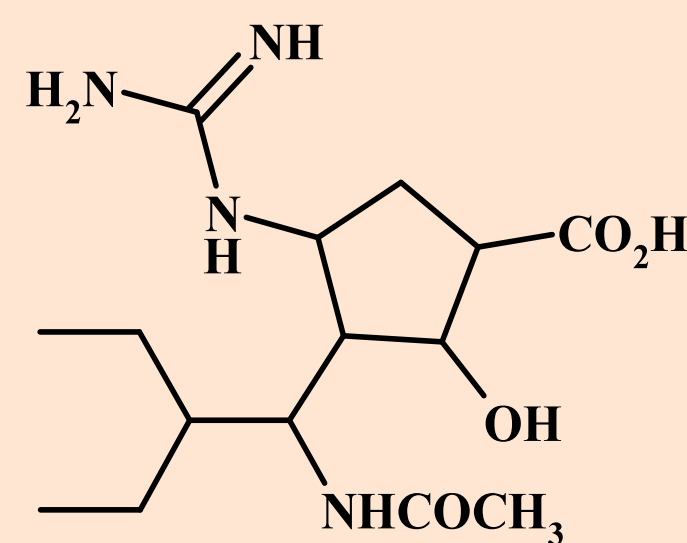


## INTRODUCTION

- Two classes of drugs are approved for prophylaxis and treatment of influenza: M2 ion channel blockers (amantadine and its derivative rimantadine) and neuraminidase (NA) inhibitors (oseltamivir and zanamivir)<sup>1</sup>. M2 ion channel blockers inhibit viral replication by blocking virus entry into the cell.<sup>2</sup> Neuraminidase inhibitors interrupt viral replication at a late stage by inhibiting the release of virions from infected cells.<sup>3</sup>
- Antiviral drugs that target different viral proteins and have different mechanisms of action when acting synergistically can provide several advantages over single agent treatment – greater potency, superior efficacy, potential for reduction of drug dosages and a higher barrier for the emergence of resistant mutants.
- Peramivir (**Figure 1**) is a specific and potent inhibitor of influenza NA and has demonstrated in vitro activity that is comparable or better than oseltamivir carboxylate and zanamivir.<sup>4</sup> Peramivir is active when administered IM and IV for both prophylaxis and treatment in mouse influenza virus infection models.<sup>5,6</sup> Intravenous administration of peramivir is currently being evaluated in clinical trials for seasonal influenza and to treat influenza in hospitalized patients.<sup>7,8</sup>
- In this study we have evaluated the efficacy of Peramivir given intramuscularly combined with rimantadine given orally against influenza A/Victoria/3/75 (H3N2) virus infection in a mouse model. The impact of combination treatment on weight loss was used as a parameter to characterize their mode of interaction.

**Figure 1:** Structure of Peramivir



## MATERIALS AND METHODS

- Influenza virus: A/Victoria/3/75 (H3N2). Animals: Female Balb/c mice 6-8 weeks old.
- Peramivir was administered by IM injection, qd and rimantadine was administered orally, bid. Five day dosing began 1 hr prior to infection with virus.
- To maintain consistency in the handling of animals, the peramivir group received vehicle orally, rimantadine group received vehicle IM, and the control group received vehicle both orally and IM.
- Parameters for evaluation of antiviral activity included weight loss, reduction in mortality and/or increase in mean day to death (MDD) determined through 16-21 days.
- Statistical analysis and Synergy determination: The t-test was used to evaluate differences in mean day to death. One way analysis of variance (ANOVA) was performed to evaluate differences in weight loss. Kaplan-Meier Survival analysis (log rank test) was applied to survival number differences. The analysis of the combined antiviral effect of rimantadine and peramivir in influenza virus A (H3N2) infection in mice was determined according to the MacSynergy three dimensional model<sup>9</sup>.

**Table 1:** Effect of Peramivir or Rimantadine on Weight Loss in Influenza A (H3N2) Infected mice (Single agent Study)\*

Drug	Dose (mg/kg/day)	Weight Change day 9** mean ± SEM (gms)
Vehicle, uninfected (n=2)	0	-0.15 ± 0.45
Vehicle (n=10)	0	-5.08 ± 0.36
Peramivir (n=10)	1	-4.01 ± 0.28 <sup>a</sup>
	3	-3.32 ± 0.6 <sup>a</sup>
	10	-0.28 ± 0.11 <sup>b</sup>
	30	-0.06 ± 0.17 <sup>b</sup>
Rimantadine (n=10)	10	-3.38 ± 0.35 <sup>b</sup>
	30	-0.75 ± 0.28 <sup>b</sup>
	100	-0.14 ± 0.22 <sup>b</sup>
	300	-0.63 ± 0.15 <sup>b</sup>
Rimantadine, uninfected (n=5)	300	0.4 ± 0.13

<sup>a</sup>Statistically significant at p<0.03; <sup>b</sup>p<0.002 vs. vehicle  
\*No deaths were observed; \*\* Maximum weight loss in the vehicle group

**Table 2.** Effect of Combinations of Peramivir and Rimantadine on Weight Loss in Influenza A (H3N2) infected mice

Compound (mg/kg/day)	Peramivir (P)			
	0.0	0.3	1.0	3.0
Rimantadine (R)				
0.0	-5.19 ± 0.16	-2.6 ± 0.75 <sup>a</sup>	-4.3 ± 0.42 <sup>a</sup>	-3.55 ± 0.35 <sup>a</sup>
5.0	-3.43 ± 0.55 <sup>a</sup>	<b>-1.97 ± 0.47<sup>ab</sup></b>	<b>-1.69 ± 0.63<sup>ac</sup></b>	<b>-1.31 ± 0.34<sup>ac</sup></b>
10.0	-2.1 ± 0.37 <sup>a</sup>	<b>-1.25 ± 0.55<sup>ad</sup></b>	<b>-0.69 ± 0.25<sup>ac</sup></b>	<b>0.05 ± 0.22<sup>ac</sup></b>
30.0	-1.64 ± 0.54 <sup>a</sup>	-1.52 ± 0.42 <sup>a</sup>	-0.41 ± 0.22 <sup>ac</sup>	<b>0.25 ± 0.14<sup>ac</sup></b>

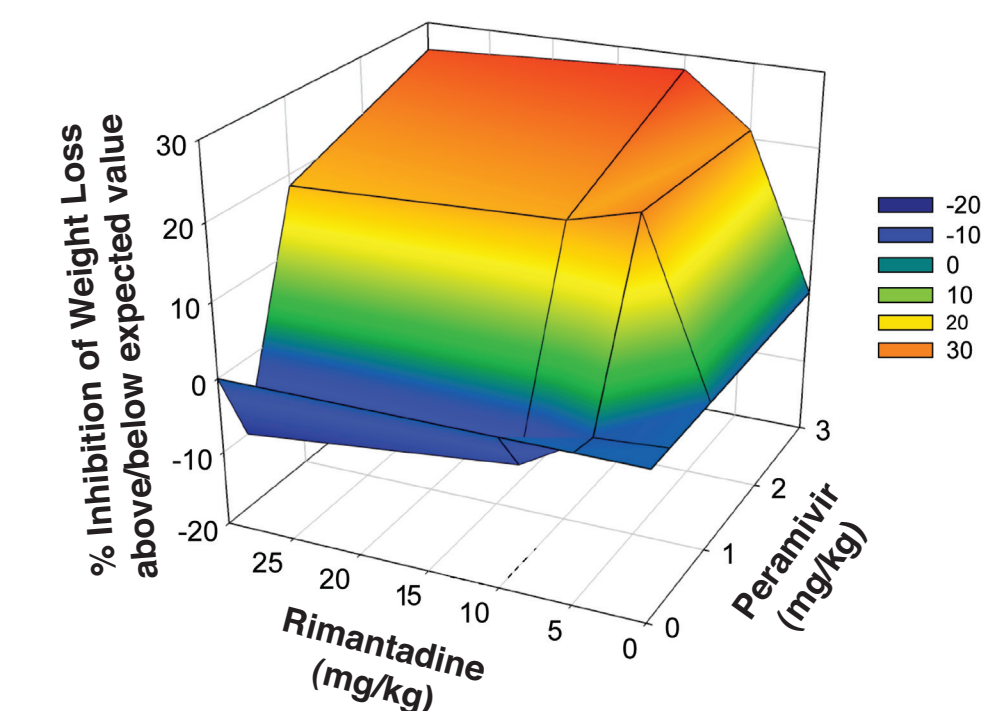
<sup>a</sup>p<0.05 vs. vehicle (0P/0R); <sup>b</sup>p<0.05 vs.0P/5R; <sup>c</sup>p<0.05 vs. either compound used alone; <sup>d</sup>p<0.05 vs. 0.3P/0R; <sup>e</sup>p<0.05 vs. 1P/0R; \*Maximum weight loss in the vehicle group with no death

**Table 3:** Effect of Combinations of Peramivir and Rimantadine on survival and mean day to death in Influenza A Infected BALB/c mice

Compound Peramivir (mg/kg/d)	Compound Rimantadine (mg/kg/d)	Mean day to death ± SEM	Survival/Total (% survival)
0	0	> 21 ± 0.0	5/5 (100%)
0	0	11.5 ± 0.34	9/15 (60%)
	5	7.5 ± 3.5	8/10 (80%)
	10	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	30	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
0.3	0	11 ± 0.0	9/10 (90%)
	5	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	10	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	30	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
1	0	12 ± 0.0	9/10 (90%)
	5	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	10	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	30	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
3	0	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	5	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	10	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>
	30	> 21 ± 0.0 <sup>a</sup>	10/10 (100%) <sup>b</sup>

<sup>a</sup>p<0.002, <sup>b</sup>p<0.03 vs. vehicle (Peramivir (0mg/kg) + Rimantadine (0mg/kg))

**Figure 2.** Three dimensional synergy plot of interactions of peramivir and rimantadine on influenza A/Victoria /3/75 mouse model



## SUMMARY

- Both rimantadine and peramivir mitigate weight loss in mice infected with influenza in a dose dependant manner (**Table 1**).
- Significant differences in weight loss were noted between combination treated groups versus groups treated with a single agent and the vehicle treated group (**Table 2 shaded area**)
- Significant improvement in survival and mean day to death was observed with single agent and combination treatment (**Table 3**)
- Combination of peramivir and rimantadine demonstrates synergistic anti-influenza effects in an influenza A mouse model (**Figure 2**).

## CONCLUSION

- Peramivir in combination with rimantadine demonstrates a synergistic anti-viral effect in mouse influenza model. These data support exploring the combination of peramivir and rimantadine for the treatment of influenza in the clinic.

## REFERENCES

- Hayden FG. Antivirals for influenza: historical perspectives and lessons learned. Antiviral Res. 2006; Sep; 71(2-3): 372-8.
- Wang C, Takeuchi K, Pinto LH, Lamb RA. Ion channel activity of influenza A virus M2 protein: characterization of the amantadine block. J Virol. 1993; Sep;67(9):5585-94
- Bhatia A, Kast RE. How influenza's neuraminidase promotes virulence and creates localized lung mucosa immunodeficiency. Cell Mol Biol Lett. 2007; 12(1):111-9.
- Bantia S, Parker CD, Ananth SL, Horn LL, Andries K, Chand P, Kotian, PL, Dehghani A, El-Kattan Y, Lin T, Hutchison TL, Montgomery JA, Kellogg DL, Babu YS. Comparison of the anti-influenza virus activity of RWJ-270201 with those of oseltamivir and zanamivir. Antimicrob. Agents Chemother 2001; 45:1162-1167.
- Bantia S, Arnold CS, Parker CD, Upshaw R, Pooran C. Anti influenza virus activity of peramivir in mice with single intramuscular injection Antiviral Research 2006; 69(1):39-45.
- Kobayashi M, Isoda N, Kodama M, Yoshida R, Sato A, Yamano Y, Sakoda Y, Kida H. Inhibitory Effect of Peramivir (S-021812, BCX-1812) against Highly Pathogenic Avian Influenza Viruses. 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2009). San Francisco, September 12-15, 2009. Abstract V-1064.
- Kohono S, Yen MY, Cheong HJ, and others. V-537a -Single-Intravenous Peramivir vs. Oral Oseltamivir to Treat Acute, Uncomplicated Influenza in the Outpatient Setting: A Phase III Randomized, Double-Blind Trial. ICAAC 2009. Abstract V-537a.
- Ison MG, McGeer AJ, Hui DS, Clezy K, O'Neil B, Flynt A, Elder J, Simon TJ, Alexander WJ. Safety and Efficacy of Multiple-Day Treatment with Intravenous Peramivir or Oral Oseltamivir in Hospitalized Adults with Acute Influenza. XI International Symposium on Respiratory Viral Infections. 2009. Oral abstract.
- Prichard MN, Shipman C Jr. A three-dimensional model to analyze drug-drug interactions. Antiviral Res. 1990; Oct-Nov;14(4-5):181-205.