Applying AIMer-Based RNA Editing Technology to Correct a Nonsense Mutation in the Lung

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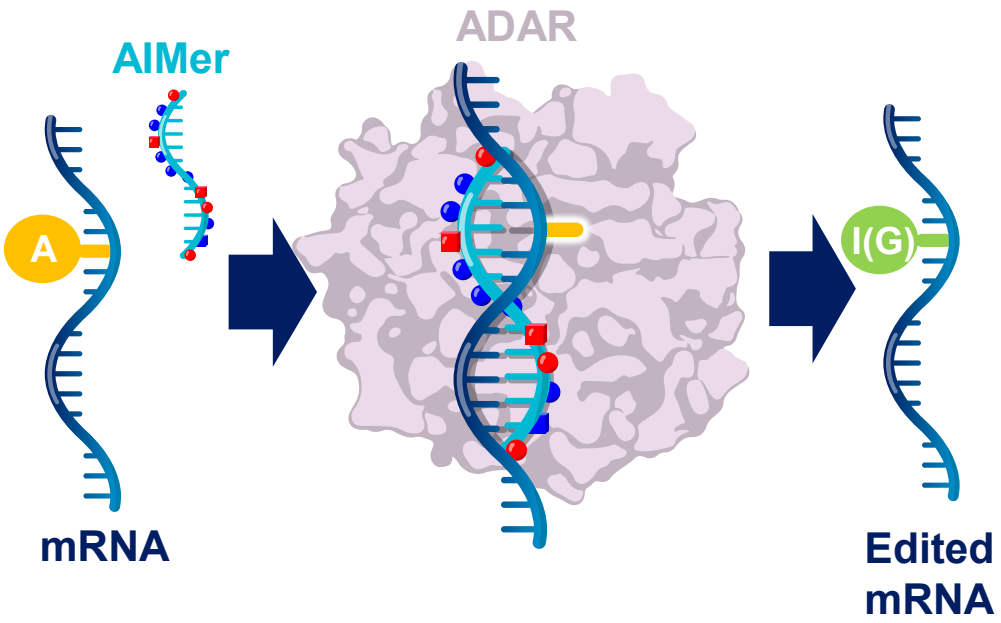
Disclosures

- **Ian Harding**, Jack Godfrey, Chikdu Shivalila, Megan Cannon, Jigar Desai, Alyse Faraone, Carolyn Grimes, Naoki Iwamoto, Pachamuthu Kandasamy, Tomomi Kawamoto, Genliang Lu, Jake Metterville, Prashant Monian, Erin Purcell-Estabrook, Stephany Standley, Hailin Yang, Ryan Yordanoff, Hui Yu, Michael Byrne, and Chandra Vargeese are employees of Wave Life Sciences
- Kevin Coote and Yi Cheng are employees of the Cystic Fibrosis Foundation

AIMers unlock A-to-I RNA editing via endogenous ADAR enzymes

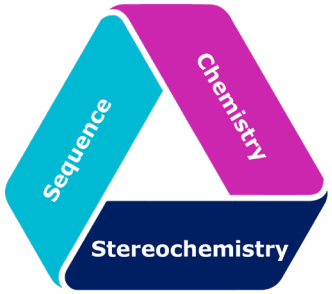
- ADAR enzymes bind double-stranded RNA and convert an adenosine (A) to inosine (I) on one strand
- Inosine is read as guanine (G) by the translation machinery and disrupts A:U pairing
- Using synthetic antisense RNA oligonucleotides to direct ADAR for therapeutic RNA editing was first proposed in 1995[1]
- AIMers are short, chemically modified oligonucleotides that engage endogenous ADAR enzymes to induce highly efficient and specific A-to-I RNA base editing[2]

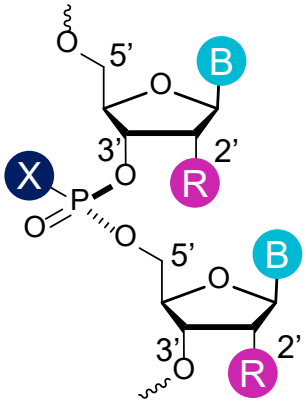


[1]Woolf *et al.*, 1995 *Proc Natl Assoc Sci* 92:8298-8302; [2]Monian *et al.*, 2022 *Nature Biotech* published online Mar 7, 2022 doi: 10.1038.s41587-022-01225-1. ADAR: Adenosine deaminase acting on RNA

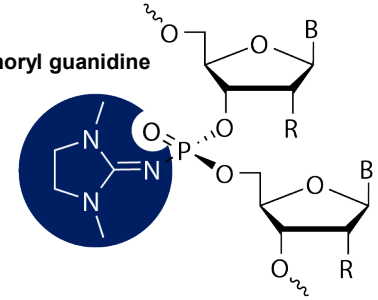
Wave's ability to rationally design stereopure oligonucleotides enables access to unique disease targets

PRISM





Phosphoryl guanidine



- **B** Base
- **R** 2'-Ribose
- **X** Stereochemistry and backbone modification

O: Phosphodiester
S: Phosphorothioate
N: Phosphoryl guanidine

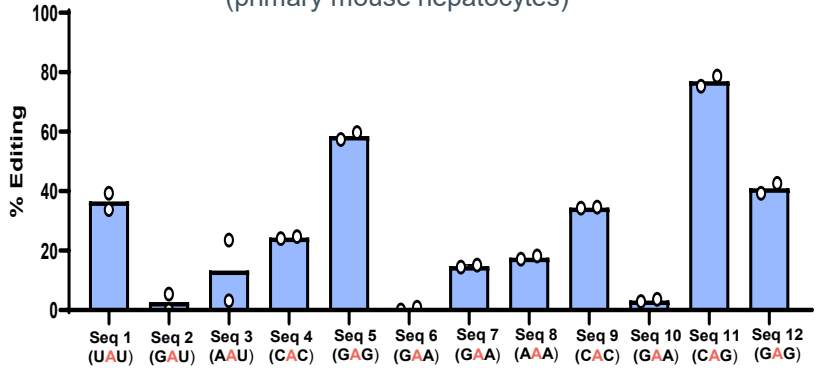
Benefits in:
- Potency
- Durability
- Tissue delivery

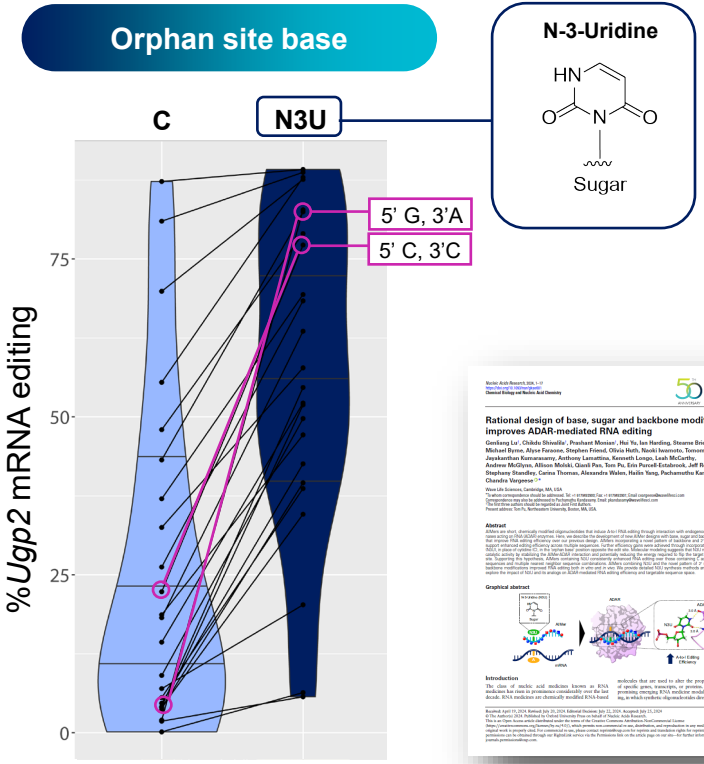
WAVE LIFE SCIENCES

Incorporating N3U at the orphan base overcomes nearest neighbor limitations of ADAR enzymes

ADAR enzymes have poor editing efficacy of adenosines with unfavorable adjacent nucleotides (nearest neighbors)

Ugp2 RNA editing
(primary mouse hepatocytes)



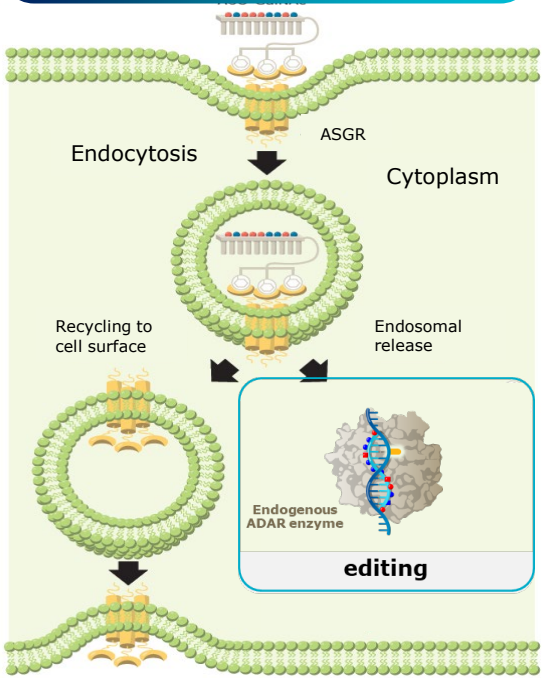


Rational design of base, sugar and backbone modifications improves ADAR-mediated RNA editing

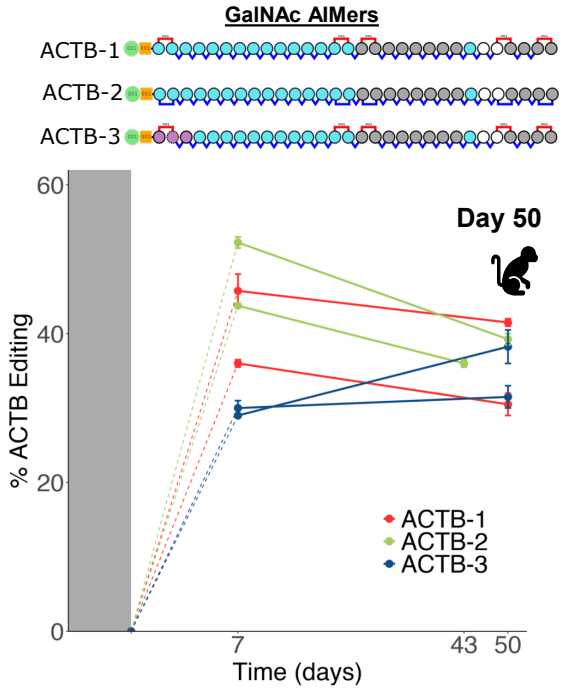
Primary mouse hepatocytes from hADAR-p110 mice were treated with 3 uM AIMers and orphan base as indicated, with varied by edit region sequence, for 72 hours. RNA editing was quantified by Sanger sequencing. n=2. Seq: Sequence; red font denotes edit site – modified base in AIMer corresponds to edit site in RNA. [1] Lu G et al., (2024) *Nucl Acid Res* https://doi.org/10.1093/nar/gkae681

Achieving RNA editing in the liver with GalNAc-conjugated AIMers

GalNAc-mediated uptake



Substantial and durable editing in NHP liver *in vivo*[1]



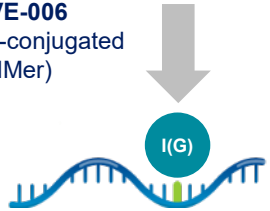
[1]Monian *et al.*, 2022 *Nature Biotech* published online Mar 7, 2022 doi: 10.1038.s41587-022-01225-1. GalNAc: *N*-Acetylgalactosamine

Investigational GalNAc-AIMer WVE-006: GalNAc-AIMer designed to correct mutant *SERPINA1* transcript and restore wild-type M-AAT protein

WVE-006 for AATD

SERPINA1 Z allele mRNA encodes Z-AAT protein with E342K mutation

WVE-006
(GalNAc-conjugated AIMer)



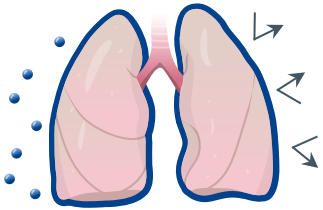
Edited *SERPINA1* mRNA enables wild-type M-AAT protein production

WVE-006 ADAR editing approach to potentially address key goals of AATD treatment:

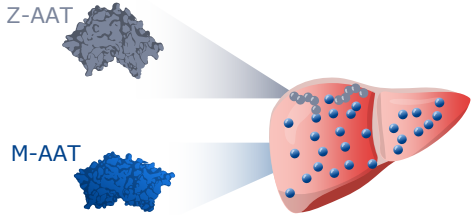
1) Restore circulating, functional wild-type M-AAT

2) Reduce Z-AAT protein aggregation in liver

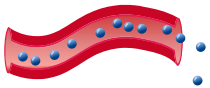
3) Retain M-AAT physiological regulation



M-AAT reaches lungs to protect from proteases



RNA correction replaces mutant Z-AAT protein with wild-type M-AAT protein



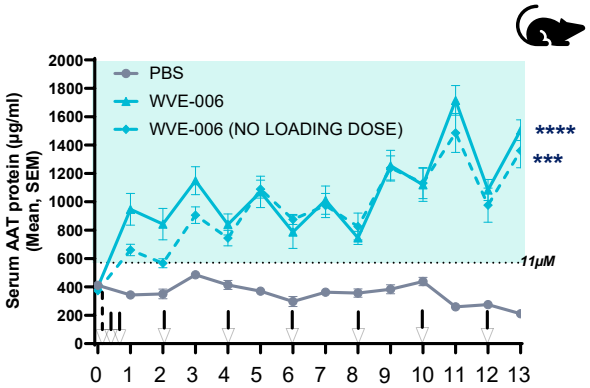
M-AAT secretion into bloodstream

AAT: Alpha-1 antitrypsin Strnad et al., 2020 N Engl J Med 382:1443-55; Blanco et al., 2017 Int J Chron Obstruct Pulmon Dis 12:561-69; Remih et al., 2021 Curr Opin Pharmacol 59:149-56.

Investigational GalNAc-AIMer WVE-006 restores functional serum M-AAT protein in mice

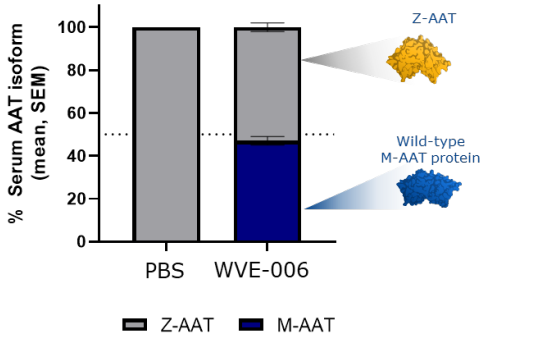
Increased AAT protein in NSG-PiZ mice

WVE-006 treatment results in serum AAT protein levels of up to 30 uM in NSG-PiZ mice



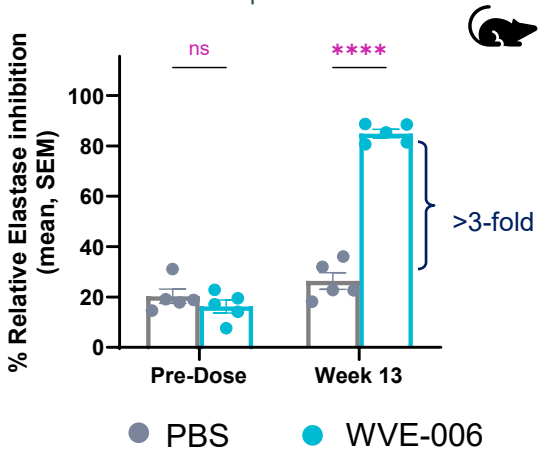
Confirmed restored wild-type M-AAT protein

WVE-006 treatment leads to M-AAT production, up to 50% of serum AAT



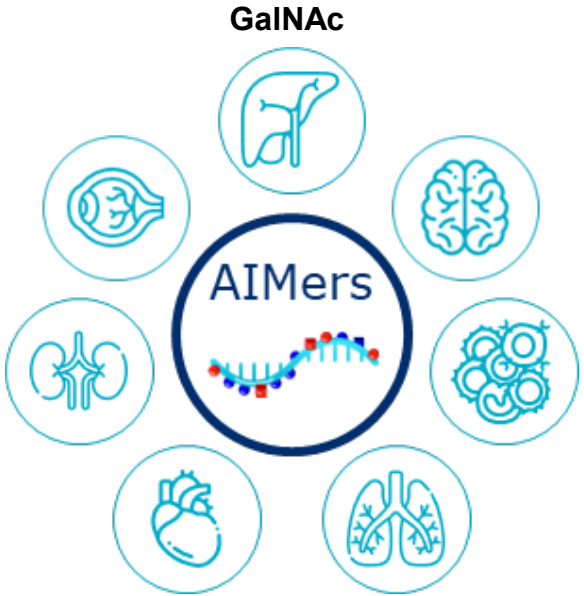
Demonstrated functionality of M-AAT protein

WVE-006 treatment increases serum inhibition of neutrophil elastase ~3-fold



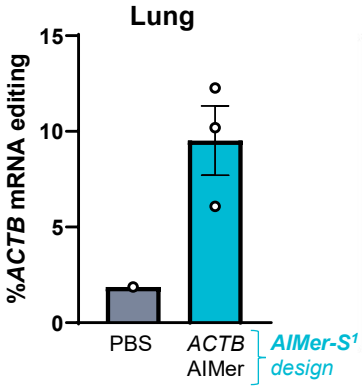
M-AAT protein: wild-type AAT protein. WVE-006 administered subcutaneously (10 mg/kg bi-weekly) in 7-week old NSG-PiZ mice (n=5 per group); Loading dose: 3 x 10 mg/kg at Day 0; total serum AAT protein quantified by ELISA; Stats, left: 2-way ANOVA with Dunnett post-hoc comparison to PBS ****<0.0001, *** <0.001. Right:; Serum collected from mice was tested for ability to inhibit fixed concentration of neutrophil elastase in an in vitro reaction; Stats, right: 2-way ANOVA with Bonferroni post-hoc for comparisons between PBS and WVE-006.

Addressing a major oligonucleotide challenge with chemistry optimization: access to extrahepatic tissues

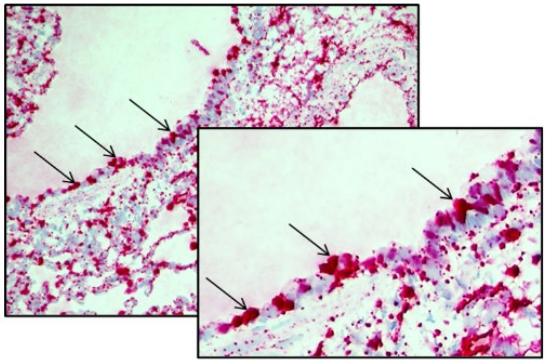


AIMers access lung tissue and support editing in the lungs of mice and NHPs

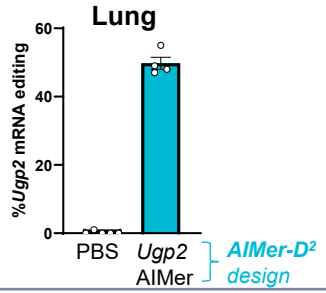
10% editing of *ACTB* in lung in NHPs 1-week post-single dose (SC)



ACTB AIMer (red) delivery to bronchial epithelial cells (arrows)

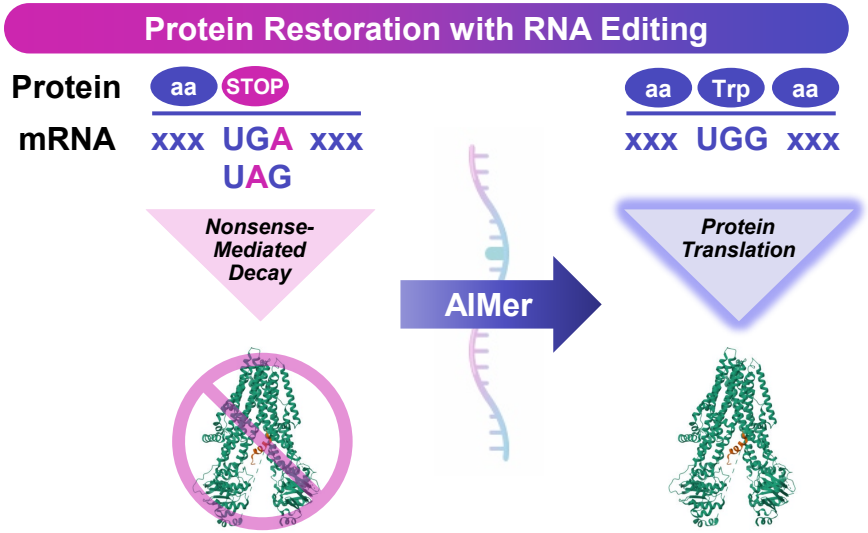


~50% editing of *Ugp2* in lung in mice 1-week post-single dose (IV)



Top, editing in NHP: [1]Monian *et al.*, 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1. Bottom: hADAR-p110 mice were dosed with 100mpk AIMer or PBS (n=5/group) by IV injection. Lung tissue was collected 7 days after dosing and Ugp2 mRNA editing was quantified by RT-PCR and Sanger Sequencing. Data expressed as mean ±SEM. IV: intravenous delivery; SC: subcutaneous delivery. [2] Lu G et al., (2024) *Nucl Acid Res* https://doi.org/10.1093/nar/gkae681

RNA editing could address an unmet need for patients with nonsense mutation-induced disease



Cystic Fibrosis → CFTR

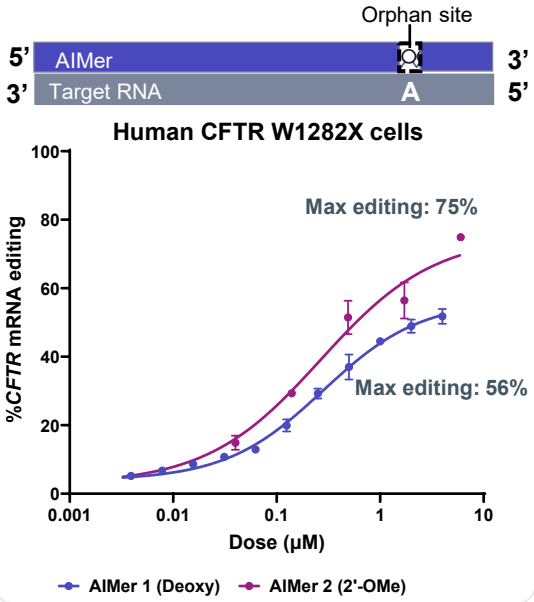
- Multi-organ disease that ultimately leads to respiratory failure due to an imbalance in epithelial ion transport
- No approved therapies for nonsense mutations, which occur in ~10% of CFTR patients[3,4]

By converting termination codons (**UGA**, **UAG**) into tryptophan (**UGG**), ADAR editing can rescue full-length protein expression

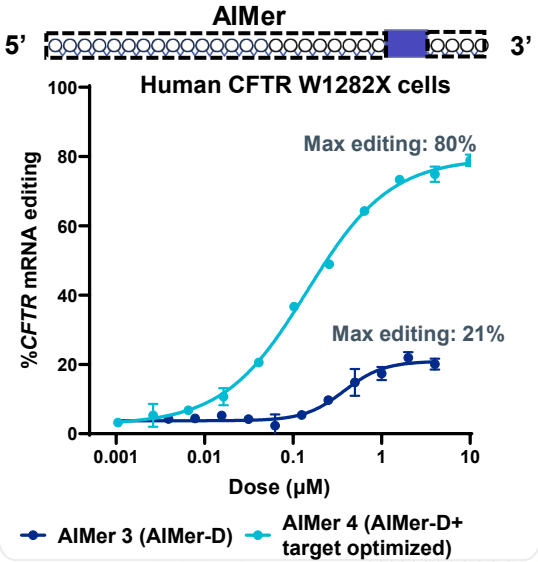
[1]Percy, A.K., Ananth, A. & Neul, J.L. 2024 CNS Drugs. [2]Percy, Alan K., et al. 2007 Journal of child neurology. [3]Fajac and Sermet. 2021 Cells; [4]Bobadilla, JL, et al. 2002 Hum. Mutat.

Chemistry optimization enhances *CFTR* RNA editing efficiency in human bronchial epithelial cells

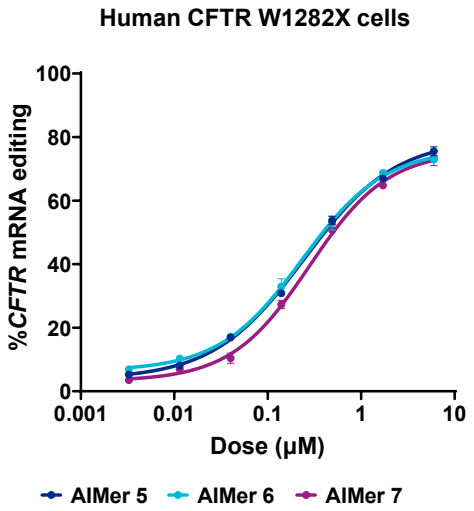
Orphan site 2’-sugar modification



Sugar and backbone modifications outside of edit region



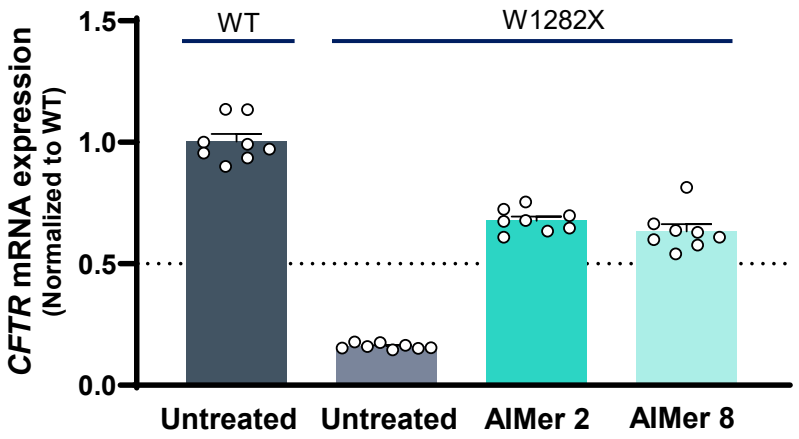
Multiple highly efficient AIMers (≥75% max editing)



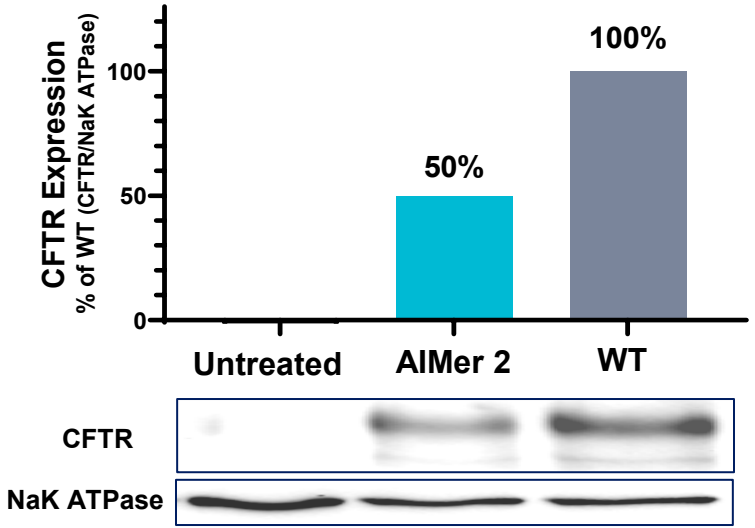
[1] Lu G et al., 2024 *Nucl Acid Res* https://doi.org/10.1093/nar/gkae681. 16HBEge CFTR W1282X cells treated with AIMers at indicated concentrations for 96h before analysis. RNA editing was quantified by RT-PCR and Sanger Sequencing, n=1-2 per condition.

CFTR AIMers increase *CFTR* mRNA and protein levels in human bronchial epithelial cells

>50% recovery of wild-type *CFTR* mRNA



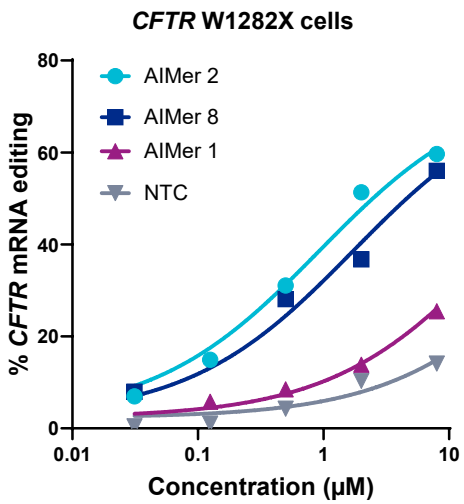
Up to 50% recovery of wild-type CFTR protein



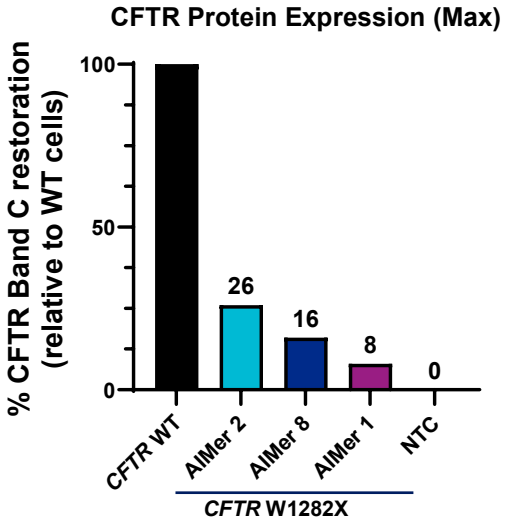
16HBEge CFTR W1282X cells treated with AIMers at indicated concentrations for 48h before analysis. Left: Cells treated with 1µM AIMers; transcript expression quantified from cDNA by qPCR. n=8, data shown as mean ±SEM. Right: Cells treated with 10µM AIMers; protein quantified by Western Blot densitometry. WT: wild-type

Editing leads to a dose dependent rescue of CFTR protein and function

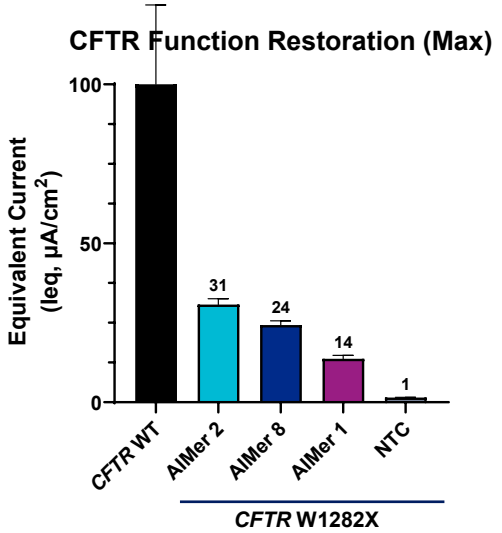
RNA editing



Protein expression



Protein function (current)



16HBEge CFTR W1282X cells treated with AIMers at indicated concentrations for 7 days before analysis; 16HBE14o- (CFTR WT) cells were used as a positive control. Left: RNA editing quantified by RT-PCR and Sanger Sequencing, n=1 per condition. Middle: Protein quantified by Western Blot densitometry for 8uM dose of AIMers. Right: Transepithelial voltage and resistance expressed in equivalents to CFTR WT current (I_{eq}) mean ± SD, *n*=3; Ieq was measured as described in Valley HC, et al. J Cyst Fibros. 2019 Jul;18(4):476-483. doi: 10.1016/j.jcf.2018.12.001. Ieq quantification is for 8uM AIMer dose. NTC: Non-targeting control; WT: wild-type.

Summary

- Wave's PRISM® platform enables rational design of AIMers that enable high efficiency A-to-I RNA editing across target sequences
- AIMer RNA base editing technology is effective in the lungs of mice and NHPs
- Disease-causing *CFTR* nonsense mutations such as *CFTR* W1282X may be amenable to correction through mRNA editing
- Target-specific AIMer optimization led to AIMers that support *CFTR* mRNA editing with high efficiency and potency in human bronchial epithelial cells
- CFTR AIMers supported restoration of *CFTR* mRNA and functional protein expression in human *CFTR* W1282X cells
- These preclinical data demonstrate proof-of-principle for use of AIMers in lung, including for cystic fibrosis

Acknowledgements

- Thanks to all colleagues and contributors from **Wave Life Sciences** and the Cystic Fibrosis Foundation for supplying the 16HBEge CFTR W1282X cells and conducting the electrophysiology experiment.
- Nicole Neuman, Wave Life Sciences, assisted in preparing this presentation.

Silencing (RNase H and Ago2) | **Splicing** | **Editing**

Selected Wave publications: PMC10201370, PMC9092894, PMID 35256816, PMC9177980, PMC9178015, PMC7870851, PMC7804567, PMID 28829437, DOI: 10.1016/j.omtn.2024.102246; DOI: 10.1093/nar/gkae681; DOI: 10.1093/nar/gkae681