A lung-targeted therapeutic siRNA against highly conserved viral M1 mRNA effectively limits highly pathogenic influenza A infection in mice

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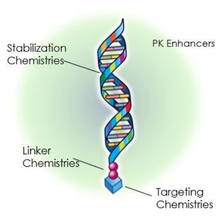
RATIONALE

- Human infections of highly pathogenic avian influenza A (bird flu) are rare but represent a significant global health concern due to the continued widespread circulation of the H5N1 virus in wild bird, poultry and more recently different mammalian populations.
- Therapeutic small interfering RNAs (siRNAs) offer an alternative approach by using the RNAi mechanism to silence viral transcripts encoding essential gene products.
- Here, an evaluation of siRNAs directed against highly conserved influenza A viruses (IAV) including highly pathogenic avian influenza (H5N1) genomic regions demonstrated a unique viral vulnerability to M1 silencing.

TRiM™ PLATFORM

- Optimized therapeutic siRNAs (RNAi triggers) for specific target gene silencing and limited potential off-target interactions.
- Rational use of modifying chemistries maximize innate stability and potency.
- Integrin αvβ6 targeting moiety facilitates delivery to pulmonary epithelium.

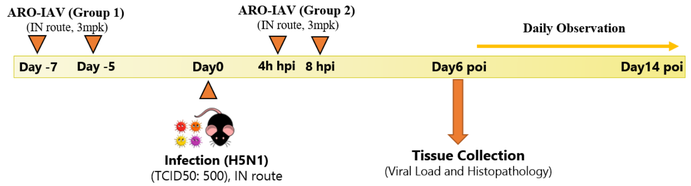


METHODS

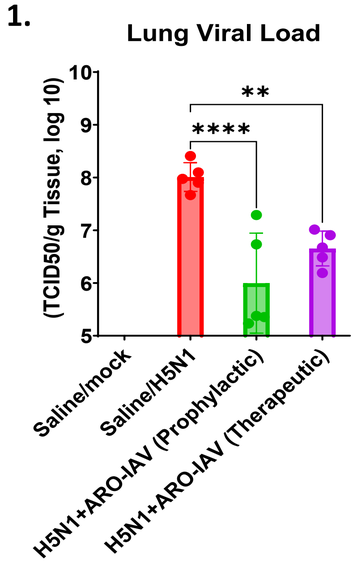
- Nine-week-old BALB/C mouse model of H5N1 (A/whooper swan/Mongolia/244/2005 strain) infection was used to assess *in vivo* efficacy of influenza M1-silencing siRNA conjugates.
- Prophylactic (pre-infection) and therapeutic (post-infection) efficacy was evaluated in two groups of mice (Groups 1 and 2)
- G1 mice received two intranasal doses (3 mg/kg each) of siRNA conjugate or vehicle (saline) on day 7 and 5 prior to infection.
- H5N1 infection was initiated by intranasal inoculation of virus.
- G2 infected mice received two intranasal doses (3 mg/kg each) of siRNA conjugate or vehicle (saline) at 4- and 8-hours post-infection.
- Lung tissue was collected 6 days post-infection for viral load analyses (TCID50 and qRT-PCR assays) and histopathological assessments.
- A subset of mice from each treatment group was reserved for clinical scoring of morbidity and mortality.
- Histological analysis of pulmonary inflammation was performed using hematoxylin and eosin (H&E) staining.

RESULTS

- Previous work evaluated over 200 candidate siRNA sequences targeting the most highly conserved regions of approximately 10,000 IAV genomes of different subtypes (H1N1, H3N2, H5N1, H5N6, H5N8, H7N2, H7N3, H7N4, H7N7, H7N9 and H9N2) **(Presented at 7th ISIRV Conference, May 2023).**
- ARO-IAV, an siRNA conjugate targeting a conserved region of the influenza A M1 gene, has been previously shown to have potent antiviral activity against **H1N1 and H3N2** viruses.
- Here, we extend observations of ARO-IAV antiviral activity to highly pathogenic **H5N1** avian influenza.



Silencing M1 expression with intranasally delivered ARO-IAV significantly reduces viral load, M1 mRNA expression in H5N1-infected mouse lung



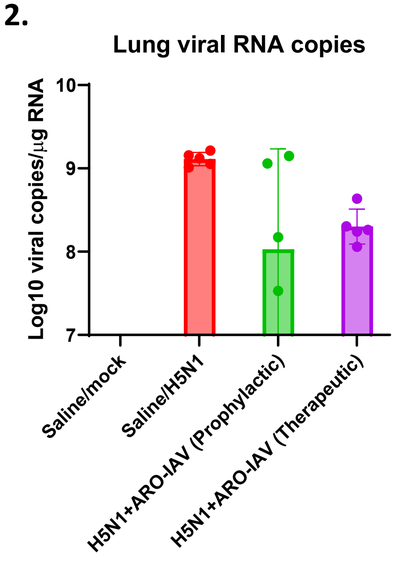


Figure 1. ARO-IAV treatment demonstrated prophylactic efficacy (reducing H5N1 viral load in the lung by >2 log of 10 vs untreated infected controls) and therapeutic efficacy (reducing H5N1 viral load in the lung by ≈ 1.5 log of 10 vs untreated infected controls).

Figure 2. ARO-IAV treatment reduced lung H5N1 genomic RNA and mRNA expression by approximately 1 log of 10 or greater compared to untreated infected controls.

Intranasal ARO-IAV significantly improves survival and clinical score in a model of severe H5N1 infection

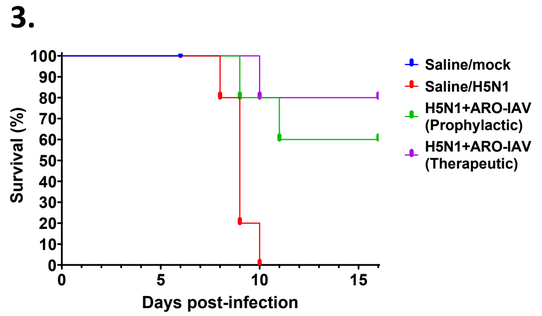


Figure 3. Survival index showed that ARO-IAV treatment significantly improved the survival rate among the infected-treated mice while by day 10 post-infection, 100% mortality was observed in untreated infected mice**.** At 14 days post-infection, 80% of mice receiving therapeutic ARO-IAV treatment survived and 60% of mice receiving prophylactic ARO-IAV treatment survived**.**

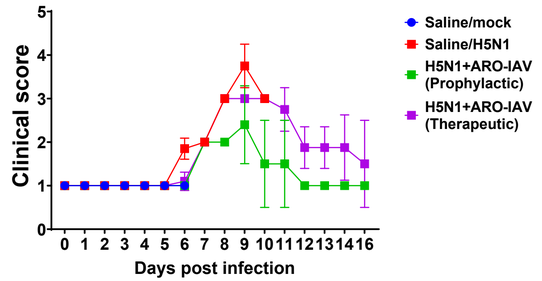


Figure 4. Clinical score index showed that treatment with ARO-IAV could improve the clinical score in mice**.** However, clinical score improvement was more significant withing the mice group which received the ARO-IAV in prophylactic mode as all remain active and considered recovered from the disease.

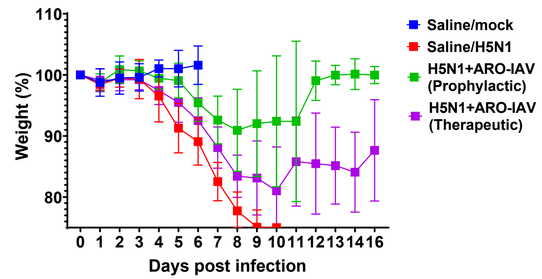


Figure 5. Body weight index showed that, infected mice receiving therapeutic or prophylactic ARO-IAV treatment lost less weight than untreated infected controls. However, weight loss prevention was more pronounced with ARO-IAV prophylaxis.

CONCLUSION

- We demonstrate a vulnerability of H5N1 avian influenza to silencing of M1, an essential viral gene product that has been challenging to drug with traditional small molecule antivirals.
- ARO-IAV, an inhaled M1-silencing therapeutic siRNA, offers a novel first-in-class approach for the potential treatment of highly pathogenic influenza A infections.