Novel Antiviral Fc-Conjugate CB-012 Demonstrates Potent Activity in Cytopathic Effect (CPE) and Viral Growth Inhibition Assays Against Influenza A and B Strains

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INTRODUCTION

A series of potent, long-acting antiviral molecules against the influenza virus has been generated using Cidara Therapeutics' Cloudbreak immunotherapy platform. These antiviral Fc-conjugates (AVCs) combine an active antiviral agent with the Fc portion of human IgG1, which engages the immune system in a multimodal MOA.

In previous studies, a novel AVC, CB-012, was evaluated with respect to PK/safety and has demonstrated efficacy in immunocompetent and immune compromised animal infection models (1-4). Herein we characterized the intrinsic activity of CB-012 in *in vitro* cytopathic effect (CPE) and viral growth inhibition assays against influenza A and B strains.

METHODS

- CPE assays were performed in Madin-Darby Canine Kidney (MDCK) cells challenged with A/California/09 (H1N1) and B/Brisbane viruses using ten, two-fold serial dilutions of CB-012 (160 – 0.3125 nM) and oseltamivir (9.6 µM – 18.75 nM). To determine the 50% effective concentration (EC_{50}), MDCK cells were infected (MOI = 0.001; 1-h incubation) and stained with crystal violet at 3- or 5-d post-infection (influenza A and B, respectively).
- Viral growth inhibition assays were performed in A549 cells using A/WSN/33 (H1N1), A/Wyoming/3/03 (H3N2), A/California/04/09 (H1N1), A/Vietnam/1203/04 (H5N1) HALo, and B/Lee/40 Victoria. Cells were pre-treated with molecules at 1 µM, 100 nM, or 10 nM for 2 h and infected with indicated strains at an MOI = 0.01 for 1-h incubation, after which cells were washed and molecules were reapplied at the same concentrations. Production of virions/viruses in the supernatant was determined via plaque assay.
- Cytotoxicity was assessed by calculating the selectivity index (SI) for MDCK cells (cytotoxicity concentration 50% [CC_{50}]/CPE EC_{50}) and by measuring viability of drug-exposed A549 cells relative to the PBS control using CellTiter-Glo™ (Promega).

RESULTS

Table 1. CPE assay and selectivity index (SI).

Compound	A/California/09 (H1N1)		B/Brisbane (Victoria)		MDCK cells
	CPE EC_{50} (nM)	SI	CPE EC_{50} (nM)	SI	CC_{50} (nM)
CB-012	4	>40	52	>3.1	>160
Oseltamivir	390	>24.6	1,065	>9	>9,600

- CPE EC_{50} values for CB-012 were 98- and 20-fold more potent against influenza A and B strains, respectively, than oseltamivir.
- SI values were positive for both drugs but could not be precisely calculated due to lack of cytotoxicity at the highest concentrations tested.

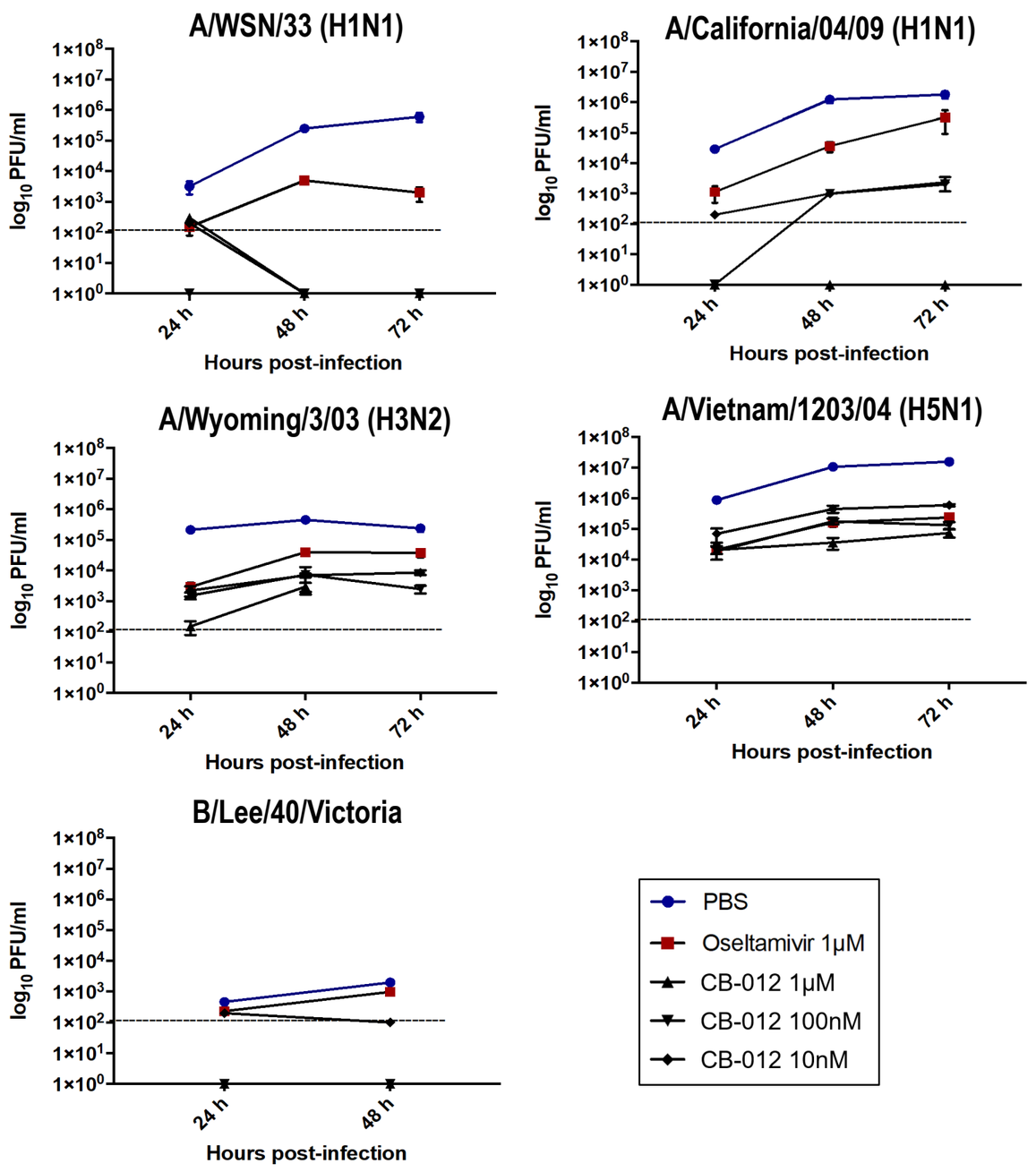
Table 2. Viral growth inhibition assay (tabular form).

Strain	Time (h)	Viral concentration (log_{10} PFU/mL)				
		PBS	Oseltamivir	CB-012		
			1 µM	1 µM	100 nM	10 nM
A/WSN/33 (H1N1)	24	3,200	150	300	0	200
	48	250,000	5,000	0	0	0
	72	603,333	2,000	0	0	0
A/California/04/09 (H1N1)	24	29,167	1,117	0	0	200
	48	1,233,333	36,500	0	1,000	1,000
	72	1,800,000	318,333	0	2,000	2,333
A/Wyoming/3/03 (H3N2)	24	215,000	2,950	150	1,533	2,233
	48	455,000	40,167	3,000	7,333	7,000
	72	240,000	37,667	0	2,500	8,667
A/Vietnam/1203/04 (H5N1)	24	890,000	21,500	21,167	19,167	70,833
	48	10,783,333	165,000	36,667	180,000	456,667
	72	15,666,667	243,333	75,000	135,000	603,333
B/Lee/40/Victoria	24	467	233	0	0	200
	48	2,000	1,000	0	0	100

*Results represent average and SD of three biological replicates

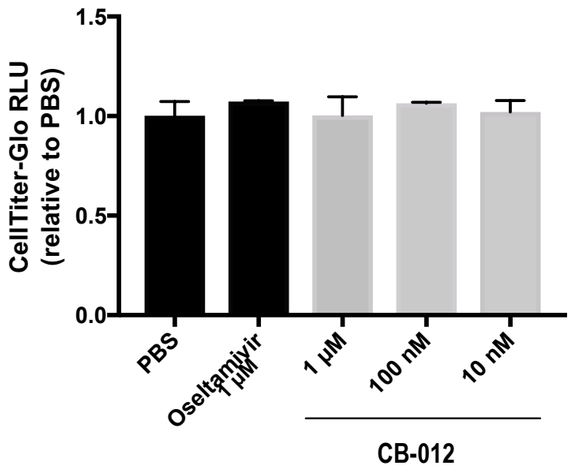
RESULTS (cont'd)

Figure 1. Viral growth inhibition assay (graphic form).



- At 10 nM, CB-012 reduced the viral titers more potently than oseltamivir at 1 µM against all strains by 72 h. As an exception, A/Vietnam/1203/04 required 100 nM of CB-012 to outperform oseltamivir.

Figure 2. Viability of drug-exposed A549 cells.



- CB-012 and oseltamivir had no impact on the viability of A549 cells across all concentrations evaluated.

CONCLUSIONS

- Both CPE and viral growth assays demonstrated more potent activity of CB-012 than oseltamivir against a variety of influenza A and B strains, in addition to showing no detectable cytotoxicity at concentrations tested, supporting further development of this novel AVC for the prevention and treatment of influenza.

REFERENCES

1. Ong, V, et al. Preclinical efficacy, pharmacokinetics, and safety of CB-012, a novel antiviral Fc-conjugate against influenza. Presented at Options X (2019). Abstract 10974.
2. Levin J, et al. Efficacy of CB-012, a novel antiviral Fc-conjugate, in lethal mouse models of oseltamivir-sensitive and -resistant influenza A H1N1 and H3N2 isolates. Presented at Options X (2019). Abstract 10979.
3. Döhrmann S, et al. Fc-mediated effector function contributes to potency of novel antiviral Fc-conjugate CB-012 Presented at Options X (2019). Abstract 11094.
4. Tari L, et al. Efficacy of CB-012, a novel antiviral Fc-conjugate, against influenza A (H1N1) in a lethal mouse model of severe combined immunodeficiency (SCID). Presented at Options X (2019). Latebreaker Abstract 11760.

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