Characterization of *In Vitro* Activity and *In Vivo* Efficacy of CD377, a Novel Antiviral Fc-Conjugate, Against 2020-2021 Northern Hemisphere Influenza Quadrivalent Vaccine Strains

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Abstract ID #269

Disclosures

- All authors are employees and shareholders of Cidara Therapeutics, Inc.

Limitations of approved influenza prophylaxis and treatment options

Vaccines

- **Vaccines are strain-specific, providing variable coverage**
  - 10%-60% effective (2004-2018)[1]
  - Less effective in elderly & immune compromised
- **~2-week lag time to achieve full protection[2]**
- **Long, complex manufacturing cycle**
  - Difficult to scale, low antigen yields can limit production capacity

- **Desired Target Product Profile:**
  - Long-acting prophylactic agent with full seasonal/pandemic coverage
  - Coverage of immune compromised subjects

[1]https://www.cdc.gov/flu/professionals/vaccination/effectiveness-studies.htm
[2]https://www.cdc.gov/flu/protect/keyfacts.htm

Antiviral Treatments

- **Short administration window**
  - 48 hours from symptom onset[3]
- **Drug resistance**
- **Effectiveness poorly defined, particularly in high-risk patients**
  - Current treatments provide modest effects in reducing symptoms, infectivity
  - Insufficient data to demonstrate that they reduce complications

- **Desired Target Product Profile:**
  - Broad-spectrum activity that reduces symptoms and infectivity with an extended treatment window
  - Minimize probability of resistance emergence

[3]https://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm

Cidara's Cloudbreak® AVCs: a new class of long-acting antivirals

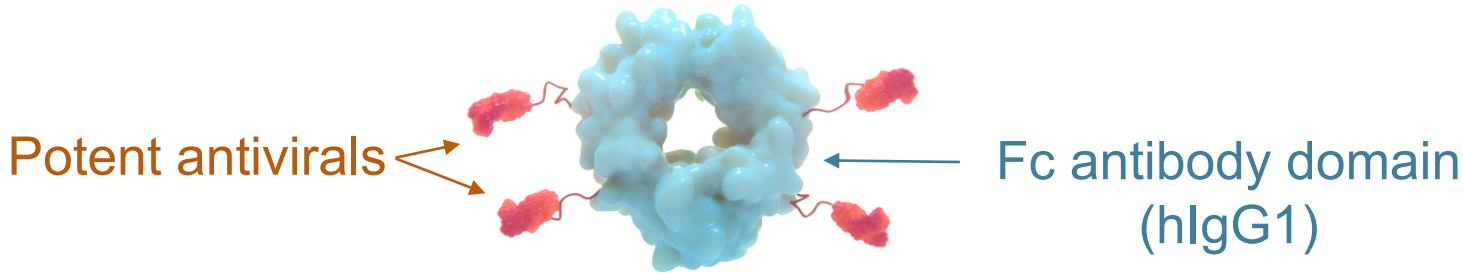
Long-acting antiviral activity and potential immune engagement

Antiviral Conjugates (AVCs)

- Comprise stable, multivalent conjugates of broad spectrum neuraminidase inhibitors with an engineered Fc antibody domain
- Multivalent presentation enhances antiviral binding and potency
- Extended half-life and immune cell engagement mediated by Fc domain

Program Goal:

- Universal influenza protection, all strains in all people, 1 SC or IM dose per season



Targeting Moiety (TM)	Fc Moiety
• *Direct antiviral activity* • *Inhibits essential surface target*	• *Extends PK* • *Engages immune system*

Influenza AVC profile summary

Preclinical development candidates include CD377 and CD388, a longer acting molecule

	Target Attribute	AVCs in Preclinical Development
Indication	Universal prevention and treatment	Data are supportive
Spectrum	A & B + drug-resistant strains, low resistance potential	Potent *in vivo* activity against all seasonal and pandemic strains
Safety/Tolerability	High safety margin for long term prevention	>50x exposure margin in rat and primate toxicity studies
Dosing Frequency	1 to 2x per flu season	Estimated 3- to 6-month coverage with single SC or IM dose
Route of Administration	SC, IM and IV dosing	Equivalent exposures and efficacy
Target Populations	Higher risk populations where vaccines are not effective	Equally effective in immune compromised & immune competent models at similar doses

Data available at: https://ir.cidara.com/presentations

Study Objective: Characterize the activity of CD377 against current influenza vaccine strains

WHO-recommended 2020-2021 N. Hemisphere quadrivalent influenza vaccine strains:

Type	Subtype/ Lineage	Strain	Updated from 2019-2020 vaccine	Egg-based vaccine	Cell- or recombinant-based vaccine	Included in this study
A	H1N1	A/Guangdong-Maonan/SWL1536/2019 pdm09	✓	✓		
		A/Hawaii/70/2019 pdm09	✓		✓	✓
	H3N2	A/Hong Kong/2671/2019	✓	✓		✓
		A/Hong Kong/45/2019	✓		✓	
B	Victoria	B/Washington/02/2019	✓	✓	✓	✓
	Yamagata	B/Phuket/3073/2013		✓	✓	✓

https://www.who.int/influenza/vaccines/virus/recommendations/2020-21_north/en/

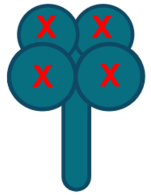
Study overview

Strains

- A/Hawaii/70/2019 (H1N1pdm09)
- A/Hong Kong/2671/2019 (H3N2)
- B/Washington/02/2019 (Victoria)
- B/Phuket/3073/2013 (Yamagata)

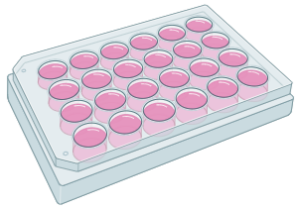
Antiviral agents

- CD377
- oseltamivir
- zanamivir
- baloxavir



In vitro and *in vivo* analyses

1) Neuraminidase Inhibition (NAI)
2) Enzyme-Linked Lectin NAI (ELLA-NAI)



3) Cytopathic Effect (CPE)
4) Plaque Reduction Assay (PRA)

5) Mouse infection model

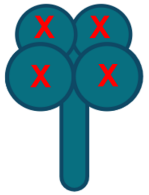
Images created with BioRender

1) Neuraminidase Inhibition (NAI) assay

Classical/Standard NA enzyme inhibition assay using small molecule neuraminidase substrate

	IC_{50} (nM)		
Strain	**CD377**	**oseltamivir**	**zanamivir**
A/Hawaii/70/2019 (H1N1pdm09)	**0.21**	0.32	0.26
A/Hong Kong/2671/2019 (H3N2)	**4.8**	0.76	1.05
B/Washington/02/2019 (Victoria)	**3.11**	16.71	2.3
B/Phuket/3073/2013 (Yamagata)	**4.58**	24.44	2.78

NAI was measured via the NA-Fluor™ kit (ABI) following pre-incubation of virus with inhibitors.



CD377 demonstrates balanced, potent activity against the NA target, comparable to zanamivir

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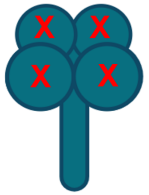
2) Enzyme-Linked Lectin Neuraminidase Inhibition (ELLA-NAI) assay

NA inhibition method using natural presentation of neuraminidase substrate with physiological relevance

	IC_{50} (nM)		
Strain	**CD377**	**oseltamivir**	**zanamivir**
A/Hawaii/70/2019 (H1N1pdm09)	**0.2**	5.9	2.7
A/Hong Kong/2671/2019 (H3N2)	**0.1**	0.3	0.4
B/Washington/02/2019 (Victoria)	**3.9**	47.9	6.6
B/Phuket/3073/2013 (Yamagata)	**2.8**	28.8	9



ELLA-NAI assays were performed using fetuin-coated plates as previously described[1]

CD377 NA target potency in the ELLA assay format demonstrates similar potency as compared to standard NAI assay and trends more potent than comparators

[1]Gao, J., Couzens, L. and Eichelberger, M. C. Measuring Influenza Neuraminidase Inhibition Antibody Titers by Enzyme-linked Lectin Assay. *J Vis Exp*, doi:10.3791/54573 (2016)

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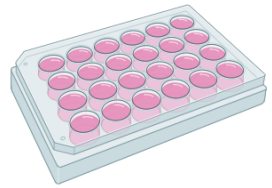
3) Cytopathic Effect (CPE) assay

Inhibition of virus-induced cell death in cell-based assay

	EC_{50} (nM)			
Strain	**CD377**	**oseltamivir**	**zanamivir**	**baloxavir**
A/Hawaii/70/2019 (H1N1pdm09)	**1.65**	326.6	686.2	11.1
A/Hong Kong/2671/2019 (H3N2)	**0.38**	7.7	170	1
B/Washington/02/2019 (Victoria)	**1.06**	1028	1.93	9.98
B/Phuket/3073/2013 (Yamagata)	**0.33**	27.6	5.2	10.01

CPE was assessed at MOI=0.01 following 3-5 d incubation and crystal violet staining/OD_{595} readings.



CD377 demonstrates potent activity in CPE with multi-log improved activity as compared to oseltamivir or zanamivir

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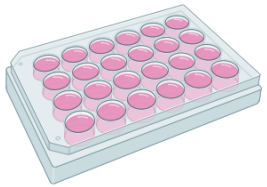
4) Plaque Reduction Assay (PRA) assay

Inhibition of plaque formation using specialized media restricting viral spread in cell-based assay

	EC_{50} (nM)			
Strain	**CD377**	**oseltamivir**	**zanamivir**	**baloxavir**
A/Hawaii/70/2019 (H1N1pdm09)	**0.14**	7.08	3.69	10.3
A/Hong Kong/2671/2019 (H3N2)*	**ND**	ND	ND	ND
B/Washington/02/2019 (Victoria)	**7.46**	28.72	9.77	18.27
B/Phuket/3073/2013 (Yamagata)	**2.61**	28.5	13.9	30.96



PRA was performed by co-incubating drugs/virus prior to addition to cells (MOI-optimized). Virus/drug was removed then cells were incubated (48 h) in the presence of drug/Avicel/dextran/DMEM/trypsin.

*ND – not determined; Strain not amenable to plaquing in MDCK or MDCK-SIAT1 cells

CD377 PRA activity was more potent than comparators

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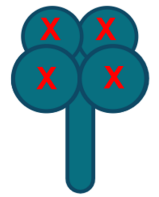
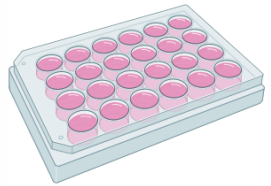
CD377 *in vitro* assay results summary

		IC_{50}/EC_{50} (nM)			
Assay	**Strain**	**CD377**	**oseltamivir**	**zanamivir**	**baloxavir**
NAI	A/Hawaii/70/2019 (H1N1pdm09)	**0.21**	0.32	0.26	ND
	A/Hong Kong/2671/2019 (H3N2)	**4.8**	0.76	1.05	ND
	B/Washington/02/2019 (Victoria)	**3.11**	16.71	2.3	ND
	B/Phuket/3073/2013 (Yamagata)	**4.58**	24.44	2.78	ND
ELLA-NAI	A/Hawaii/70/2019 (H1N1pdm09)	**0.2**	5.9	2.7	ND
	A/Hong Kong/2671/2019 (H3N2)	**0.1**	0.3	0.4	ND
	B/Washington/02/2019 (Victoria)	**3.9**	47.9	6.6	ND
	B/Phuket/3073/2013 (Yamagata)	**2.8**	28.8	9	ND
CPE	A/Hawaii/70/2019 (H1N1pdm09)	**1.65**	326.6	686.2	11.1
	A/Hong Kong/2671/2019 (H3N2)	**0.38**	7.7	170	1
	B/Washington/02/2019 (Victoria)	**1.06**	1028	1.93	9.98
	B/Phuket/3073/2013 (Yamagata)	**0.33**	27.6	5.2	10.01
PRA	A/Hawaii/70/2019 (H1N1pdm09)	**0.14**	7.08	3.69	10.3
	A/Hong Kong/2671/2019 (H3N2)	**ND**	ND	ND	ND
	B/Washington/02/2019 (Victoria)	**7.46**	28.72	9.77	18.27
	B/Phuket/3073/2013 (Yamagata)	**2.61**	28.5	13.9	30.96

ND – not determined





CD377 demonstrates consistent, potent activity, similar to or better than comparators, that's most differentiated in cell-based assays

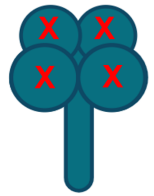
Images created with BioRender

CD377 NAI and CPE assay data against WT influenza A and B strains

CD377 in vivo data from non-2020-2021 vaccine strain studies

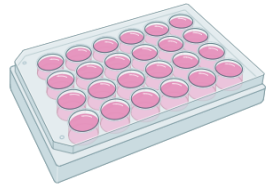
NAI

			Median IC_{50} (nM)		
Type	Subtype/Lineage	Strains (n)	CD377	oseltamivir	zanamivir
A	H1N1	9	**1.5**	1.5	0.6
	H3N2	6	**4.5**	0.4	1
B	Victoria & Yamagata	7	**3.1**	28.5	5.9



CPE

			Median EC_{50} (nM)			
Type	Subtype/Lineage	Strains (n)	CD377	oseltamivir	zanamivir	baloxavir
A	H1N1	10	**1**	925	343	3
	H3N2	6	**1**	3,190	112	2
B	Victoria & Yamagata	6	**3.9**	654.8	67	11.5



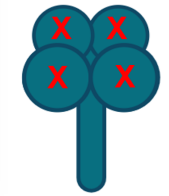
CD377 has universal activity against influenza A and B *in vitro*

CD377 NAI and CPE assay data against NA mutant strains

CD377 in vivo data from non-2020-2021 vaccine strain studies

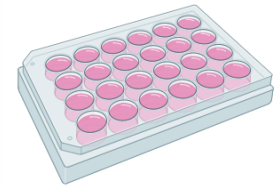
NAI

Strain (NA mutation)	Subtype	IC_{50} (nM)		
		CD377	oseltamivir	zanamivir
A/Texas/23/2012 (**H275Y**)	H1N1pdm09	**1.7**	507.1	1.1
A/Texas/12/2007 (**E119V**)	H3N2	**2.6**	148.9	3.8
A/Bethesda/956/2006 (**R292K**)	H3N2	**1.2**	>1000	22.18



CPE

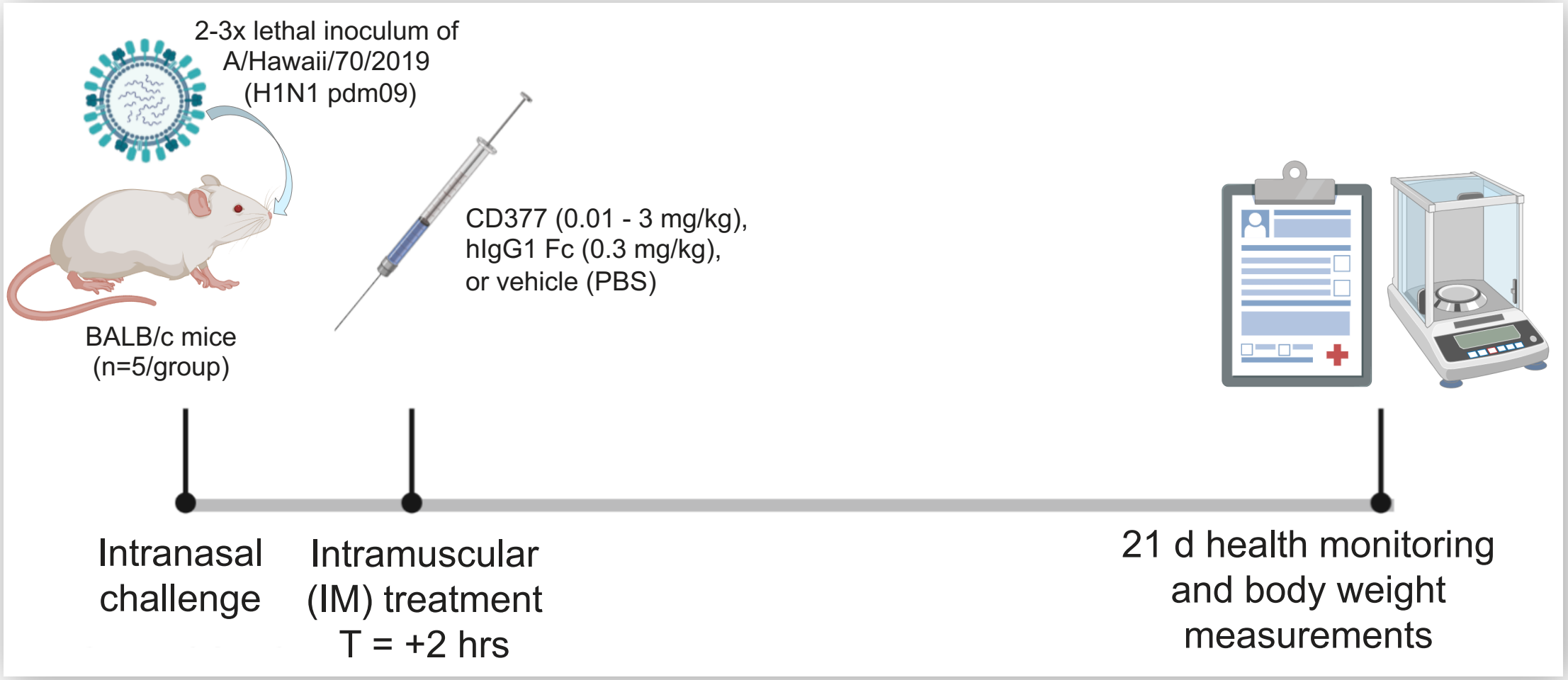
Strain (NA mutation)	Subtype	EC_{50} (nM)		
		CD377	oseltamivir	zanamivir
A/Texas/23/2012 (**H275Y**)	H1N1pdm09	**2.1**	>10,000	327.7
A/Texas/12/2007 (**E119V**)	H3N2	**0.7**	351.4	2.1
A/Bethesda/956/2006 (**R292K**)	H3N2	**1.8**	>10,000	>10,000



CD377 retains potent activity against influenza NA mutants with reduced susceptibility to approved neuraminidase inhibitors

Images created with BioRender

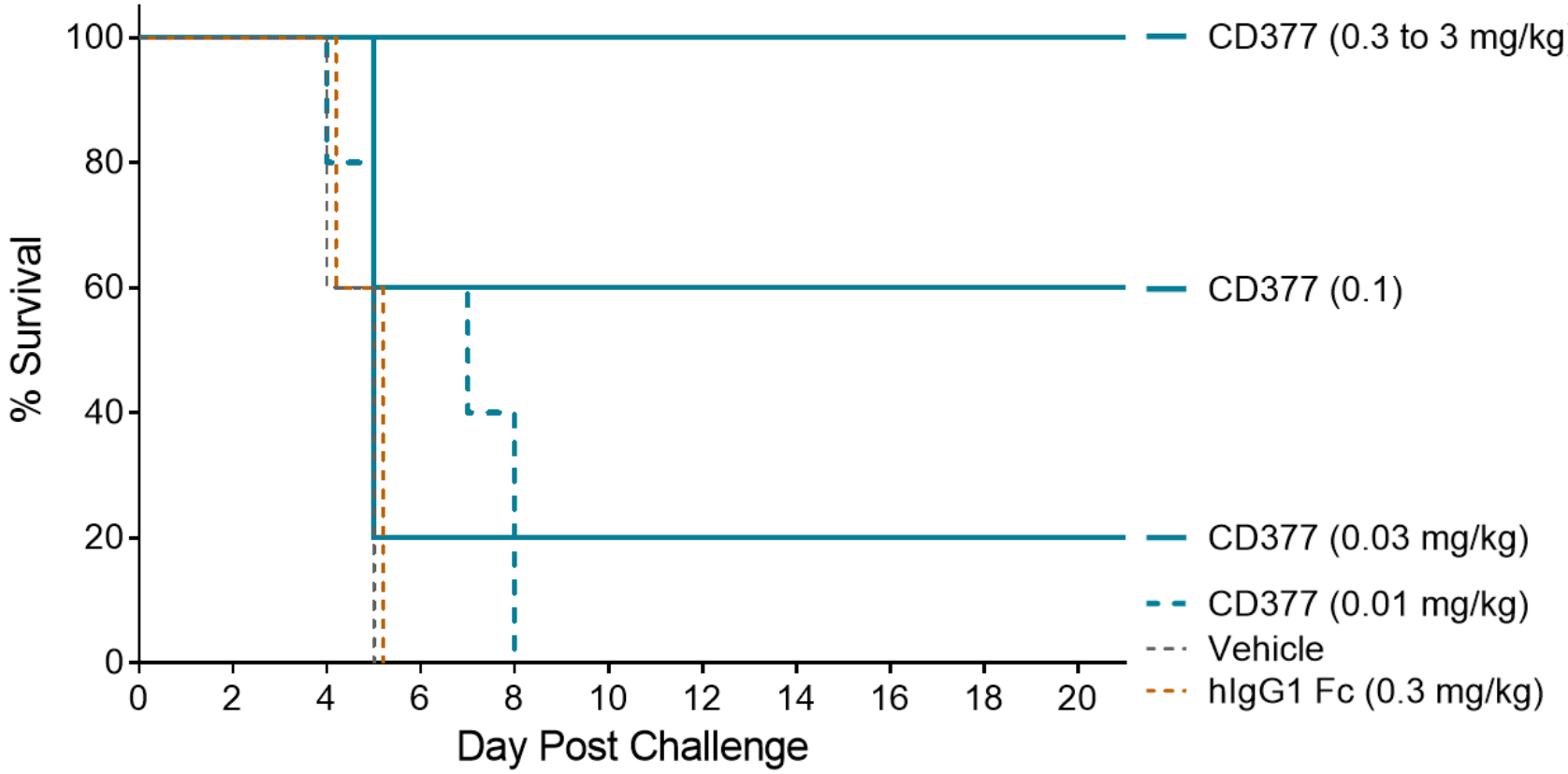
5) CD377 activity in a lethal H1N1 pdm09 mouse infection model



Images created using BioRender

Lethal H1N1 pdm09 mouse infection model – Survival

A/Hawaii/70/2019 (H1N1 pdm09)

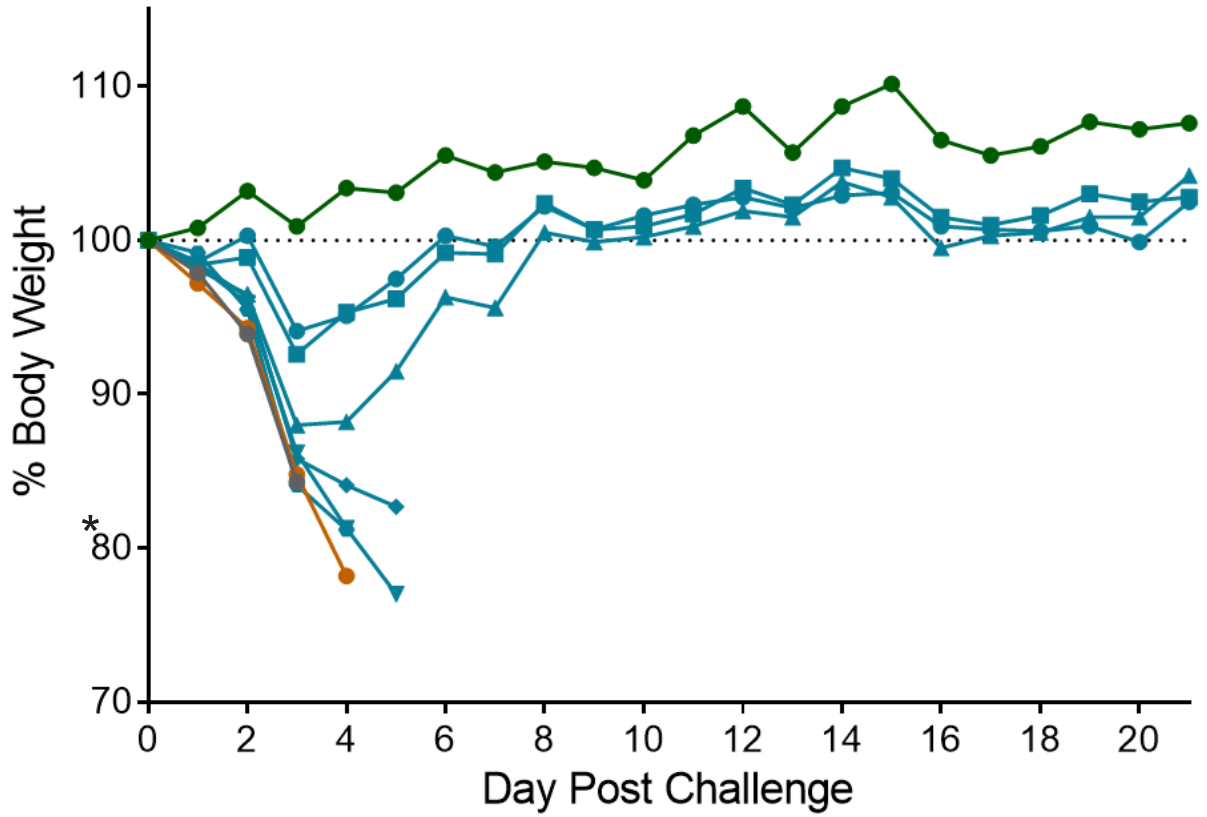


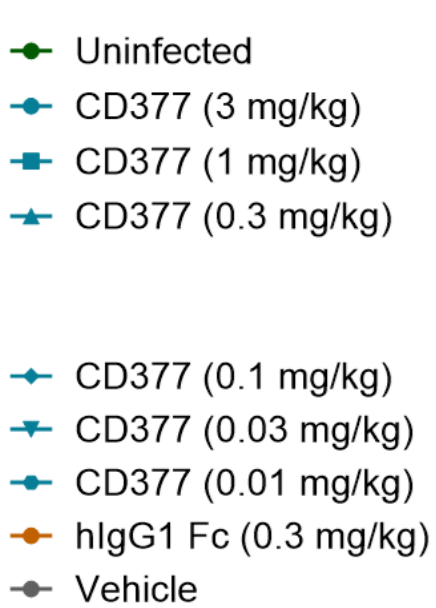
CD377 is 100% protective at single doses of ≥0.3 mg/kg

Image created using BioRender

Lethal H1N1 pdm09 mouse infection model – Body weights

A/Hawaii/70/2019 (H1N1 pdm09)





*BW losses >20% were recorded as death.

Efficacious doses of CD377 minimize impact of infection on body weight loss

Image created using BioRender

CD377 efficacy data against other H1N1 isolates

CD377 in vivo data from non-2020-2021 vaccine strain studies

Lethal mouse infection model
(BALB/c mice, T+2h dosing)

H1N1 Strain	Dose range (mg/kg)	Route	Fully protective dose (significance)
A/Puerto Rico/8/1934	0.01 - 1.0	SC	0.1 mg/kg (P = 0.0031)
A/California/07/2009 pdm	0.1 - 1.0	SC	0.1 mg/kg (P = 0.0126)
A/WSN/1933	0.03 - 3.0	SC	0.3 mg/kg (P = 0.0023)
A/Texas/23/2012 (**H275Y**)	0.03 - 3.0	SC	0.3 mg/kg (P = 0.0144)
A/California/12/2012 pdm	0.03 - 3.0	IM	0.3 mg/kg (P = 0.0015)
A/Hawaii/66/2019 pdm	0.03 - 1.0	IM	0.03 mg/kg (P = 0.0035)
A/Perth/261/2009 (**H275Y**)	0.03 - 10.0	SC	0.3 mg/kg (P = 0.0035)

CD377 is highly efficacious against all H1N1 strains tested, including those containing the predominant oseltamivir-R H275Y NA mutation. Similar potency is also observed against H3N2 and both B lineages.

Image created using BioRender

Conclusions

- CD377 demonstrates uniform, potent activity across a variety of NA and cell-based assays for all 2020-2021 vaccine strains
- Single doses of ≥0.3 mg/kg CD377 conferred 100% protection in a lethal H1N1 pdm09 mouse infection model
- CD377 data in this study are consistent with prior data showing potent *in vitro* activity and high *in vivo* efficacy across all influenza strain types

CD377 demonstrates potential to serve as a broad-spectrum agent for the prevention and treatment of seasonal influenza

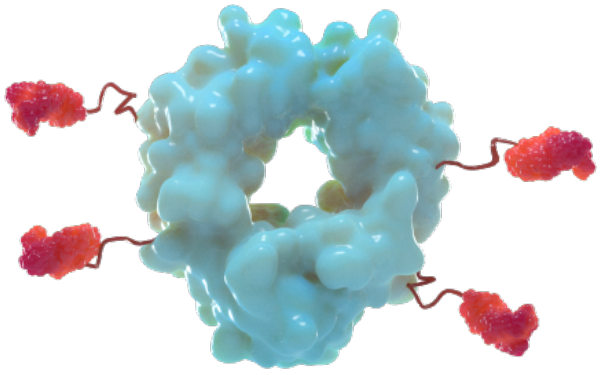
Additional CD377 and AVC platform data presented at ESWI 2020

Beyond Vaccines and mAbs: Progress Towards Universal Influenza Protection with Cloudbreak® Influenza Antiviral Conjugates (AVCs)
Talk ID: Keynote lecture / 7th December / 6 pm CET
Presenters: Mike Ison, MD and Les Tari, PhD

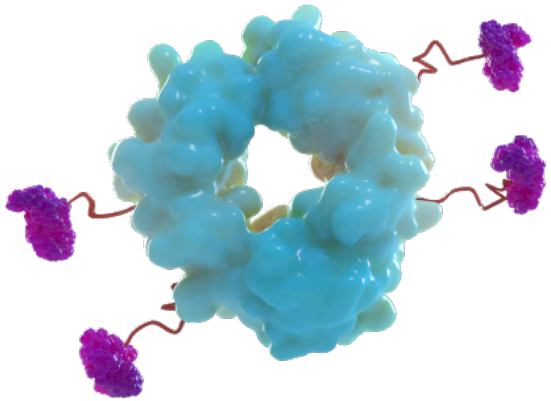
In Vitro Potency and In Vivo Efficacy of CD377, a Novel Antiviral Fc-Conjugate, Against Highly Pathogenic Avian Influenza (HPAI)
Poster ID: 139
Presenter: James Levin, PhD

CD377, a Novel Antiviral Fc-Conjugate, is Equipotent by Different Dosing Routes and Active Against Oseltamivir-Resistant Isolates of Influenza A (H1N1) in Lethal Mouse Infection Models
Poster ID: 159
Presenter: James Levin, PhD

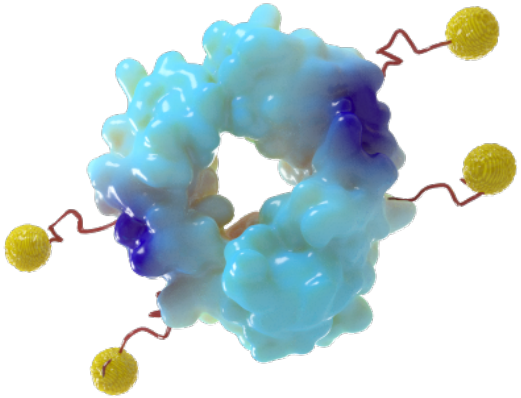
Cidara Cloudbreak® AVC platform: Expansion to other viruses



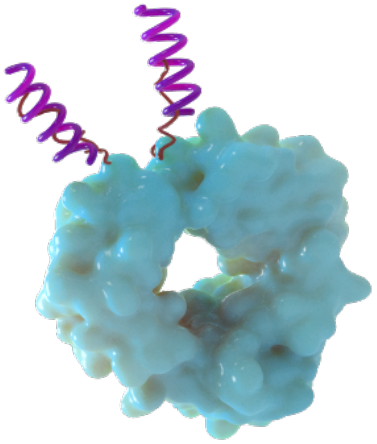
Influenza



RSV



HIV



SARS-CoV-2

For more information, visit: https://www.cidara.com/cloudbreak/

Acknowledgments

- Cidara Team
- Centers for Disease Control and Prevention
- ESWI 2020 Conference Organizers