Optimized RESTORE+ oligonucleotides for an efficacious and safe RNA base editing treatment for Alpha-1 Antitrypsin Deficiency

#1106

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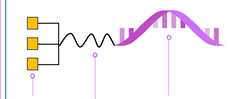
Executive summary

- Endogenous ADAR-mediated RNA editing is a transformative technology enabling precise A-to-I editing of mRNA.
- AIRNA's RESTORE+ platform utilizes proprietary chemical modifications and optimized GalNAc delivery to enhance RNA editing potency and durability in vivo.
- AIRNA's research candidates (rAIR-100 family) target the *SERPINA1* mRNA to treat Alpha-1 Antitrypsin Deficiency (AATD) for precise A-to-I editing to correct the PiZ mutation responsible for liver and lung pathology in AATD.
- rAIR-100 achieved >90% editing efficiency with 20 nM in vitro in primary mouse hepatocytes, as well as potent editing in iPSC-derived hepatocytes.
- rAIR-100 demonstrated >50% RNA editing and >30 µM M-AAT production with subcutaneous GalNAc molecule, which led to a ~9-fold decrease in liver aggregates and >17-fold increase in neutrophil elastase inhibition in hPiZ mouse model.
- Pharmacokinetics (PK) and safety in non-human primates (NHPs) show prolonged exposure compared to mice, with no observable toxicity.
- AIRNA's RESTORE+ platform enables rapid development of potent and safe RNA editing therapies for diseases with high unmet need.
- AIR-001 is further optimized for improved potency and durability, expected to file CTA in H2 2025.

AIRNA target product profile to provide a functional cure for all AATD patients

AIRNA AATD family of research candidates (rAIR-100 family)

Proprietary RESTORE+ modifications optimize potency, safety, and stability



rAIR-100 research candidates

AIRNA target product profile for all AATD patients

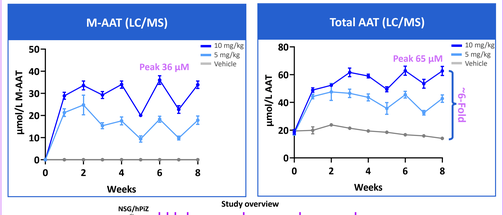
Potency	Physiological levels (MZ phenotype)
Precision	No detectable off-target editing
Administration	Subcutaneous, infrequent
Safety	Well tolerated

GalNAc delivery of oligos provides a preferred product profile for patients and favorable translation into the clinic

	GalNAc	LNP
Administration	Subcutaneous	Intravenous
Frequency	2-6 months[1,4]	3-4 weeks[2]
Tox	Well tolerated	Potential liver tox, steroid pre-treatment[3]
Clinical Setting	Self-administer / out-patient	At physician
Translation[4]	Mouse < NHP < Human	Mouse : NHP : Human

1 Amvuttra, Zilebesiran, Inclisiran, Givosiran, Fitusiran, Fazisiran, Nedosiran, Olpasiran, Plozasiran, Zodasiran, Zerlasiran, Imdusiran, Olezarsen, Eplontersen, Vupanorsen, Pelacarsen, Vir-2218; 2 Qnpattro, NTLA-2001, MRT5005, ARCT-810, ARB-1740; 3 NTLA-2001; Qnpattro; 4 Brown et al., Nucleic Acids Research (NAR), 2020

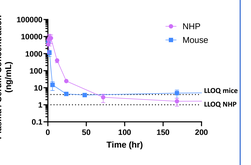
rAIR-100 demonstrates restoration of serum M-AAT (>30 µM) in long-term mouse study with subcutaneous GalNAc molecule



NSG-PiZ mice were treated with 3 loading doses of PBS (vehicle) or 5 or 10 mg/kg rAIR-100 (subcutaneous; dosed on days 0, 2, 4, and 7), followed by 4 biweekly maintenance doses (n = 5/group). Dosing and blood collection are represented by arrows and asterisks, respectively. Serum levels of M-AAT (*left*) and total AAT (*right*) were measured weekly by LC-MS/MS. Data are expressed as mean ± SEM.

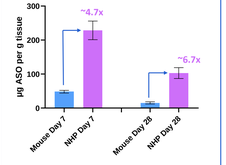
Pharmacology of rAIR-100 in NHP demonstrates durable liver exposure for >1 month after a single dose

AIRNA molecule rapidly cleared from plasma in NHP and mouse



Single 10 mg/kg sub-q administration in both mice and NHP

AIRNA molecule accumulates in the liver with a prolonged half-life in NHPs

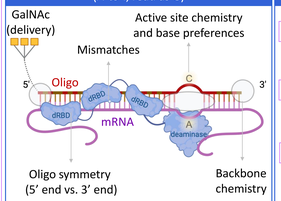


Single 10 mg/kg sub-q administration in both mice and NHP

Left: Pharmacokinetics of rAIR-100 in NSG-PiZ mice (n = 3/group) and cynomolgus monkeys (n = 2/group) after single-dose treatment at 10 mg/kg. *Right*: rAIR-100 levels were determined by an electrochemiluminescent (ECL) hybridization assay. Data are expressed as mean ± SEM.

AIRNA RESTORE+ platform optimizes key features of ADAR-directed oligonucleotides for A-to-I (read as G) RNA editing

Features of ADAR-oligo RNA editing complex (A-to-I, read as G)



Adopted from Aquino-Jarquin, 2020

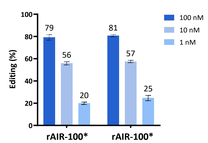
Key elements of RESTORE+ platform

1. **Backbone and base modifications**
   Enhance stability and reduce non-specific protein binding
2. **Optimized GalNAc**
   Maximize delivery to liver, without inhibiting ADAR potency
3. **Structure-based design**
   Maximize ADAR1 p110 isoform engagement, minimize degradation

rAIR-100 optimally engages endogenous ADAR1 isoforms

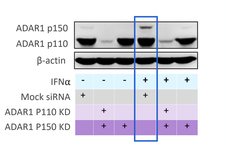
Strong *SERPINA1* RNA editing in vitro in the absence of exogenous IFNα

Editing in hPiZ iPSC-derived hepatocyte like cells



* without GalNAc

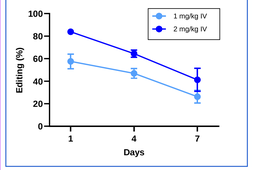
Induction of ADAR1 p150 by IFNα



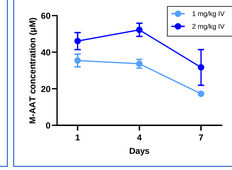
Left: PiZ patient-derived iPSCs were transfected with 1-100 nM unconjugated rAIR-100 in the presence or absence of IFNα (1U/µL). RNA editing levels were determined by NGS 48 hours after transfection. Data are expressed as mean ± SEM. *Right:* Induction of ADAR1 p150 in HeLa cells treated with IFNα, analyzed by Western blot.

LNP formulated rAIR-100 demonstrates >80% editing and high M-AAT (>50 µM) levels in a human PiZ NSG transgenic mouse model

***SERPINA1* editing levels**



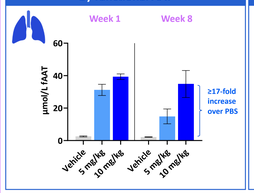
M-AAT levels



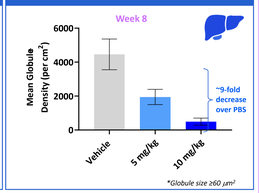
NSG-PiZ mice (n = 3/group) were treated intravenously (IV) with a single dose of 1 or 2 mg/kg MC3-LNP-formulated, rAIR-100 without GalNAc. *Left:* Liver RNA editing levels were quantified on days 1, 4, and 7 by NGS. *Right:* Serum M-AAT levels were quantified by LC-MS/MS. Data are expressed as mean ± SEM.

rAIR-100 demonstrates improvement across both lung and liver disease-relevant endpoints in long-term mouse study

Inhibition of neutrophil elastase by functional AAT



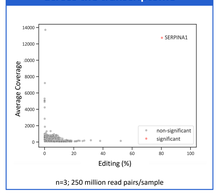
Liver Z-AAT accumulation



Left: Functional AAT (fAAT) levels after 3 initiation doses (Week 1) or after 3 initiation doses with biweekly maintenance dosing (Week 8), quantified by a neutrophil elastase inhibition kit. The dashed line indicates the LLOQ of 2.05 µmol/L. *Right*: Decrease in density of large (≥60 µm²) PAS-D-stained globules after 8 weeks of treatment with rAIR-100. n = 5/group. Data are expressed as mean ± SEM.

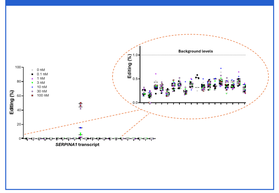
rAIR-100 shows target specificity with no detectable bystander edits

No significant off-target editing across the transcriptome



n=3; 250 million read pairs/sample

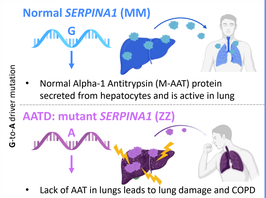
No significant bystander edits in *SERPINA1*



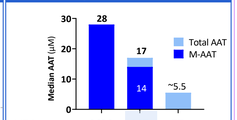
PiZ patient-derived iPSCs were treated with 100 nM (left) or 0.1-100 nM (right) unconjugated rAIR-100, or RNAiMAX (control). *Left:* Off-target editing was evaluated by RNA-Seq. Mean editing levels of 3 samples are shown. *Right:* Bystander editing of adenosines within a 66-nt window surrounding the target site, evaluated by NGS. All surrounding sites exhibited RNA editing levels below 1%.

Alpha-1 antitrypsin deficiency (AATD) biology is particularly suited for RNA editing, and human genetics inform clinical efficacy benchmarks

Severe disease caused by G-to-A mutation



Human genetics informs target protein levels

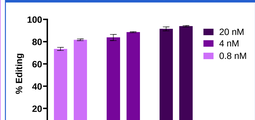


AAT Genotype	MM	MZ	ZZ
Odds ratio of disease progression			
• COPD	1.0	1.0 (0.95-1.1)	8.8 (5.8-13.3)
• Cirrhosis	1.0	1.5 (1.1-1.8)	7.8 (2.5-24.4)

Adapted from Nakanishi et al., 2020; AAT levels calculated from Bornhorst et al., 2013, and Donato et al., 2015

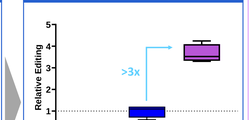
AIRNA RESTORE+ platform chemistry and delivery optimized for in vivo editing with GalNAc-conjugated molecules

***SERPINA1* editing in vitro at low doses** (Primary PiZ mouse hepatocytes)



- Standard: Traditional chemistry and delivery (borrowed from siRNA)
- RESTORE+: AIRNA proprietary chemistry and optimized GalNAc delivery

Improved *SERPINA1* editing in vivo (hPiZ B6 mice)

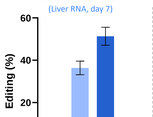


- Standard: Traditional chemistry and delivery (borrowed from siRNA)
- RESTORE+: AIRNA proprietary chemistry and optimized GalNAc delivery

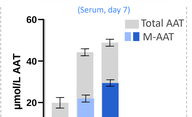
Left: Primary hepatocytes isolated from hPiZ B6 mice were transfected with 0.8-20 nM rAIR-100. RNA editing levels were determined 24 hours after transfection. *Right:* hPiZ B6 mice (n = 3/group) were treated with PBS or 10 mg/kg GalNAc-conjugated rAIR-100 on days 0, 2, and 4. Day-7 RNA editing levels were quantified by NGS. Data are expressed as mean ± SEM.

rAIR-100 demonstrates >50% RNA editing and 30 µM M-AAT with subcutaneous GalNAc molecule

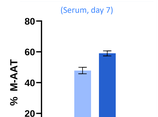
RNA Editing %



AAT (LC/MS)



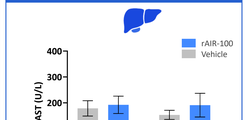
% M-AAT



NSG-hPiZ mice (n = 5/group) were treated subcutaneously with three doses of PBS or 5 or 10 mg/kg of GalNAc-conjugated rAIR-100. *Left:* Day-7 liver RNA editing levels were determined by NGS. *Middle:* Serum levels of total AAT and M-AAT were quantified by LC-MS/MS. *Right:* Percentage of M-AAT relative to total serum AAT, determined by LC-MS/MS. Data are expressed as mean ± SEM.

No elevation of liver and kidney tox parameters in mice over 8 weeks of treatment with rAIR-100

Liver tox: AST



Kidney tox: BUN

Evaluation of liver (*left*) and kidney (*right*) function parameters as assessed by serum chemistry in NSG-hPiZ mice dosed with PBS or 10 mg/kg rAIR-100 over 8 weeks (n = 5/group). Data are expressed as mean ± SEM.

Summary of AIRNA RESTORE+

Platform

Mechanism

AIRNA RNA editing molecules are optimized for in vivo potency, effectively engage p110 isoform of ADAR1, and precisely edit the PiZ mutation with no detectable off-target edits.

Safety & Pharmacology

GalNAc-conjugated **rAIR-100 research molecules** did not result in significant safety findings at high doses in mouse or NHPs, and showed up to 6.7x increase in liver exposure from mouse to NHPs.

Pipeline

AATD

GalNAc-conjugated **rAIR-100 research molecules** demonstrated >50% RNA editing and >30 µM M-AAT production, which led to a ~9-fold decrease in liver aggregates and >17-fold increase in neutrophil elastase inhibition in hPiZ NSG mouse model.

Future

AIRNA's product candidate, **AIR-001**, is further optimized for improved potency and durability, expected to file CTA in H2 2025.

Presented at 28th ASGCT Annual Meeting May 14th, 2025, at New Orleans, LA, USA