Utility of Astodrimer Sodium 1% Nasal Spray for Pandemic Preparedness Demonstrated by Broad-Spectrum Antiviral Effects and Protection against SARS-CoV-2 Omicron, and Influenza A and B Virus Infection

RP4

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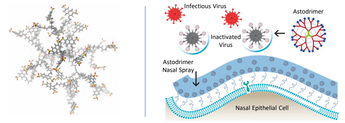
Background

- Astodrimer sodium (SPL7013) is a broad-spectrum antiviral dendrimer that has been developed as a topical nasal spray (VIRALEZE™) to provide pre- and post-exposure prophylaxis against infection and transmission of SARS-CoV-2 Variants of Concern and other pandemic-causing respiratory viruses, such as influenza viruses.
- Astodrimer acts as a barrier between the viral 'spike' proteins and the cell membrane, trapping and irreversibly inactivating viral particles, blocking virus from attaching to and entering cells.



Representation of astodrimer sodium (left) and mechanism of action in trapping and blocking viruses away from the cell membrane (right)

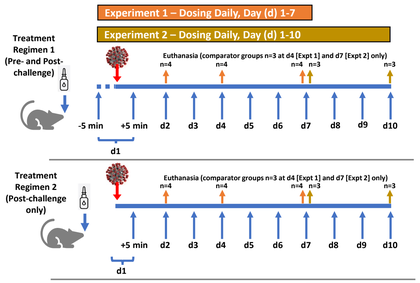
Methods

***IN VITRO* ASSAYS**

- Astodrimer was evaluated in dose-response antiviral and virucidal assays against:
  - SARS-CoV-2 Omicron BA.1 (hCoV-19/USA/MD-HP20874/2021) in Vero E6-TMPRSS2-ACE2, and Calu-3 (lung epithelial) cells
  - Influenza A (A/Wisconsin/629-D02452/2009 (H1N1)pdm09) (IAV) and Influenza B virus (Victoria lineage) (IBV) in Madin-Darby canine kidney cells (MDCK) cells
- Time-of-exposure virucidal evaluation:
  - Astodrimer (10 mg/mL) incubated with virus ($2.5x10^4$ PFU/250,000 cells, multiplicity of infection [MOI] 0.1) for 1, 5, 15 and 30 mins
  - Astodrimer neutralized by pelleting the pre-incubated mixture through 20% sucrose cushion (Beckman SW40 Ti rotor) and removing astodrimer-containing supernatant[1]
  - Amount of residual infectious virus quantified by plaque assay; virucidal efficacy measured by percent reduction of progeny infectious virus vs untreated virus control[1]

***IN VIVO* SARS-CoV-2 OMICRON CHALLENGE STUDIES**

- 6 to 8-week-old K18-hACE2 mice (Jackson Laboratory, stock #034860)
- 10^3 PFU SARS-CoV-2 Omicron BA.1 intranasally (50 µL; 25 µL / nostril)
- Animals maintained under isoflurane anaesthesia for intranasal (IN) dosing and virus inoculation
- Studies conducted at Department of Animal Resources, The Scripps Research Institute (TSRI) (San Diego, CA, USA) in strict adherence with protocols approved by TSRI ethics committee



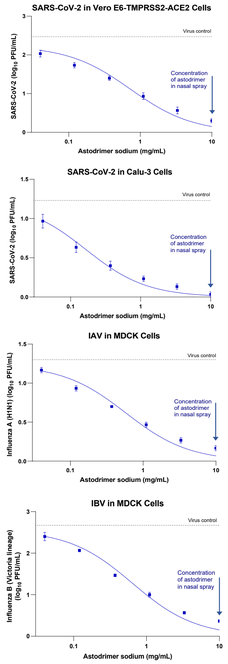
Methods and Results

***IN VITRO* ASSAYS**

Astodrimer demonstrated broad-spectrum antiviral and virucidal activity against SARS-CoV-2 Omicron, influenza A (H1N1) and influenza B (Victoria lineage) *in vitro*

- Antiviral assays used a MOI of 0.1 and the amount of infectious virus in cell culture supernatant was measured by plaque analysis in MDCK, Vero E6-TMPRSS2-ACE2 or Calu-3 cells at Day 2 (SARS-CoV-2 Omicron) or Day 7 (IAV or IBV) post-infection; astodrimer added 1 hr prior to infection

Astodrimer reduced SARS-CoV-2 Omicron, IAV and IBV replication in a dose-dependent manner *in vitro* (graphs below show mean of triplicate assays ± standard error of the mean [SEM])



Astodrimer 1% (10 mg/mL) demonstrated rapid and potent virucidal activity against SARS-CoV-2 Omicron, IAV and IBV (see table below)

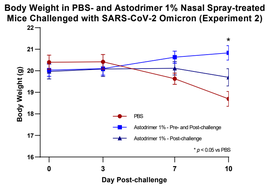
Virus:SPL7013 Incubation Time	Percent Reduction* in Infectious Influenza A vs Virus Control^	Percent Reduction* in Infectious Influenza B vs Virus Control^	Percent Reduction* in Infectious SARS-CoV-2 Omicron vs Virus Control^
1 minute	92.1%	99.4%	96.3%
5 minutes	95.0%	99.7%	98.6%
15 minutes	93.7%	99.6%	99.6%
30 minutes	95.0%	99.4%	99.6%

^virus not treated with astodrimer
*maximal possible reduction in these assays was 96% for IAV, 99.8% for IBV and 99.6% for SARS-CoV-2 Omicron

***IN VIVO* SARS-CoV-2 OMICRON CHALLENGE STUDIES**

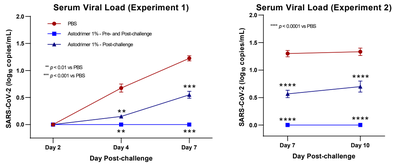
BODY WEIGHT

- In Experiment 1, no notable changes in body weight to d7 in mice challenged with SARS-CoV-2 Omicron (data not shown)
- In Experiment 2, SARS-CoV-2 Omicron-infected mice treated with PBS experienced reduced body weight at d7 and d10
- In contrast, treatment with Astodrimer 1% Nasal Spray protected animals from weight loss; animals treated pre- and post-challenge gained weight to d10, at which point the difference to PBS was statistically significant (graph shows mean ± SEM)



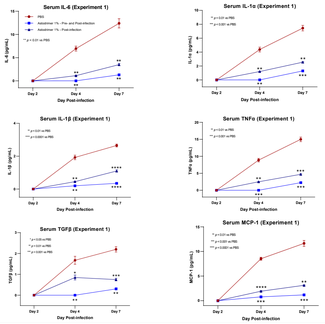
VIRAL LOAD – SERUM

- Viral load determined by quantitative-reverse transcriptase polymerase chain reaction (qRT-PCR); primers detected conserved SARS-CoV-2 nucleocapsid region[2]
- SARS-CoV-2 Omicron copies were detectable in serum from d4 post-challenge in the PBS-treated group, and viral load increased to d7 (Experiment 1), remaining steady to d10 (Experiment 2)
- Animals treated with Astodrimer 1% Nasal Spray (Regimens 1 and 2) had statistically significant lower viral load in serum on d4, d7 and d10 post-challenge compared with PBS-treated animals (graphs show mean ± SEM)



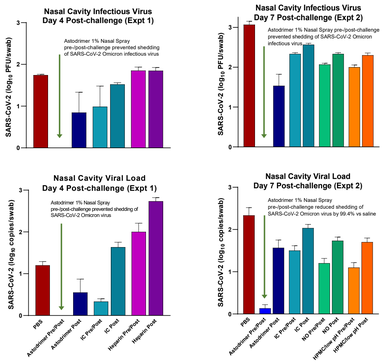
PROINFLAMMATORY CYTOKINES AND CHEMOKINE – SERUM

- Proinflammatory cytokines (IL-6, IL-1α IL-1β, TNF-α and TGF-β) and chemokine (MCP-1) in serum quantified by ELISA[3]
- Cytokine profiles mirrored occurrence and increase in serum viral load; Astodrimer 1% Nasal Spray protected against production of proinflammatory cytokines in mice challenged with SARS-CoV-2 Omicron (graphs show mean ± SEM)



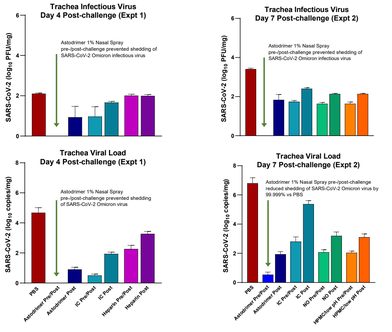
VIRAL LOAD – NASAL CAVITY

- Amount of infectious virus and viral copies (load) quantified from nasal swabs by plaque assay and qRT-PCR, respectively
- Treatment with Astodrimer 1% Nasal Spray 5 mins pre- and post-challenge followed by daily treatment (Regimen 1) prevented infectious viral shedding at d4 (Experiment 1) and d7 (Experiment 2)
- Treatment with Astodrimer 1% Nasal Spray 5 mins post-challenge followed by daily treatment (Regimen 2) reduced infectious viral shedding by ~50% in both experiments
- In general, application of nasal sprays prior to and post-challenge resulted in better protection than when applied only post-challenge
- Astodrimer 1% Nasal Spray was more effective at preventing or reducing SARS-CoV-2 shedding from the nasal cavity than 0.12% iota-carrageenan (IC), 1% heparin, nitric oxide (NO) and 1% hydroxypropyl methylcellulose (HPMC)/low pH comparator nasal spray products (graphs show mean ± SEM)



VIRAL LOAD – TRACHEA

- 25 mg of trachea tissue homogenized and amount of infectious virus and viral load quantified by plaque assay and qRT-PCR, respectively
- Treatment with Astodrimer 1% Nasal Spray pre- and post-challenge protected the trachea from infection
- In general, application of nasal sprays prior to and post-challenge was more efficacious than when applied only post-challenge
- Astodrimer 1% Nasal Spray was more efficacious than the comparator products at reducing infectious virus and viral load in trachea (graphs show mean ± SEM)

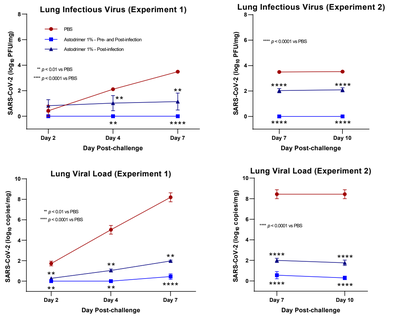


VIRAL LOAD – LUNG

- **I**N challenge with SARS-CoV-2 Omicron resulted in 100% infection of the lung of PBS-treated animals; viral load increased 4-fold between d2 and d7 in Experiment 1
- Treatment with Astodrimer 1% Nasal Spray pre- and post-inoculation resulted in protection from infection, as indicated by absence of detectable infectious virus in the lung (see table below)

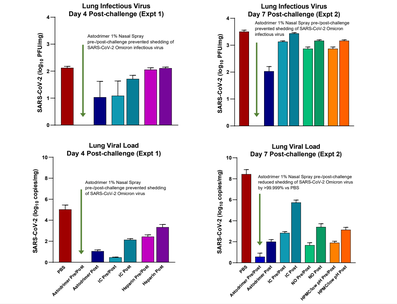
Treatment (Regimen 1)	Number of Animals Tested	Number of Animals Infected	Percentage of Animals Infected
Experiment 1 (d7)			
PBS	12	12	100%
Astodrimer 1% Nasal Spray	12	2	16.7%
Experiment 2 (d7/10)			
PBS	6	6	100%
Astodrimer 1% Nasal Spray	6	0	0%

- Astodrimer 1% Nasal Spray post-inoculation significantly reduced amount of virus in lung at all time points in Experiments 1 and 2 (graphs show mean ± SEM)



- Of all comparator nasal sprays, only Astodrimer 1% Nasal Spray was protective against SARS-CoV-2 Omicron infection of the lung in this challenge model (graphs show mean ± SEM)

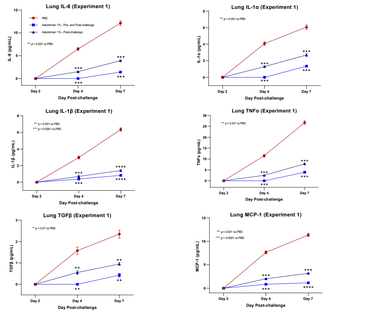
Nasal Spray Product / Regimen	Percent Reduction in Infectious SARS-CoV-2 Omicron in Lung vs PBS at Day 7 (Experiment 2)
Astodrimer 1% / Pre- and Post-challenge	**>99.9%**
Astodrimer 1% / Post-challenge	**96.8%**
Iota-carrageenan / Pre- and Post-challenge	49.9%
Iota-carrageenan / Post-challenge	20.6%
Nitric Oxide / Pre- and Post-challenge	74.9%
Nitric Oxide / Post-challenge	49.9%
HPMC/low pH / Pre- and Post-challenge	74.9%
HPMC/low pH / Post-challenge	49.9%



Nasal Spray Product / Regimen	Tissue	Percent Reduction in SARS-CoV-2 Omicron Viral Load vs PBS at Day 7 (Experiment 2)
Astodrimer 1% / Pre- and Post-challenge	Lung	>99.999%
Astodrimer 1% / Post-challenge		>99.999%
Astodrimer 1% / Pre- and Post-challenge	Trachea	>99.999%
Astodrimer 1% / Post-challenge		99.999%
Astodrimer 1% / Pre- and Post-challenge	Nasal Cavity	99.4%
Astodrimer 1% / Post-challenge		82.9%

PROINFLAMMATORY CYTOKINES AND CHEMOKINE – LUNG

- Generally, cytokines first detected in lungs of SARS-CoV-2 Omicron-infected mice on d4 post-challenge (PBS treatment group)
- Significantly reduced in Astodrimer 1% Nasal Spray treatment groups
- Lowest levels in lungs found in animals treated with Astodrimer 1% Nasal Spray pre- and post-challenge (graphs show mean ± SEM)



Discussion and Conclusions

Astodrimer 1% Nasal Spray offered protection against infection by the highly transmissible SARS-CoV-2 Omicron variant and outperformed comparators in an *in vivo* viral challenge model, even when product was applied only after exposure to virus

Broad-spectrum antiviral effects and ability to protect against infection *in vivo* indicate Astodrimer 1% Nasal Spray has a potential role in future pandemic preparedness strategies

- Astodrimer demonstrated potent antiviral and virucidal activity against SARS-CoV-2 Omicron, IAV and IBV *in vitro*
- All animals treated with Astodrimer 1% Nasal Spray before and after SARS-CoV-2 Omicron challenge had no detectable virus in lung, trachea or nasal cavity up to 4 days after exposure to virus
- Viral load in lung and trachea of astodrimer-treated animals was effectively eliminated (≥99.999%) compared with virus levels in PBS-treated animals 7 days after viral challenge
- Astodrimer 1% Nasal Spray pre- and post-challenge resulted in 84.3% (Expt 1) and 100% (Expt 2) of animals having no evidence of virus replication in lung, trachea, nasal secretion, and serum at d7
- Astodrimer 1% Nasal Spray applied only after IN viral challenge also effectively prevented (≥99.999%) virus in lung and trachea at 7 days after viral challenge compared with PBS
- Astodrimer-treated animals also exhibited normal body weight gain and a significant reduction in proinflammatory cytokines compared with PBS, indicating reduced severity of disease

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References

1. Paull, JRA, *et al. Antiviral Res.* **191**, 1050892021 (2020);
2. Winkler, ES, *et al. Nat Immunol* **21**, 1327-35 (2020)
3. Paull, JRA, *et al. Viruses* **13**, 1656 (2021)

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