54:4568-74.

NAI resistance mutations generated in vitro (OSE, ZAN or PVR)

NA Mutation	Virus subtype	Catalytic Residues	Framework Residues	NAI
→ R292K*	H3N2, H5N1, B	Yes		OSE, PVR, ZAN
D151E	H3N2	Yes		OSE
R152K	H3N2	Yes		ZAN, OSE
R118K	H3N2, B	Yes		OSE
R224K	H3N2	Yes		OSE
E276D	H3N2	Yes		OSE
R371K	H3N2	Yes		OSE
→ H274Y/275Y**	H1N1, H3N2, H5N1		Yes	OSE, PVR, ZAN
N294S	H1N1, H5N1		Yes	OSE
E119V	H3N2, B		Yes	ZAN, OSE
E119D	H3N2, B		Yes	ZAN
E119G	H3N2, H5N1, B		Yes	ZAN
E119A	H3N2, B		Yes	ZAN

A/PR/8/34 (H1N1), X121 (a reassortant virus with H3N2 surface glycoproteins), **B/Yamagatta/16/88, *A/Singapore/1/57 (H2N2), A/Shandong/09/92 (H3N2), A/Charlottesville/31/95 (H1N1) and **A/WSN/33 (H1N1).

Baz M, Abed Y, Boivin G. *Antiviral Research*, 2007;74:159-62. Lackenby A, et al., *Current opinion in infectious diseases*, 2008, 21:626-638

NAI resistance in treated patients (mainly OSE, some ZAN and PVR)

NA Mutation	Virus subtype		NAI
→ H275Y	H1N1, H5N1	Observed in adults, children and immunocompromised adults	OSE, PVR
R292K	H3N2	Observed in Japanese children	OSE
R152K	B	Observed in Immunocompromised child	ZAN
E119V	H3N2	Observed in Immunocompromised child and adult	OSE, ZAN
N294S	H3N2, H5N1	Observed in Children	OSE
D198N	B	Observed in immunocompromised child	OSE
G402S	B	Observed in children	OSE
I222V	H3N2	Observed in immunocompromised children	OSE, ZAN
I223R	H3N2	Observed in immunocompromised child	ZAN
Q136K	H1N1	Observed in immunocompromised child	ZAN

Lackenby A, et al., *Current opinion in infectious diseases*, 2008, 21:626-638 Hurt AC, Holien JK, Parker M, et al. Zanamivir-resistant influenza viruses with a novel neuraminidase mutation. *J Virol* 2009; 83:10366–10373. Thorlund K, Awad T, Boivin G and Thabane L., *BMC Infect Dis*. 2011; 11: 134. doi: [10.1186/1471-2334-11-134](https://doi.org/10.1186/1471-2334-11-134)

Methods

- Four completed randomized phase 2 and 3 studies conducted from December 2006 to October 2010 were analyzed
- Eligible patients were randomly assigned to treatment with PVR IV or IM, oral OSE, or matched placebo (PBO)
- Virus was collected from NP swabs at BL and post treatment (Rx)
- Influenza A subtype and B infection was determined by RT-PCR, culture, or serology and H275Y status (WT or mutant) was assessed by pyrosequencing in H1N1 specimens (1 hospital study)
- NA inhibition assay was performed on virus MDCK culture supernatants by MUNANA technique at BL & post Rx
- NA genotypes at BL and post Rx were performed in 2 populations:
 - SD subset: Paired isolates with Δ in NAI IC_{50} from BL to last timepoint $>$ mean + 2 SD, or $>$ 3 fold
 - Delayed clearance subset: Subjects with positive culture post-Rx at days 5 or 9

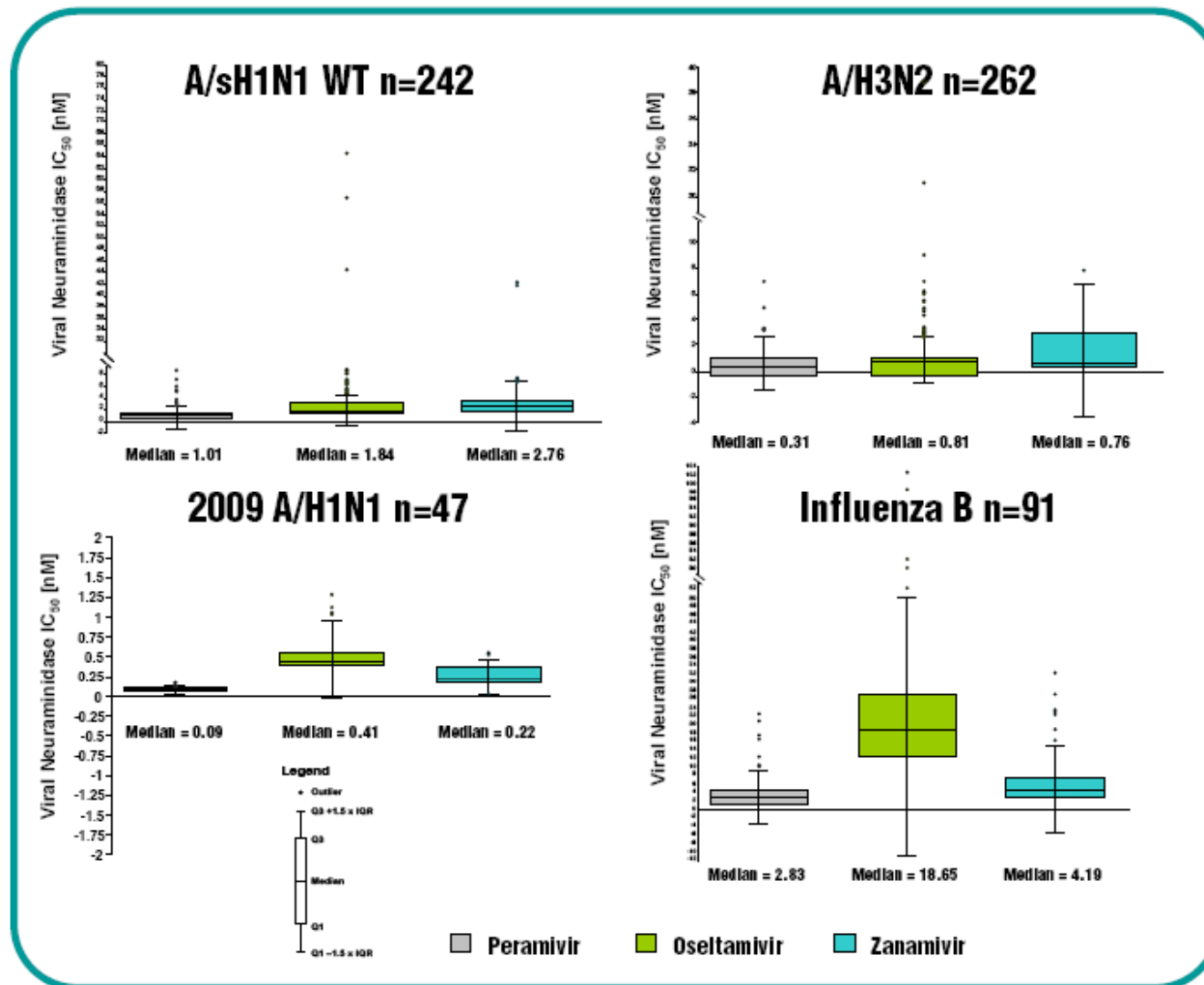
Study Designs and Treatments

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 = 113 300 mg IM = 115	300 mg IV = 99 600 mg IV = 99	200 mg IV = 45 400 mg IV = 46	300 mg IV BID = 114 600 mg IV QD = 116
Control	Placebo = 114	Placebo = 100	OSE 75 mg BID = 46	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

Peramivir Phase 2 and 3 Trials - Baseline IC₅₀ values were lowest for peramivir, then zanamivir, then oseltamivir (2006 – 2010)



BCX1812- 211 (2006/7) Little change in susceptibility from BL to last culture after a single PVR IM dose in outpatients

Viral Susceptibility, Median (Min, Max)

Influenza Virus Type	Treatment Group		NAI		
			Peramivir	Oseltamivir	Zanamivir
Influenza A (H1N1) Wild Type	PBO, n=27	IC ₅₀ of last positive culture	0.10 (0.01, 1.05)	1.13 (0.23, 13.02)	0.62 (0.06, 32.64)
		Fold change from baseline*	0.65 (0.04, 14.00)	1.04 (0.19, 7.92)	1.00 (0.12, 14.00)
	PVR 150, n=18	IC ₅₀ of last positive culture	0.22 (0.01, 2.34)	0.94 (0.10, 95.45)	0.62 (0.02, 10.39)
		Fold change from baseline*	1.97 (0.21, 69.50)	1.15 (0.12, 28.81)	0.73 (0.08, 14.60)
	PVR 300, n=19	IC ₅₀ of last positive culture	0.14 (0.01, 9.62)	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)
Influenza A (H3N2)	PBO, n=44	IC ₅₀ of last positive culture	0.18 (0.01, 2.63)	0.58 (0.06, 20.19)	0.50 (0.03, 8.30)
		Fold change from baseline*	1.12 (0.16, 18.00)	1.04 (0.16, 23.60)	1.19 (0.14, 25.38)
	PVR 150, n=37	IC ₅₀ of last positive culture	0.17 (0.02, 1.78)	0.41 (0.02, 3.99)	0.62 (0.05, 3.56)
		Fold change from baseline*	1.18 (0.11, 53.00)	1.00 (0.07, 6.23)	1.28 (0.25, 5.98)
	PVR 300, n=40	IC ₅₀ of last positive culture	0.16 (0.04, 4.65)	0.51 (0.11, 13.09)	0.82 (0.04, 14.15)
		Fold change from baseline*	1.45 (0.28, 37.75)	1.22 (0.26, 36.36)	1.40 (0.08, 36.15)
Influenza A (Indeterminate)	PBO, n=2	IC ₅₀ of last positive culture	0.06 (0.01, 0.11)	0.67 (0.37, 0.96)	0.79 (0.57, 1.00)
		Fold change from baseline*	5.67 (0.33, 11.00)	0.70 (0.23, 1.17)	1.03 (0.67, 1.39)
	PVR 150, n=3	IC ₅₀ of last positive culture	0.92 (0.69, 20.00)	17.47 (1.36, 42.75)	4.57 (0.67, 13.83)
		Fold change from baseline*	0.85 (0.69, 20.00)	1.73 (1.16, 2.52)	0.91 (0.51, 1.66)
	PVR 300, n=1	IC ₅₀ of last positive culture	0.98	15.65	2.37
		Fold change from baseline*	0.66	1.01	1.02
Influenza B	PBO, n=18	IC ₅₀ of last positive culture	2.53 (0.02, 6.30)	17.40 (0.06, 54.63)	2.74 (0.02, 11.97)
		Fold change from baseline*	1.12 (0.50, 3.04)	1.15 (0.24, 2.52)	1.02 (0.30, 2.41)
	PVR 150, n=14	IC ₅₀ of last positive culture	2.75 (1.02, 11.69)	17.87 (4.42, 32.95)	5.56 (3.16, 14.91)
		Fold change from baseline*	1.03 (0.21, 2.08)	0.96 (0.24, 1.19)	1.12 (0.68, 2.71)
	PVR 300, n=19	IC ₅₀ of last positive culture	1.74 (0.29, 3.41)	14.80 (6.36, 32.57)	2.99 (1.07, 8.80)
		Fold change from baseline*	0.95 (0.03, 1.87)	0.94 (0.13, 1.44)	0.92 (0.37, 3.09)

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

Two genotyping subsets were defined: paired isolates with NAI IC₅₀ values > the BL mean + 2 SD (n=20), and subjects culture positive at day 9 (n=18). **Two viruses for which the NA sequence was determined had an emergent known resistance mutation (both H275Y).**

BCX1812- 201 (2007/8) No change in susceptibility from BL to last culture after 5 days of daily IV PVR therapy in hospital

NAI Assay of Influenza Isolates: Fold Change from Baseline to Last Positive Culture

Influenza Virus Type	Treatment Group	Median (Min, Max) IC ₅₀ Fold Change from Baseline to Last Positive Culture		
		Peramivir	Oseltamivir	Zanamivir
Influenza A H1N1 Wild Type n = 5	OSE, n=2	3.4 (1.5, 5.2)	0.7 (0.2, 1.3)	0.6 (0.1, 1.1)
	PVR 200, n=1	1.0	1.3	0.8
	PVR 400, n=2	0.8 (0.6, 1)	0.8 (0.3, 1.3)	1.0 (0.6, 1.4)
Influenza A H1N1 H275Y n=2	OSE, n=1	0.52	1.39	0.62
	PVR 200, n=1	0.72	0.72	0.62
	PVR 400, n=0			
Influenza A H3N2 n = 23	OSE, n=8	0.7 (0.3, 2.7)	0.8 (0.1, 4.5)	0.8 (0.2, 25.8)
	PVR 200, n=6	0.9 (0.4, 21.9)	4.0 (0.5, 36.4)	2.6 (0.5, 5.1)
	PVR 400, n=9	1.1 (0.1, 2.7)	1.1 (0.2, 7.6)	1.1 (0.5, 6.8)
Influenza B n = 19	OSE, n=6	0.7 (0.1, 5.4)	0.9 (0.5, 22.7)	1.4 (0.4, 5.4)
	PVR 200, n=8	1.0 (0.6, 2.9)	0.9 (0.6, 1.2)	1.0 (0.6, 2.0)
	PVR 400, n=5	0.9 (0.02, 3.8)	0.9 (0.5, 2.0)	1.0 (0.5, 1.7)

Two genotyping subsets were defined: paired isolates with NAI IC₅₀ values > the BL mean + 2 SD (n=12), and subjects culture positive at day 5 (n=18). **No virus for which the NA sequence was determined had an emergent known resistance mutation.**

BCX1812-303 (2009/10): Little Change in 2009 H1N1 influenza A virus susceptibility to NAIs, median IC₅₀ nM (Range)

Treatment Group	Peramivir	Oseltamivir	Zanamivir
Peramivir 300 BID			
Baseline, Mdn (min,max)	0.09 (0.01, 0.14)	0.37 (0.32, 1.15)	0.22 (0.16, 0.55)
Last +, Mdn (min,max)	0.09 (0.06, 31.02)	0.37 (0.23, 282.4)	0.23 (0.08, 0.30)
Median Fold Change	No change*	No change*	No change
Peramivir 600 QD			
Baseline, Mdn (min,max)	0.09 (0.05, 0.14)	0.43 (0.18, 1.26)	0.22 (0.02, 0.57)
Last +, Mdn (min,max)	0.10 (0.07, 0.13)	0.38 (0.28, 0.93)	0.25 (0.11, 0.34)
Median Fold Change	No change	No change	No change

Two genotyping (GT) subsets were defined: paired isolates with NAI IC₅₀s > BL mean + 2 SD and subjects culture positive at day 5. ***Only one isolate (high post-BL IC₅₀) had H275Y, prior oseltamivir therapy**

Genotypic data from peramivir Phase 2 and 3 clinical trials (2006 to 2010) - Eight isolates with post therapy H275Y identified

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 isolates from **single dose studies** (SD subset n=31; delayed clearance, n=18) found **7 subjects (1.6%) with H275Y substitutions in NA.**

In the **5-10 day studies**, genotyping found **only 1 subject (0.5%) with the H275Y mutation** (SD subset, n=13; delayed clearance, n=18); that subject had received prior oseltamivir treatment.

No additional known resistance mutations were identified.

Conclusions

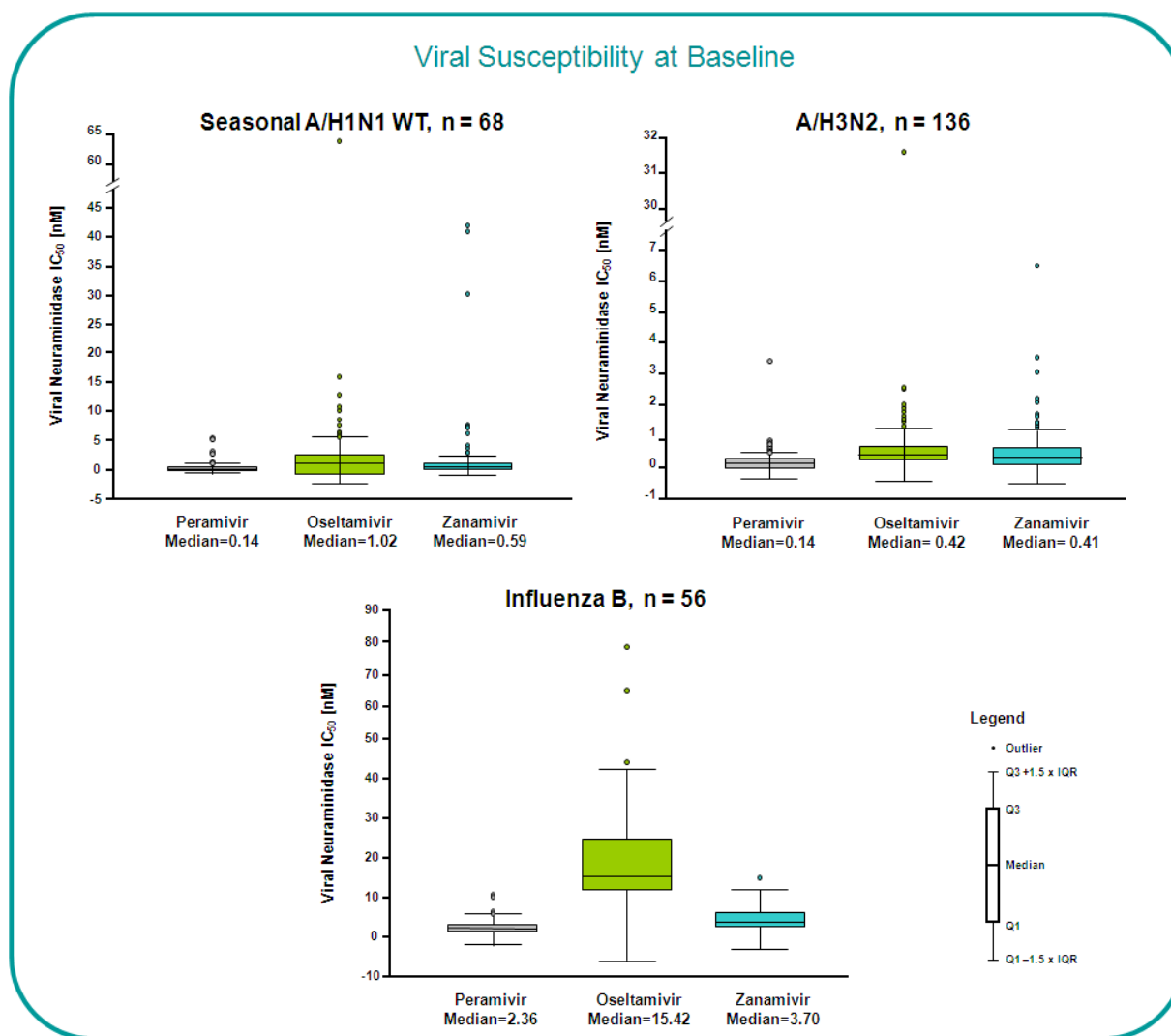
- Baseline IC₅₀ values for influenza A/H1N1 (wild type), A/H3N2, 2009 A/H1N1, and influenza B were as previously reported with the order being peramivir<zanamivir<oseltamivir.
- In general, little change in IC₅₀s post-BL was seen.
- Few subjects shed virus at day 5-10.
- Few paired isolates had post-BL IC₅₀ values > 2 SD from the mean.
- When genotypes were obtained:
 - Only 7 viruses (1.6%, SD subset) in the single dose studies
 - Only one (<1%, SD subset) in the 5-10 day studies
 - had a post-BL known resistance mutation (all H275Y).

Acknowledgements

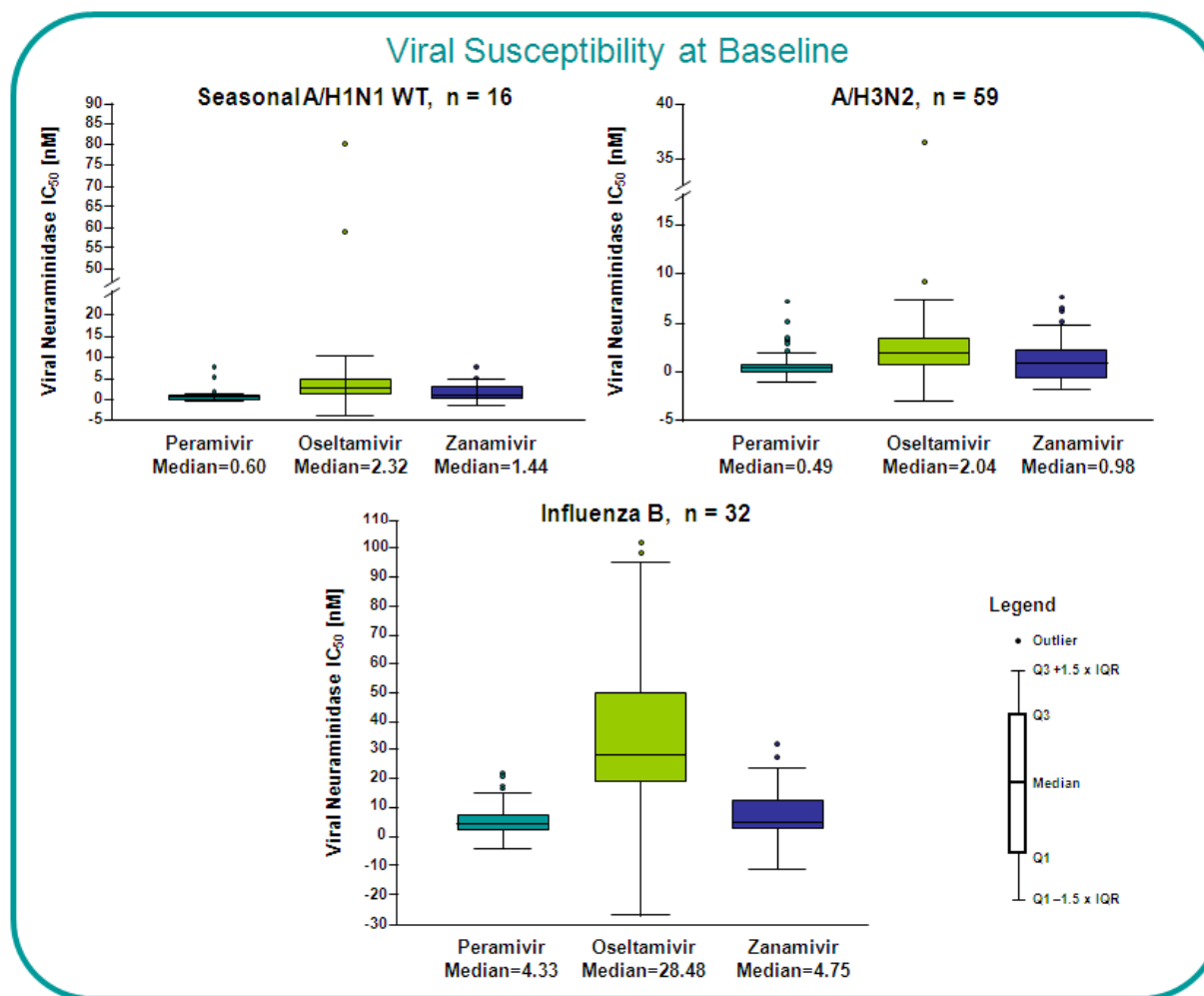
- The authors acknowledge the study participants, investigators, study site personnel, and BioCryst/Shionogi study teams who participated in these 4 clinical trials
- The US department of HHS/BARDA support the development of peramivir (Advanced Development Contract HHS0100200700032C)
- ViroClinics Biosciences BV, Rotterdam, The Netherlands, performed all the virology work analyzed and presented here

Back-up Slides

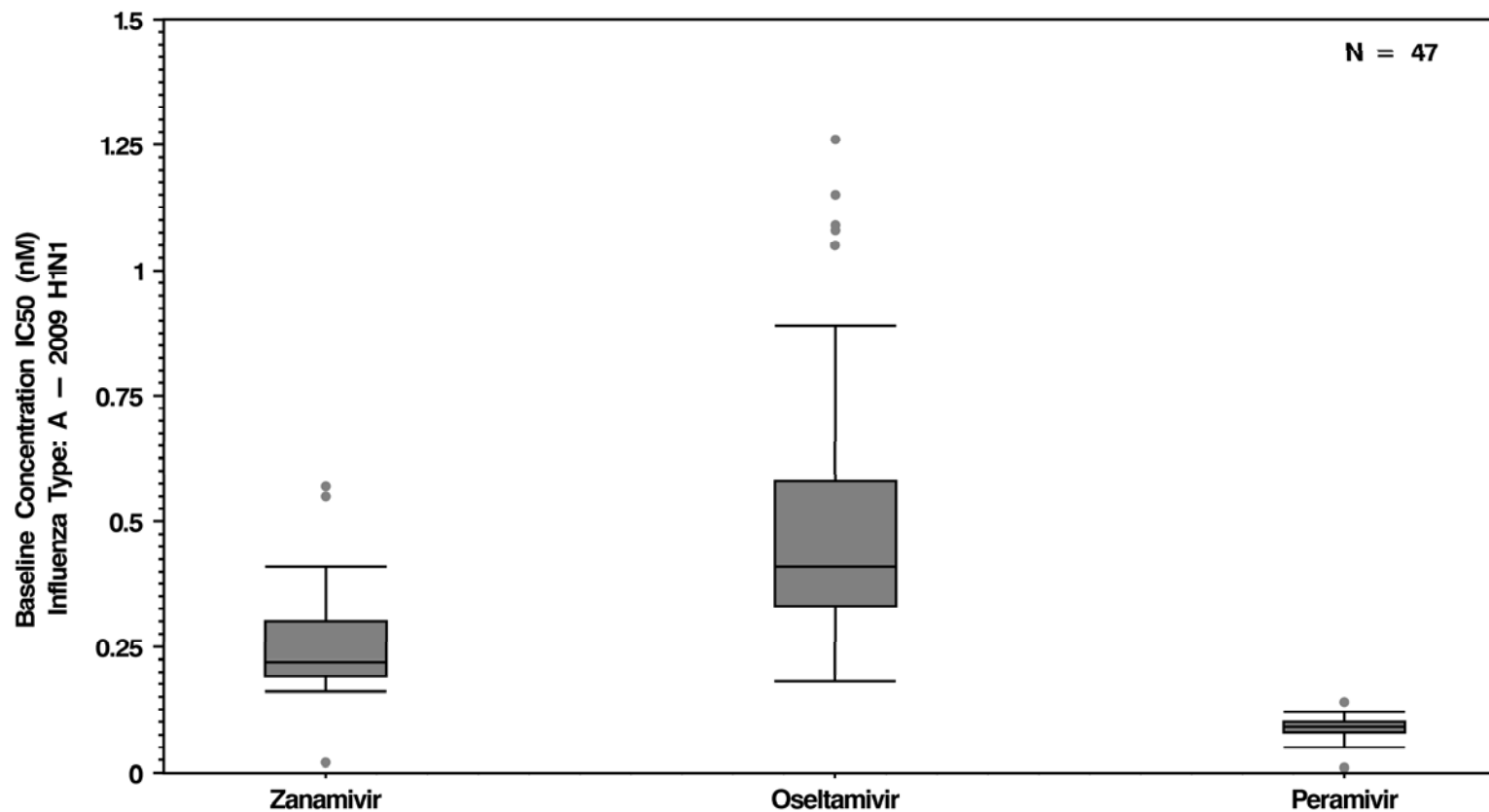
BCX1812-211 – Baseline IC₅₀ values were lowest for peramivir, then zanamivir, then oseltamivir (2006/2007 and 2007 seasons)



BCX1812-201 - Baseline IC₅₀ values were lowest for peramivir, then zanamivir, then oseltamivir (2007 and 2007/2008 seasons)

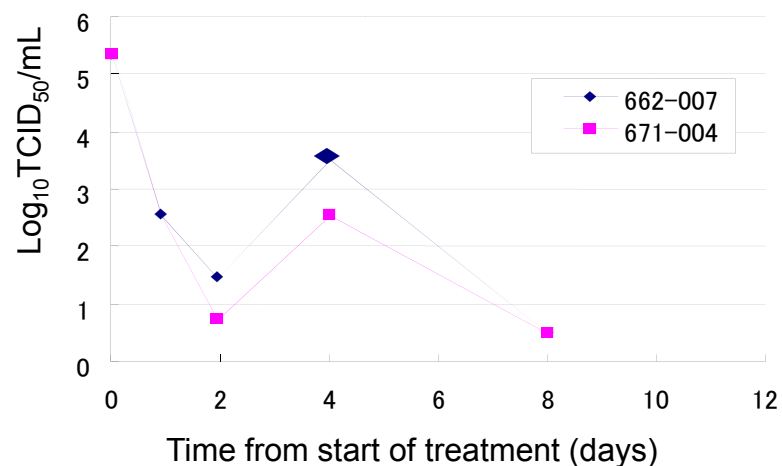
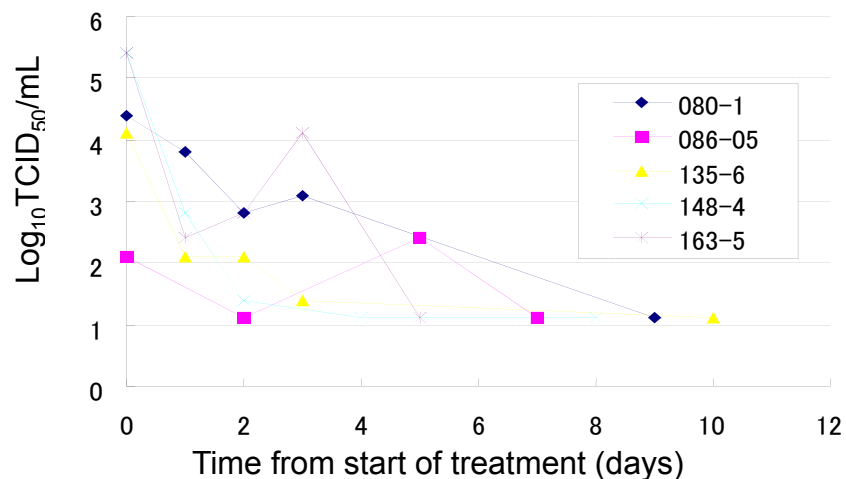


BCX1812- 303 baseline susceptibility of isolated influenza viruses (2009/10 and 2010 seasons)



Change in influenza virus titer for isolates with the H275Y mutation post therapy

Shionogi study 621, single IV dose 300 or 600mg BioCryst study 211, single IM dose 150 or 300mg



BioCryst study 303, 5-10d IV dose 300mg BID or 600mg QD

