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Towards the Clinic: Spotlight on RNA Editing for AATD

Virtual event | September 28, 2022

Forward-looking statements

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Today's Agenda

September 28, 2022
10:00 - 11:30 am EST

Presentation	Speaker
Opening Remarks and AATD Market Perspectives	**Paul Bolno, MD, MBA** President and CEO
WVE-006: First-in-Class RNA Editing Approach for AATD	**Paloma Giangrande, PhD** VP, Platform Discovery Sciences, Biology and WVE-006 Program Lead
Clinical Perspectives on AATD	**D. Kyle Hogarth, MD, FCCP** University of Chicago, Professor of Medicine in the Section of Pulmonary and Critical Care Medicine
Future Applications of AIMers	**Chandra Vargeese, PhD** Chief Technology Officer
Q&A	All speakers

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Paul Bolno, MD, MBA
President and Chief Executive Officer
Wave Life Sciences

Opening Remarks

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Wave is developing oligonucleotide therapeutics to target the transcriptome and modulate gene expression

Innovative RNA-targeted therapeutics portfolio

WVE-003 HD (SNP3)	*Silencing*
WVE-004 ALS and FTD (C9orf72)	*Silencing*
WVE-N531 DMD (Exon 53)	*Splicing*
WVE-006 AATD (SERPINA1)	*RNA editing*

Innovative RNA platform PRISM Stereochemistry, PN chemistry

SELECT HD and FOCUS-C9 data: Continued clinical validation of PRISM platform and PN stereochemistry

- ✓ **PN chemistry translation:** WVE-003 and WVE-004 clinical data indicate target engagement in CNS
- ✓ **Platform validation:** Successfully predicted target engagement with PK/PD modeling, all pipeline programs leverage similar *in vivo* modeling work
- ✓ **Adaptive clinical trial:** Identify target engagement and adapt to optimize dose level and frequency

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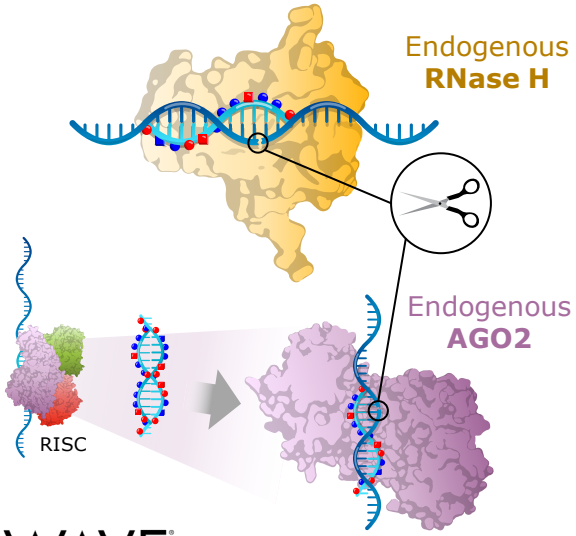
Kandasamy et al., NAR Breakthrough Article, 2022 doi: 10.1093/nar/gkac037; Kandasamy et al., 2022; doi: 10.1093/nar/gkac018

Harnessing the biological machinery in our cells to treat genetic diseases

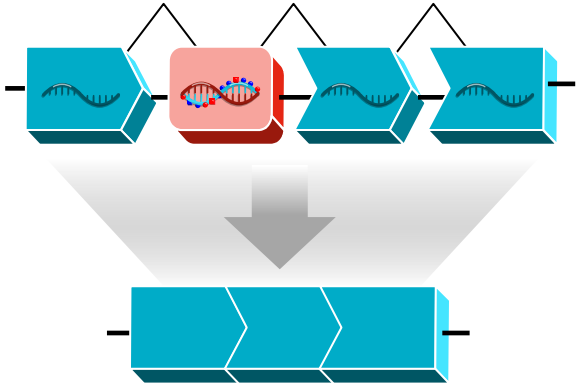
Silencing

- Degradation of RNA transcripts to **turn off** protein production



Splicing

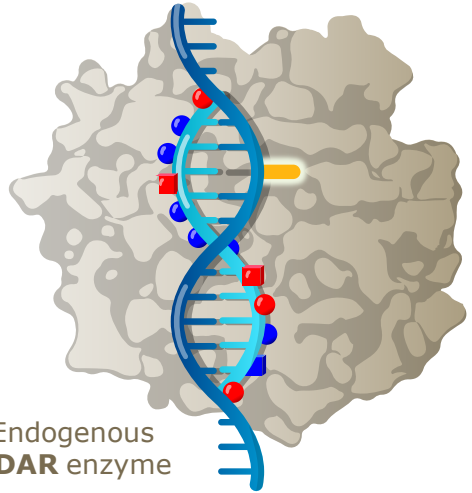
- Restore RNA transcripts and **turn on** protein production



Restored **Reading Frame**

RNA Base Editing

- Efficient editing of RNA bases to **restore** or **modulate** protein production



Endogenous **ADAR** enzyme

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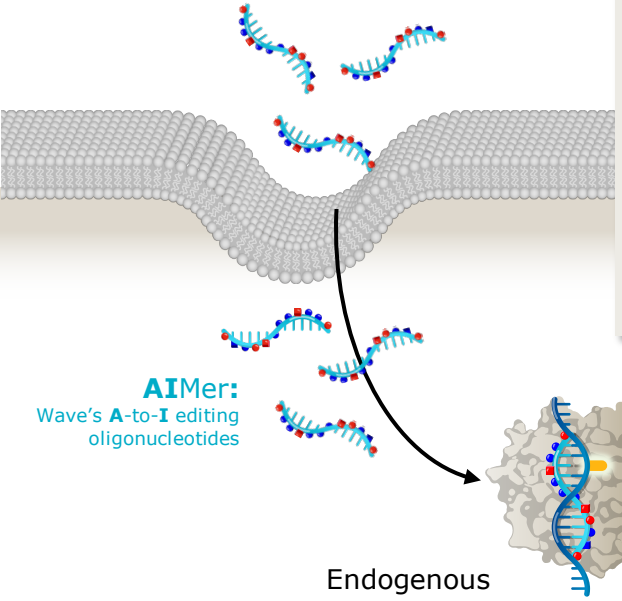
Unlocking therapeutic RNA editing with AIMers

Free-uptake of chemically modified oligonucleotides
(No need for LNPs or viral vectors)



AIMer:
Wave's **A**-to-**I** editing oligonucleotides

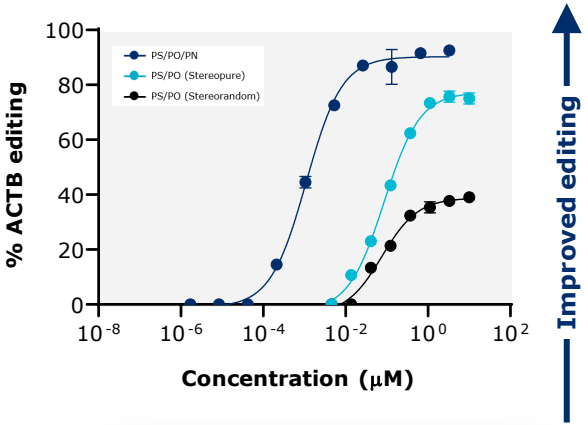
Endogenous ADAR enzymes

ADAR enzymes

- First publication (1995) using oligonucleotide to edit RNA with endogenous ADAR[1]
- Catalyze conversion of A-to-I (G) in double-stranded RNA substrates
- A-to-I (G) edits are one of the most common post-transcriptional modifications
- ADAR1 is ubiquitously expressed across tissues, including liver and CNS

- ✓ Learnings from biological concepts
- ✓ Applied to ASO structural concepts
- ✓ Applied PRISM chemistry

Stereochemistry and PN chemistry enhance potency and editing efficiency of GalNAc AIMers in primary human hepatocytes




nature biotechnology ARTICLES

Endogenous ADAR-mediated RNA editing in non-human primates using stereopure chemically modified oligonucleotides

Prashant Monian[1,2], Chikdu Shivalila[1,2], Genliang Lu[1], Mamoru Shimizu[1], David Boulay[1], Karley Bussow[1], Michael Byrne[1], Adam Bezigian[1], Arindom Chatterjee[1], David Chew[1], Jigar Desai[1], Frank Favaloro[1], Jack Godfrey[1], Andrew Hoss[1], Naoki Iwamoto[1], Tomomi Kawamoto[1], Jayakanthan Kumarasamy[1], Anthony Lamattina[1], Amber Lindsey[1], Fangjun Liu[1], Richard Looby[1], Subramanian Marappan[1], Jake Metterville[1], Ronelle Murphy[1], Jeff Rossi[1], Tom Pu[1], Bijay Bhattarai[1], Stephany Standley[1], Snehlata Tripathi[1], Hailin Yang[1], Yuan Yin[1], Hui Yu[1], Cong Zhou[1], Luciano H. Apponi[1], Pachamuthu Kandasamy[1] and Chandra Vargeese[1]✉

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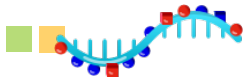
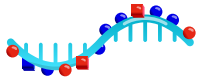
[1]Woolf et al., PNAS Vol. 92, pp. 8298-8302, 1995; Right: Data from independent experiments;
Total RNA was harvested, reverse transcribed to generate cDNA, and the editing target site was amplified by PCR and quantified by Sanger sequencing

AIMers: Realizing potential of therapeutic RNA editing by harnessing endogenous ADAR

Solved for key therapeutic attributes for potential best-in-class RNA editing therapeutics

Efficient ADAR recruitment	Stability	Delivery and intracellular trafficking	Beyond liver
• AIMer design principles • SAR developed to design AIMers for different targets	• Decade of investment and learnings to improve stability of single-stranded RNAs	• GalNAc compatible for targeted liver delivery • Endosomal escape and nuclear uptake	• AIMer design also works for delivery to CNS and other tissue types
Potent and specific editing *in vivo*	**Potential for infrequent dosing**	**Subcutaneous dosing**	**IT, IVT, systemic dosing**





- **Systematized AIMer design enables rapid advancement of new targets**
- **Strong and broad IP including chemical and backbone modifications, stereochemistry patterns, novel and proprietary nucleosides**

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SAR: structure-activity relationship

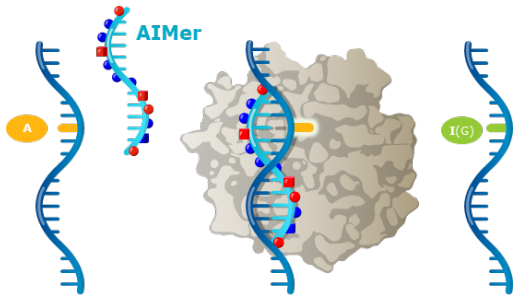
Opportunity to apply AIMers to correct G-to-A mutations and to modulate protein interactions

Correct G-to-A driver mutations with AIMers

Modulate protein interactions with AIMers

Restore or correct protein function

WVE-006 (GalNAc AIMer)
AATD



Modulate protein-protein interaction

Upregulate expression

Modify function

Post-translational modification

Alter folding or processing

AATD and additional hepatic diseases

Neurological disorders

Recessive or dominant genetically defined diseases

Renal, cardiometabolic, immunology

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Paul Bolno, MD, MBA
President and Chief Executive Officer
Wave Life Sciences

Alpha-1 Antitrypsin Deficiency (AATD) Market Perspectives

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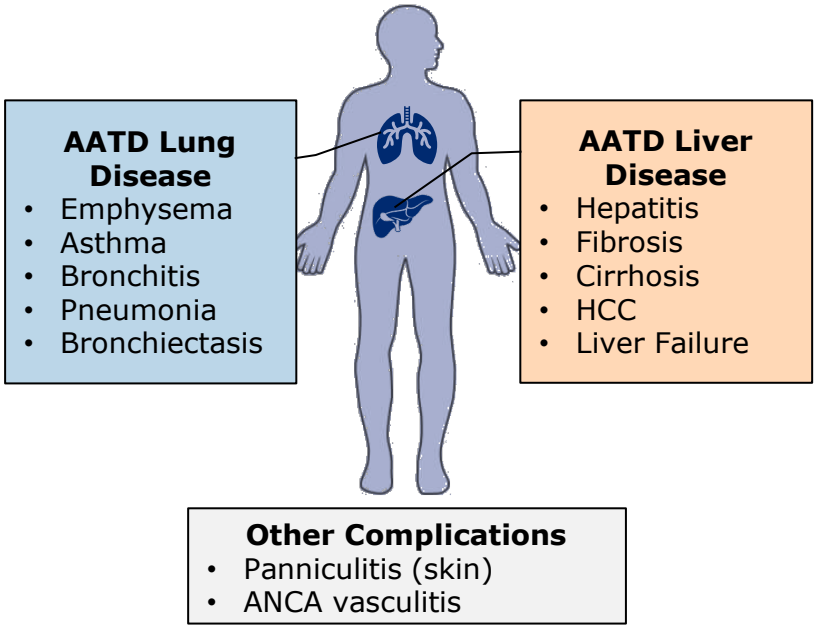
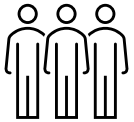
AATD may result in lung and liver disease and has limited treatment options

- ***SERPINA1* Z mutation** (E342K) is most common cause of AATD
- **~200,000 Pi*ZZ patients** in US and Europe[2]
- **Augmentation therapy** is only treatment option for AATD lung disease and requires weekly IV infusions
- **No treatment for AATD liver disease**, other than liver transplant
- Average age of diagnosis of AATD lung disease is **46 years**[3] and average age of adult-onset liver disease is **61 years**[4]



1. Greene, C.M., *et al*., 2016 Nat Rev Dis Primers 2, 16051; 2. Blanco *et al*., 2017 *Int J Chron Obstruct Pulmon Dis* 12:561-69; 3. Strnad, *et al*. 2020 *N Engl J Med* 382:1443-55; 4. Tanash and Piitulainen 2019 *J Gastroenterol* 54:541-548

Patient insights highlight burden of AATD



> *It's an **invisible disease.** It seems like it tricks you - I look healthy and then I tell someone I can't help them grab something 20 feet away. That's the disconnect.*

> *I used to own a salon and with my lungs going bad so quickly, I had to do away with that…I get **short winded**…I lay around a lot, I'm sick a lot.*

> *It makes it harder for us to travel and go do things, because I have to be home once a week for my infusions. It's definitely an **inconvenience**.*

> *I now have very **high elevated liver enzymes and fatty liver** just in the last year. I get a lot of pain on that side. They believe it is related to AATD.*

> *I have back issues, anytime they do a CAT scan or MRI there is always a notation about fatty liver disease and scarring on my liver. **I know it's there. I know there's a problem.** It's just part of my everyday.*

Engaging patient community to inform clinical development plans

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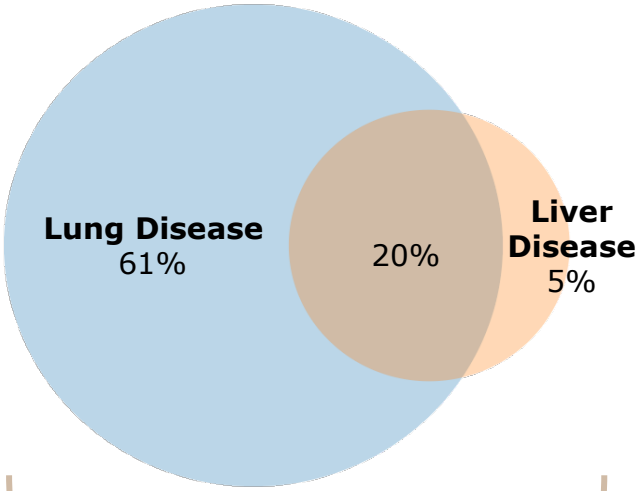
Lumanity AATD Patient Research 2022

AATD landscape is poised to evolve but most approaches focus on lung or liver disease

Selected Therapeutic Strategies in Development for AATD

- Augmentation therapy (Plasma derived, IV) ***[Approved]***
- Recombinant Fc-AAT (IV)
- Inhaled AAT (nebulized)
- Neutrophil elastase inhibitors (oral)

*Frequency of Lung or Liver Disease of Pi*ZZ Patients at Diagnosis**



- RNAi (subcutaneous)
- **WVE-006 (subcutaneous)**
- Gene editing
- Small molecule folding correctors (oral)

**From Alpha One International Registry (n=3,405)*[3] (13% reported no disease at diagnosis)

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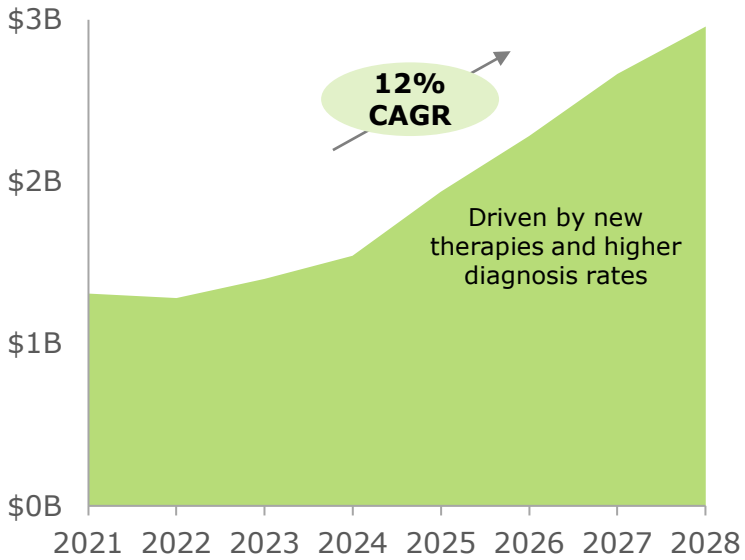
1. Rahaghi FF. 2021 Therapeutic Advances in Chronic Disease. 12_suppl.; 2. Fazleen A and Wilkinson T. 2021 ERJ Open Research 7, 00494-2021;
3. McElvaney, *et al.*, 2020 Eur Respir J 55:1902410

AATD market estimated to grow to ~$3B by 2028

AATD Market Overview

- **AATD market today is estimated at ~$1.3B worldwide[1] despite limitations of current treatment**
  - Market consists entirely of plasma-derived augmentation therapy for AATD-lung disease
  - Augmentation therapy requires weekly IV and is not reimbursed in some markets
- **Market expected to grow to ~$3B by 2028**
  - Treatment for AATD-liver disease in development
  - Opportunity to improve upon treatments for AATD-lung disease (efficacy, administration)
  - Potential to increase diagnosis (e.g., for liver disease, through direct-to-consumer genetic testing)

Global AATD Market Value (2021 – 2028)[1]



1. Evaluate Pharma; 2. Lumanity KOL Market Research 2022

Paloma Giangrande, PhD
VP Platform Discovery Sciences and Biology; WVE-006 Program Lead

WVE-006: First-in-Class RNA Editing Approach for AATD

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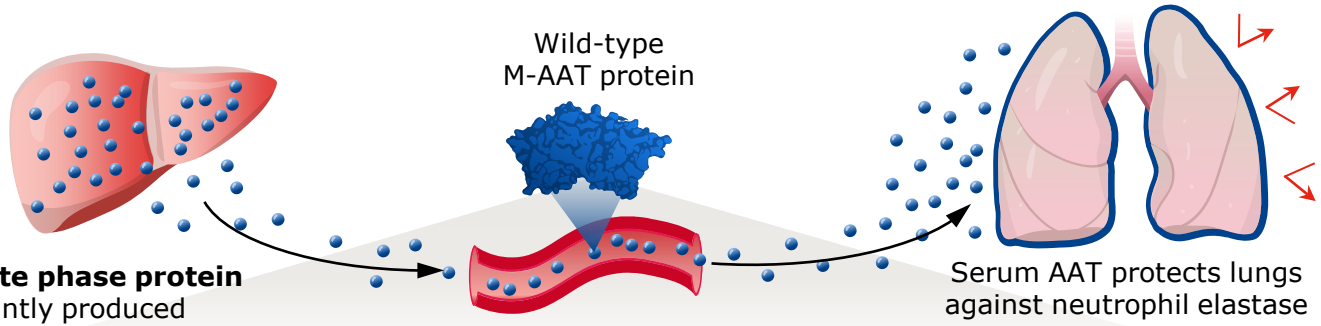
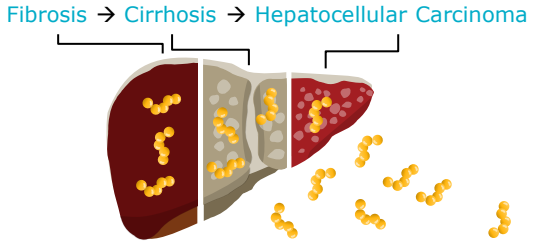
SERPINA1 Z mutation: The most common cause of alpha-1 antitrypsin deficiency (AATD)

Healthy

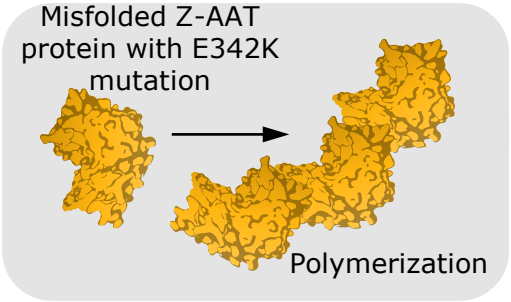


AATD

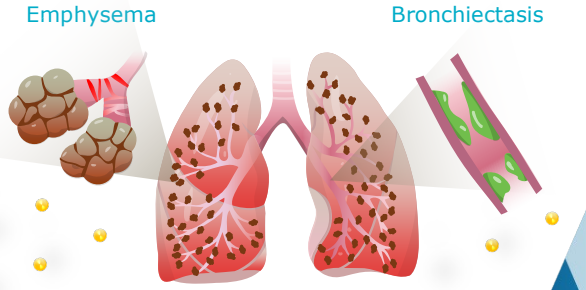
Gain of Function: Liver Disease



Z protein causes AAT proteotoxic stress, leading to progressive liver disease



Loss of Function: Lung Disease



Low serum AAT leads to lung disease

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Strnad *et al.*, 2020 *N Engl J Med* 382:1443-55; Stoller et al., 1993 *Alpha-1 Antitrypsin Deficiency* GeneReviews

WVE-006: Designed to correct mutant SERPINA1 transcript to address both liver and lung manifestations of AATD

WVE-006 corrects the Z allele mRNA to enable M-AAT protein to be produced

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

WVE-006
(GalNAc-conjugated AIMer)

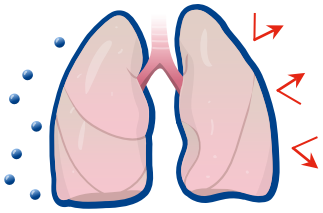
Edited *SERPINA1* mRNA enables wild-type M-AAT protein production

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

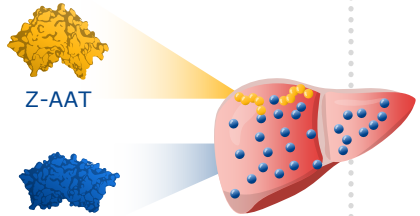
1) Restore circulating, functional wild-type M-AAT

2) Reduce Z-AAT protein aggregation in liver

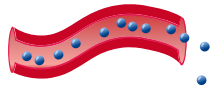
3) Retain M-AAT physiological regulation



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

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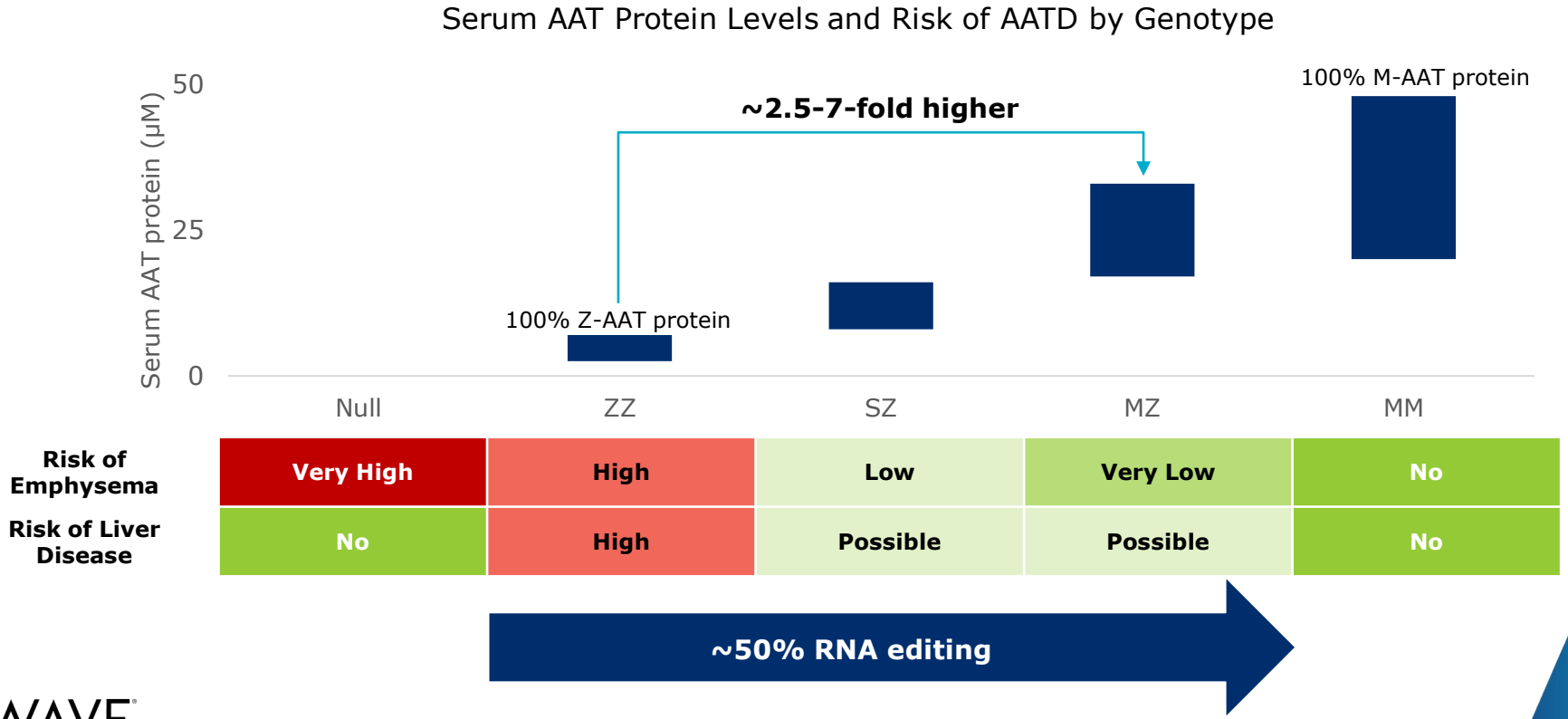
AAT: Alpha-1 antitrypsin Strnad *et al*., 2020 *N Engl J Med* 382:1443-55; Blanco *et al*., 2017 *Int J Chron Obstruct Pulmon Dis* 12:561-69; Remih *et al*., 2021 *Curr Opin Pharmacol* 59:149-56.

~50% RNA editing expected to increase PI*ZZ patient serum AAT levels to PI*MZ levels, with low risk of disease

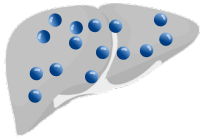


1. Brode, et al. 2012 CMAJ 184:1365-1371; 2. ATS/ERS. 2003 Am J Respir Crit Care Med 168:818–900.

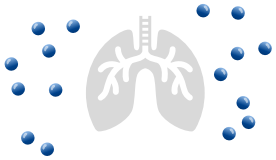
Wave's approach is uniquely positioned to address AATD lung and liver disease

Untreated patients

Healthy

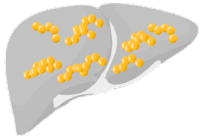


M-AAT secreted from liver

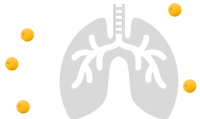


M-AAT protects lungs

AATD (Pi*ZZ)



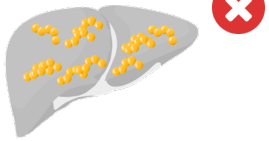
Z-AAT aggregates in liver



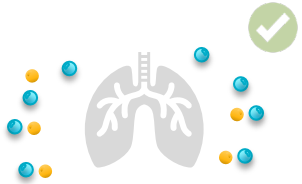
Limited protection from Z-AAT

Pi*ZZ patient treatment approaches

Augmentation Therapy

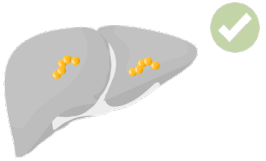


No impact on liver disease



AAT protein replacement

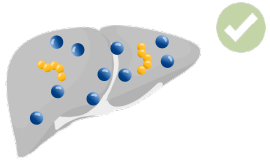
RNAi



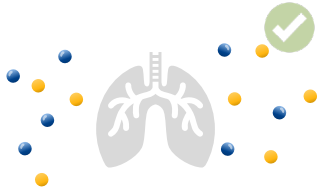
Knockdown of Z-AAT production to allow aggregate clearance

AAT knockdown may further reduce Z-AAT reaching lungs

Wave AIMer



Correction of mRNA transcript enables M-AAT production and clearance of Z-AAT aggregates



M-AAT protects lungs

M-AAT Z-AAT AAT from augmentation therapy Z-AAT aggregate

Manifestation addressed Manifestation not addressed

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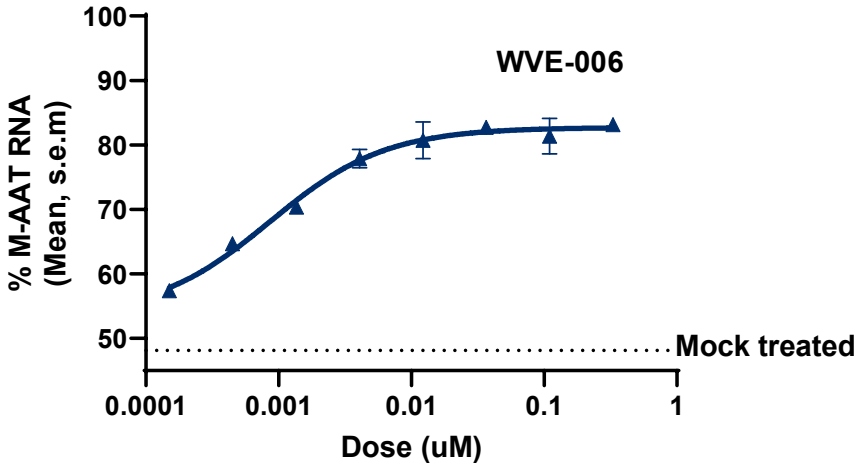
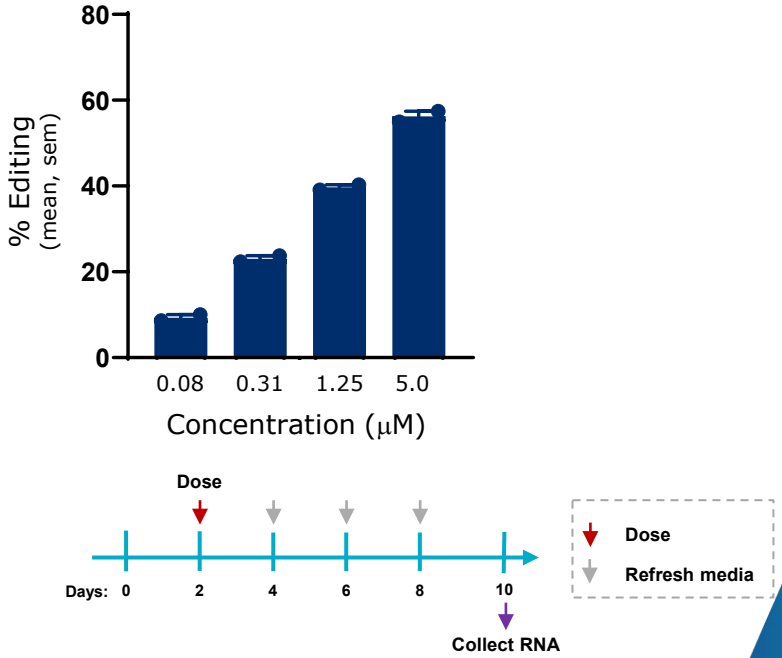
WVE-006 supports dose-dependent RNA editing in human preclinical model systems

Efficient SERPINA1 editing in donor-derived primary human hepatocytes with WVE-006 (MZ genotype)



Note: Due to MZ genotype, Y-axis ranges from ~50-100%

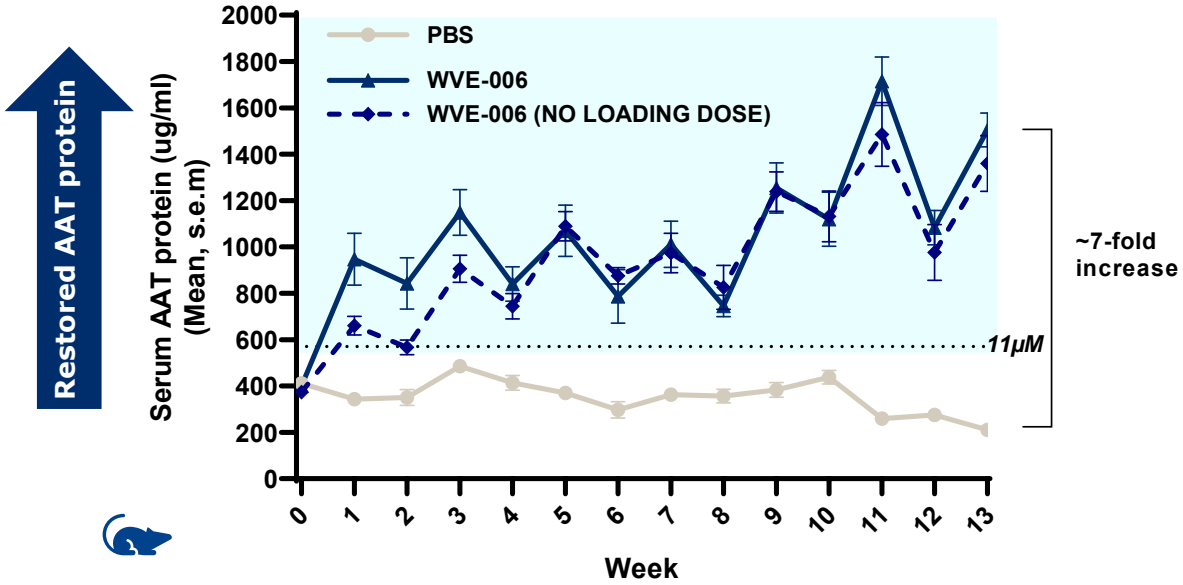
iPSC-derived human hepatocytes (ZZ genotype)



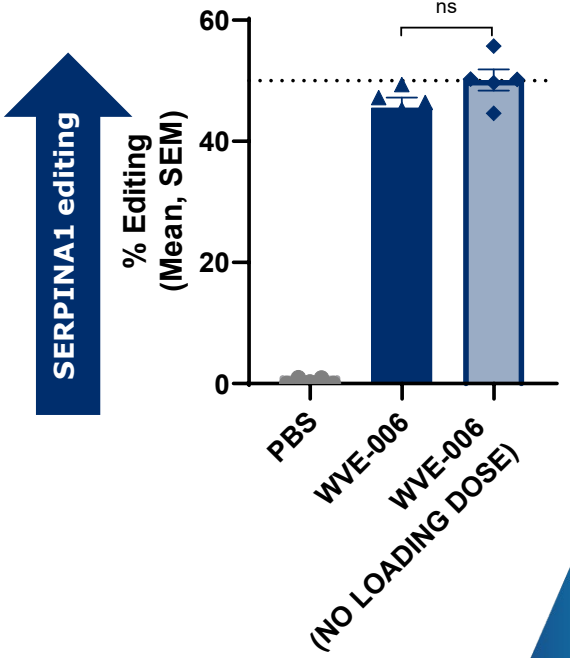
Left: MZ-donor derived primary human hepatocytes treated with WVE-006 at indicated concentrations for 48 hours
Right: Patient-iPSC derived hepatocytes (ZZ genotype) plated on day 0 and treated on day 2 with WVE-006 at indicated concentrations. Media refreshed every 2 days (days 4, 6, 8). RNA was collected on day 10. In each experiment, RNA editing was quantified by Sanger sequencing (n=2 biological replicates)

WVE-006 results in circulating AAT protein levels 7-fold above PBS control, well above established 11µM threshold

WVE-006 treatment results in serum AAT protein levels >11 uM in AATD mouse model (NSG-PiZ mice)

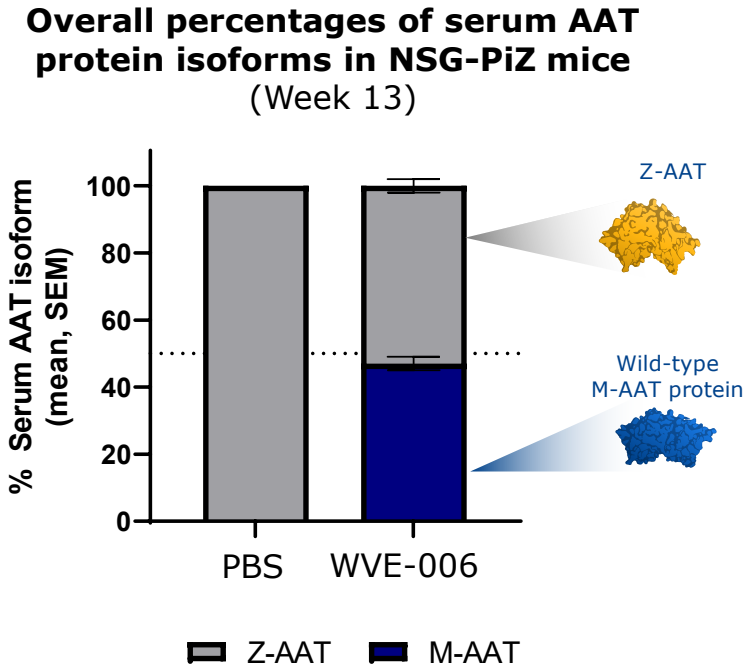


SERPINA1 mRNA editing in liver of AATD mouse model (NSG-PiZ mice) (Week 13)



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. Left: Liver biopsies collected at week 13 (one week after last dose) and SERPINA1 editing was quantified by Sanger sequencing; Stats: One-way ANOVA with adjustment for multiple comparisons (Tukey); Right: Total serum AAT protein quantified by ELISA; Stats: Two-Way ANOVA with adjustment for multiple comparisons (Tukey)

WVE-006 leads to restoration of confirmed, wild-type M-AAT protein in serum



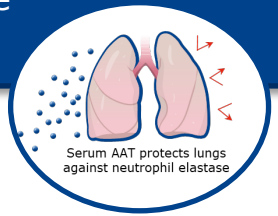
- Mass spectrometry confirms restoration of circulating healthy M-AAT protein *in vivo* after WVE-006 treatment
- Consistent with RNA editing of mutant transcript

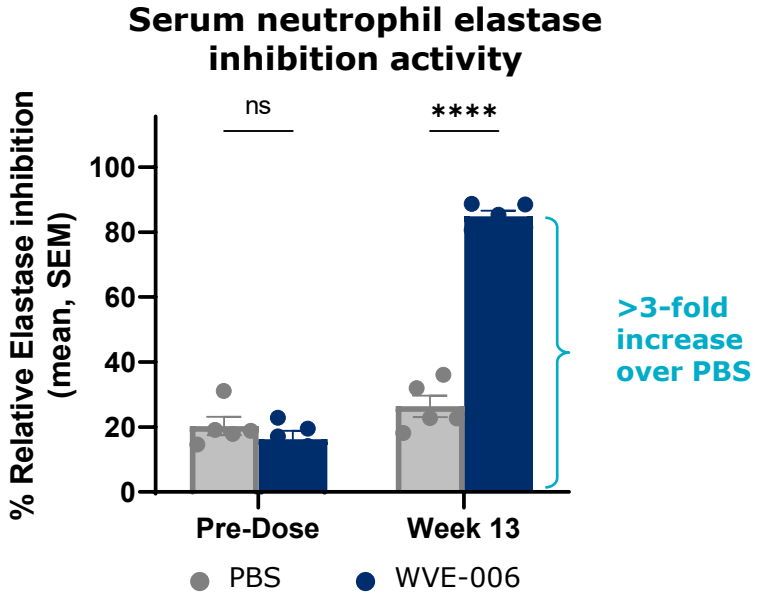
WVE-006 administered in 7-week old NSG-PiZ mice (n=5 per group). Relative proportion of M- vs. Z-AAT protein in serum collected from animals at week 13 (one week after last dose) was measured by mass spectrometry

Significant increase in neutrophil elastase inhibition activity indicates restored M-AAT protein is functional

Increased neutrophil elastase inhibition activity demonstrates functionality of AAT protein

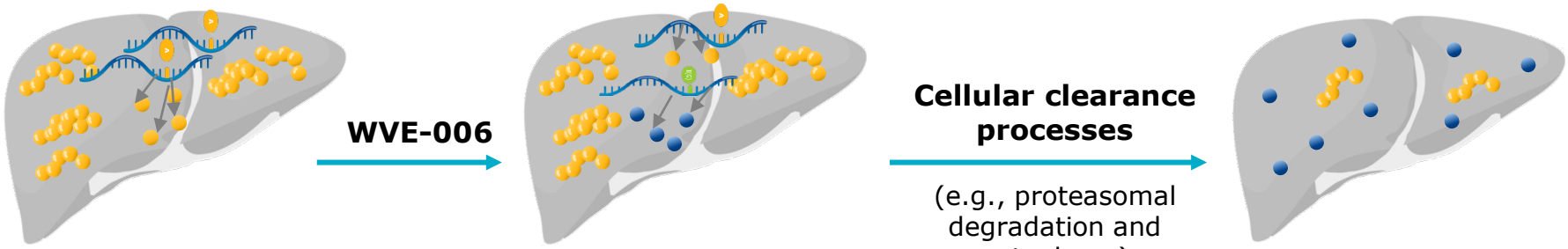
- Increases in neutrophil elastase, a proteolytic enzyme, may cause emphysema and damage the surrounding lung tissue
- Main function of AAT protein is to neutralize/control neutrophil elastase





GalNAc-conjugated AIMers administered in 7-week old NSG-PiZ mice (n=5 per group). Serum collected from mice was tested for ability to inhibit fixed concentration of neutrophil elastase in an *in vitro* reaction. Stats: Two-way ANOVA with adjustment for multiple comparisons (Bonferroni)

Replacing Z-AAT with M-AAT enables clearance of liver aggregates



Continual production / aggregation of Z-AAT protein prevents cells from clearing aggregates

WVE-006 reduces production of Z-AAT protein and enables production of M-AAT protein

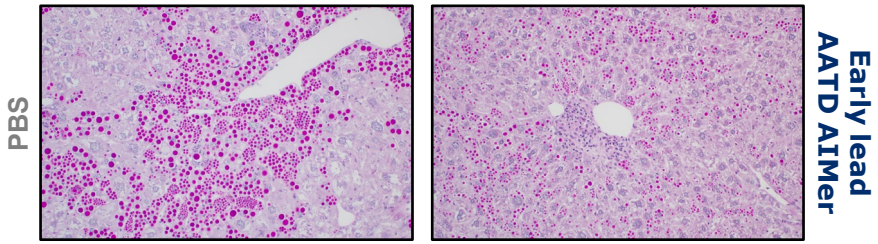
Reduction in Z-AAT production enables cells to clear aggregates

Healthy M-AAT | Mutant Z-AAT | Z-AAT aggregate | *SERPINA1* mRNA

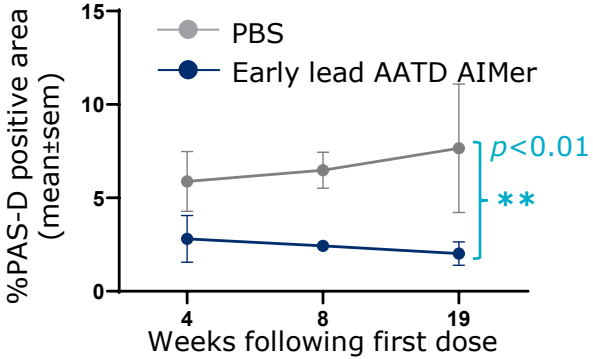
WAVE LIFE SCIENCES

Early lead (pre-optimization) AATD AIMer alleviates aggregation of Z-AAT and inflammation in mouse liver

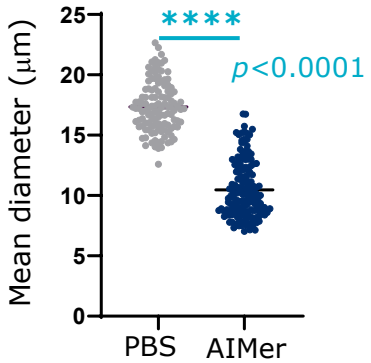
PAS-D staining
(19 weeks)



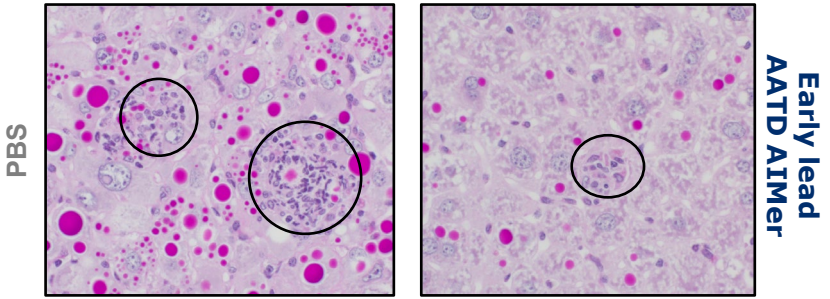
PAS-D-positive area



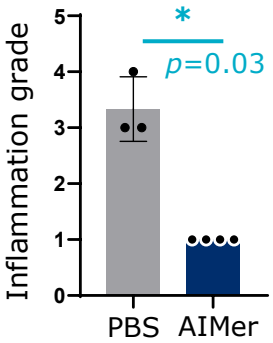
PAS-D globule size
(19 weeks)



Lobular inflammation
(19 weeks)



Lobular inflammation
(19 weeks)



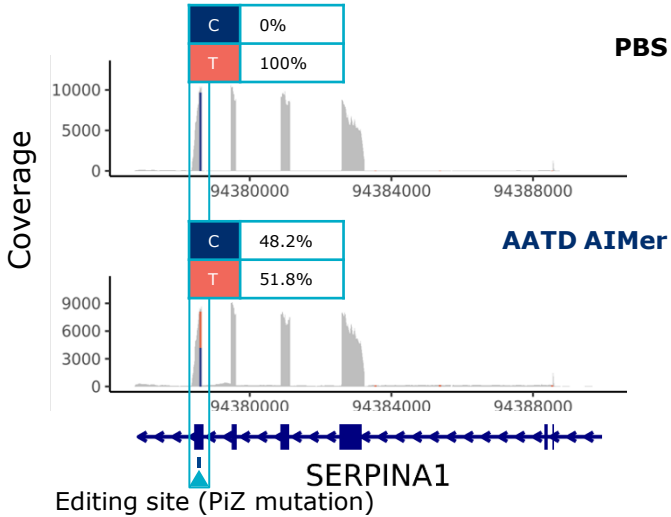
WAVE LIFE SCIENCES

Early lead pre-optimization AATD AIMer (SA1-5) administered in hu*ADAR/SERPINA1* mice (8–10 wKs old); lower left: 20x liver images PAS-D stained, 19 weeks; Quantification of PAS-D positive staining, Stats 2-way ANOVA; Right: Quantification lobular inflammation grade (Grade based on # of inflammatory foci in lobules: Grade 0: 0; G1 1-5; G2 6-10; G3 11-15; G4 ≥16) and mean globular diameter (40 largest globules/ animal) with HALO. Stats Wilcox rank-sum tests

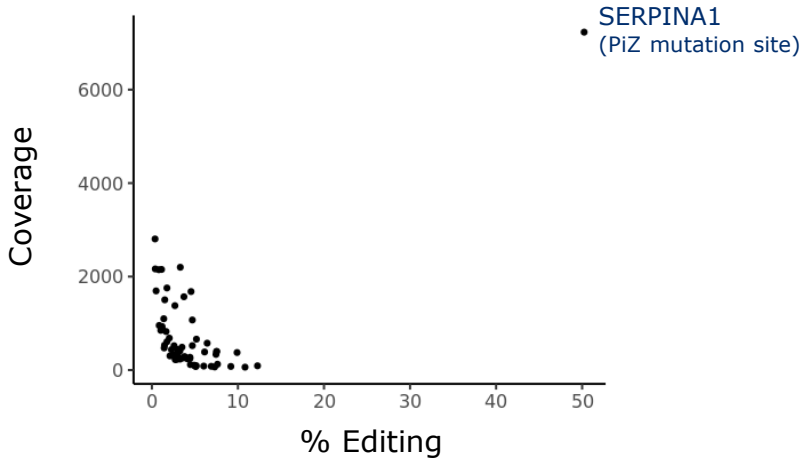
AIMer-directed editing is highly specific in mice

No bystander editing observed on SERPINA1 transcript

RNA editing only detected at PiZ mutation site in SERPINA1 transcript
(mouse liver)



RNA editing across transcriptome
(mouse liver)



Dose 3x10 mg/kg (days 0, 2, 4) SC with AATD AIMer (SA1 – 4). Liver biopsies day 7. RNA-seq to quantify on-target SERPINA1 editing, to quantify off-target editing reads mapped to entire mouse genome; plotted circles represent sites with LOD>3 (N=4), SERPINA1 edit site is indicated

WVE-006 is a potential first- and best-in-class candidate for AATD

- **Correct Z-allele mRNA to replace mutant Z-AAT protein with functional wild-type M-AAT protein**
  - RNA editing levels show potential to support conversion of a patient from ZZ to MZ mRNA expression
  - M-AAT protein can address lung disease
  - Reduction of Z-AAT protein enables clearance of protein aggregates in liver
- **M-AAT protein produced with WVE-006 would remain under physiological regulation**
- **mRNA editing is highly specific**
- **Potentially applicable across AATD patient subpopulations**
- **Convenience of subcutaneous administration**

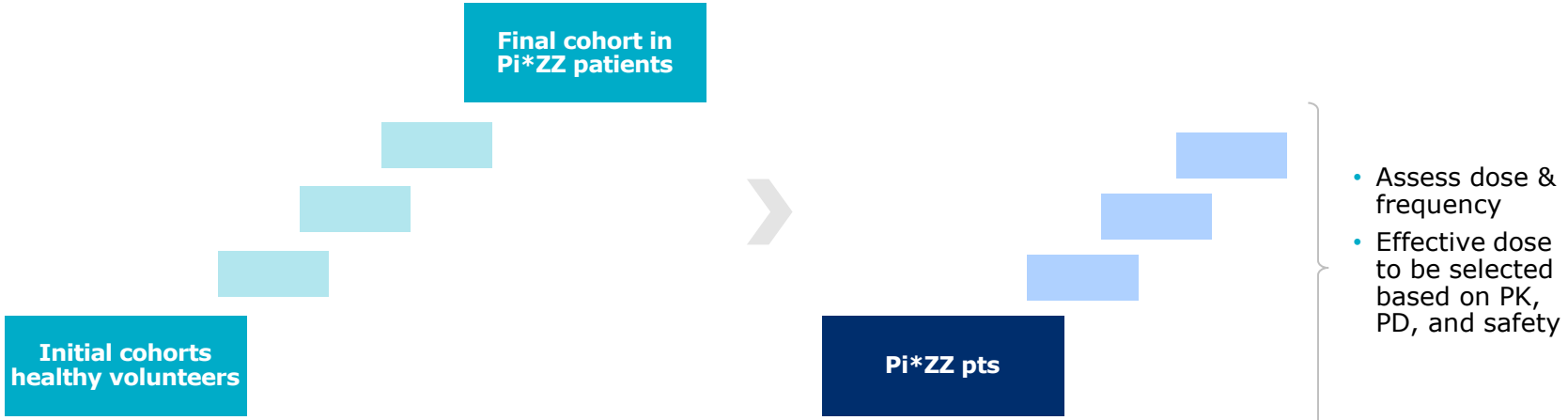
WAVE LIFE SCIENCES

Planning for clinical development for WVE-006 underway

Phase 1/2 placebo-controlled study to establish dose and evaluate target engagement

Single-ascending dose (SAD) cohorts

Multiple-ascending dose (MAD) cohorts



Safety, tolerability, PK, change in relevant biomarkers, including serum AAT

CTA submissions for WVE-006 expected in 2023

WAVE LIFE SCIENCES

D. Kyle Hogarth, MD, FCCP

Professor of Medicine and Director
of the Alpha-1 Clinical Resource Center
University of Chicago

Clinical Perspectives on AATD

Professor of Medicine, Section of Pulmonary and Critical Care Medicine
University of Chicago
Director of Bronchoscopy
Director of the Alpha One Antitrypsin Deficiency Clinical Resource Center
Director of the Pulmonary Rehab
Co-Director of Lung Cancer Screening Program

D. Kyle Hogarth, MD, FCCP, is a Professor of Medicine in the Section of Pulmonary and Critical Care Medicine at The University of Chicago. He is the Director of Bronchoscopy and is heavily involved in the field of advanced bronchoscopy and interventional pulmonary with numerous publications. He also runs the Alpha One Antitrypsin Deficiency Clinical Resource Center, one of the largest in the Midwest with over 250 patients.
Dr. Hogarth received his medical degree at Case Western Reserve University School of Medicine in Cleveland, Ohio. He then completed a residency in Internal Medicine and a fellowship in Pulmonary and Critical Care at The University of Chicago. He has been published in the New England Journal of Medicine, Chest, ERJ and many other journals. He previously served on the editorial board of CHEST, where he was the section editor for the podcast section and giants in chest medicine series. He helped write the 2016 Alpha One Antitrypsin deficiency Clinical Practice Guidelines. He has won numerous clinical awards and teaching awards from his University. He is a founding member and a Past President of the Society for Advanced Bronchoscopy.

Alpha One - Antitrypsin Deficiency

D. Kyle Hogarth, MD, FCCP
Professor of Medicine
Director - Alpha One Clinical Resource Center
University of Chicago

Current Conflict of Interest (as of 9/22/22)

•Stock Option holder/Stock holder:

–Body Vision, Broncus, Eolo, Eon, Gravitas, Imbio, Lanier, Magnisity, Noah Medical, LX-Medical, Med-Opsys, Monogram Orthopedics, Preora, Preview Med, Prothea-X, Ryme, Ruby Robotics, Spesana, VIDA

•Individually Purchased Shares on open market (does NOT include mutual funds/retirement accounts):

–J&J, Exact Sciences

•Consultant within last 3 years:

–Alpha Sights, Ambu, Atheneum, Auris, Body Vision, Boston Scientific, Broncus, Coleman, CSL, Deerfield, Eolo, Fluidda, Gala, Gilman Capital, GLG, Grand Rounds, Guidepoint Global, Imbio, Intuitive, J&J, Lanier, Level-Ex, Magnisity, MediFind, Morgan-Stanley, Mosaic, Noah Medical, NovaScan, Olympus (Spiration), Oncocyte, Patients Like Me, Preora, Preview Med, Prothea-X, PulmonX, Qure.ai, Ryme, Ruby Robotics, Serpex, Spesana, Takeda, TSC, Veracyte, Volv, Wave Life Sciences.

•Research Dollars/Contracted Research (past 3 years and present):

–Ambu, Boston Scientific, Gala, Medtronic, Nuvaira, Olympus (Spiration), PulmonX, Shire

•DSMB member (past and present)

–InhibRx (past)

•Lectures Given (Honoraria received) within the last 3 years:

–Astra-Zeneca, Biodesix, B.I., Boston Scientific, Broncus, Genentech, Grifols, PulmonX, Spiration (Olympus), Takeda, Veracyte

Intellectual Conflict of Interest

"Intellectual conflicts of interest are completely ubiquitous" and have generally been ignored.

- Gordon Guyatt, Professor of Medicine at McMaster University

- Intellectual conflicts occur when clinicians or researchers may be too deeply embedded in their own area of expertise to objectively look at a research question "with an open mind".
- "Even when money is not involved … we get very attached to our ideas."

- This is compounded by university culture, which rewards researchers if their work is highly referenced by others and is perceived to be influential.
- This environment creates an incentive for those participating in guideline development to highlight their own research in clinical practice guidelines.

History

- In 1962, Dr. Carl-Bertil Laurell (1919-2001) at the University of Lund, Sweden discovered the absence of the alpha-one band in 2 serum electrophoresis gels during his protein studies
- Then, further investigation by Dr. Sten Eriksson demonstrated 4 more with missing alpha-one bands
- 4 of the 6 patients had advanced emphysema at early ages.

Laurell C-B, Eriksson S. Scand J Clin Lab Invest 1963; 15:132-140.
Eriksson S, Laurell C-B. Acta Chem Scand 1963;17:150-153.

Normal Alpha Protein

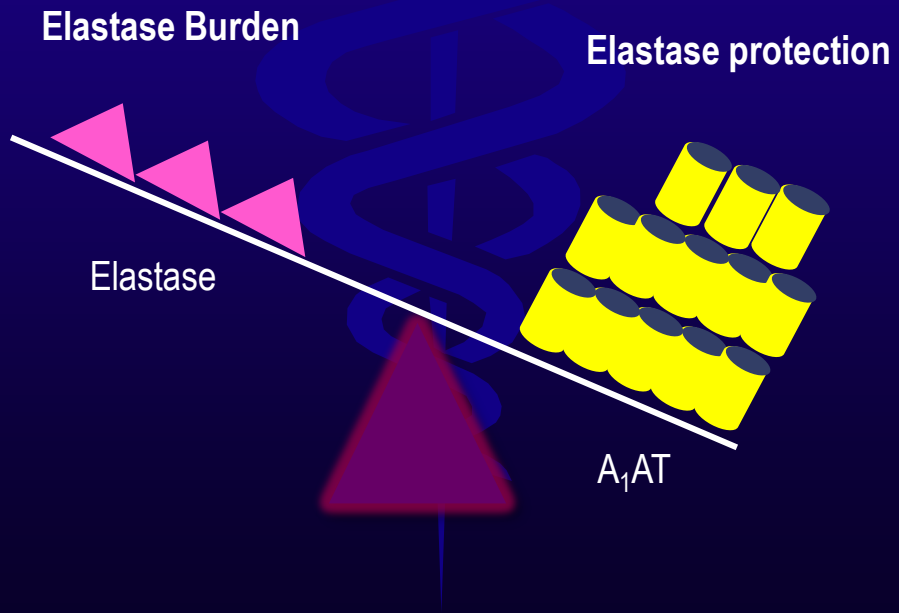
- Majority of alpha one antitrypsin is synthesized by the liver, though some is made by lung epithelial cells and monocytes
- Main function is to neutralize/control Neutrophil Elastase.

Neutrophil Elastase

- Neutrophil Elastase is discovered in 1967
- Elastase-Antielastase hypothesis: first viable hypothesis to explain emphysema
  - Papain, a plant enzyme with elastinase properties, can cause emphysema in animal models
  - Antitrypsin keeps elastin activity “in-check” and prevents lung tissue breakdown

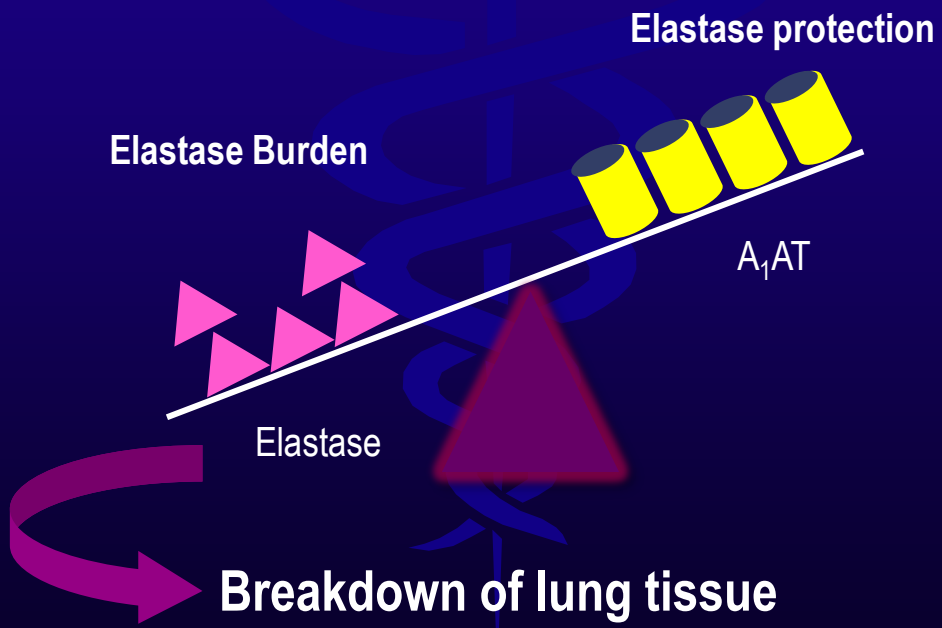
Balance of Neutrophil Elastase & A_1AT

Healthy



Balance of Neutrophil Elastase & A_1AT

A_1AT Deficient



Deficient Variants

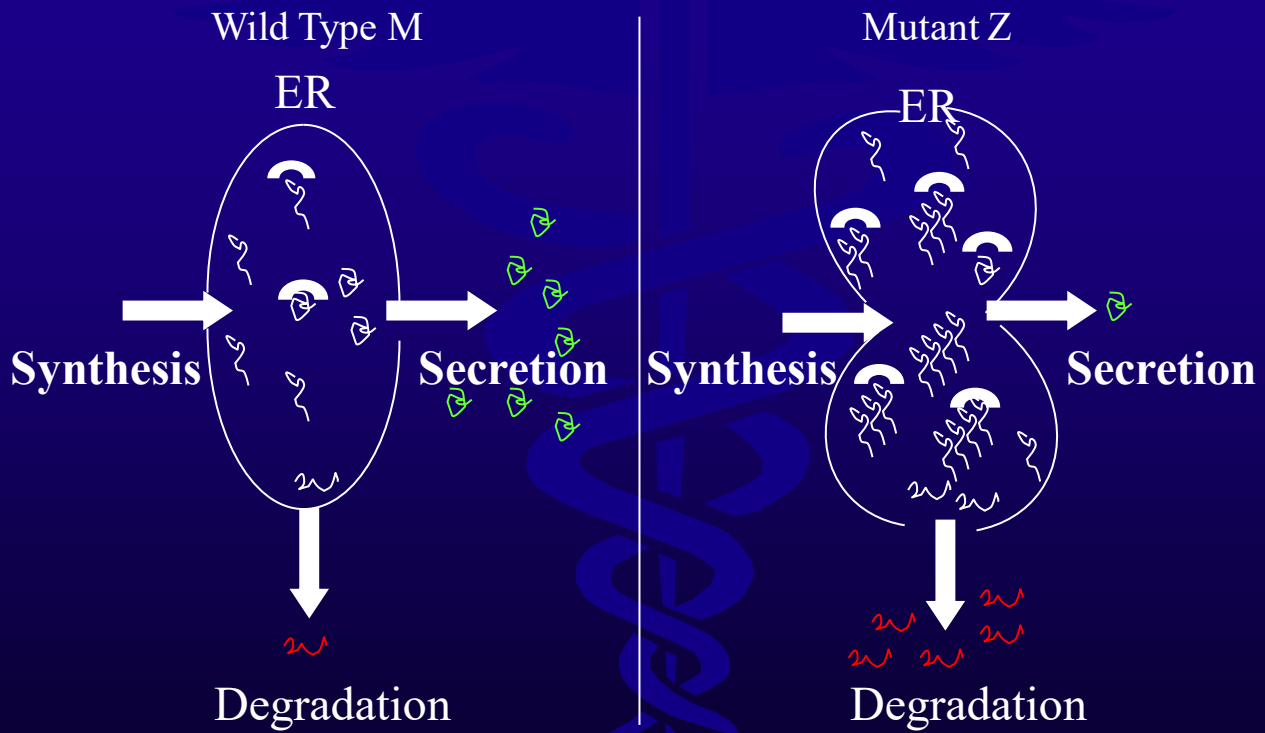
- Z allele (Glutamic acid-342-Lysine)
  - Variant found in 95% of deficient individuals who present clinically
  - A_1AT cannot be released effectively from the liver cells
- S allele (Glutamic acid-264-Valine)
  - Variant associated with a milder deficiency
  - Not associated with A_1AT accumulation within liver cells
- More than 120 other less common mutations exist

AATD is a Liver Disease

- Dr. H.L. Sharp described the association between alpha one deficiency and liver disease in 1969.
- Sveger's work demonstrated 10% of Pi*ZZ develop neonatal cholestasis and 1-2% progress to cirrhosis as children.
- Up to 20% of Pi*ZZ develop slow progressive portal fibrosis with 20% of Pi*ZZ developing cirrhosis in later adult life.

Sharp HL, Bridges RA, Krivit W, Freier EF. J Lab Clin Med 1969;73:934-9
Sveger T. N Eng J Med 1976; 294:1316–1321.

Model of a1AT protein processing in the ER of Hepatocytes



Liver Disease

- In the late 1970s, work on the etiology of the plasma deficiency of alpha one is discovered to be due to blockage of release of the protein from the liver.
  - Jeppson JO, Larsson C, Eriksson S. NEJM 1975; 293: 576-9.
- Liver injury from the disease is likely due to $alpha_1$-antitrypsin accumulation in liver cells

Liver Disease

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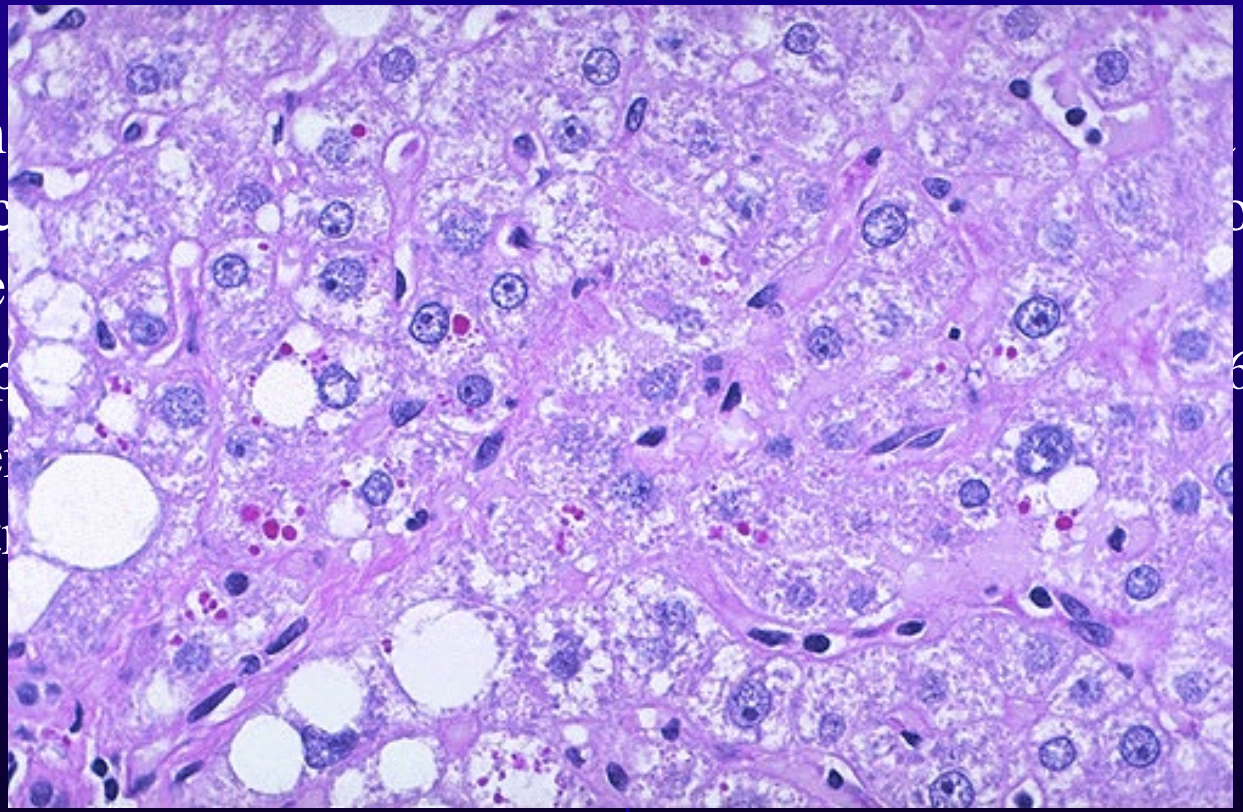

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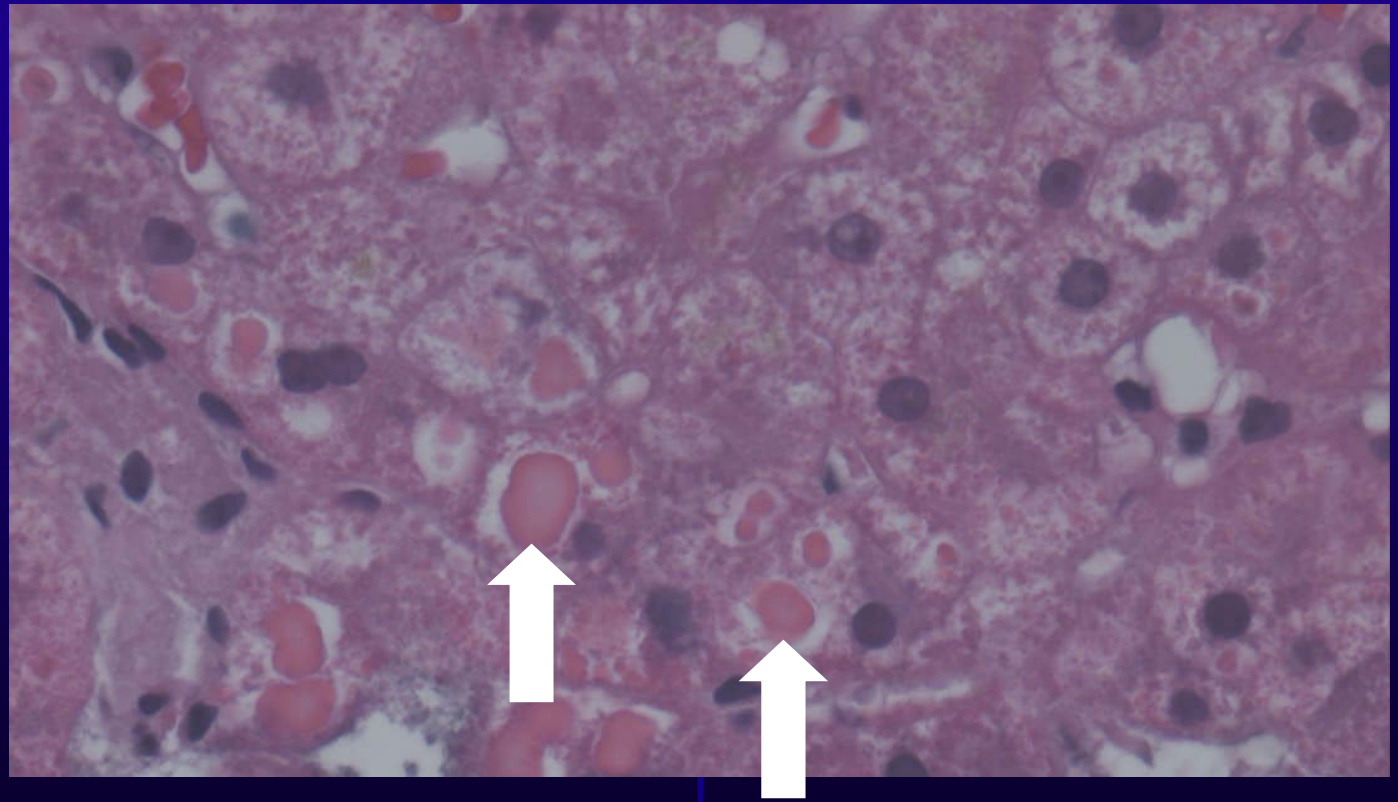
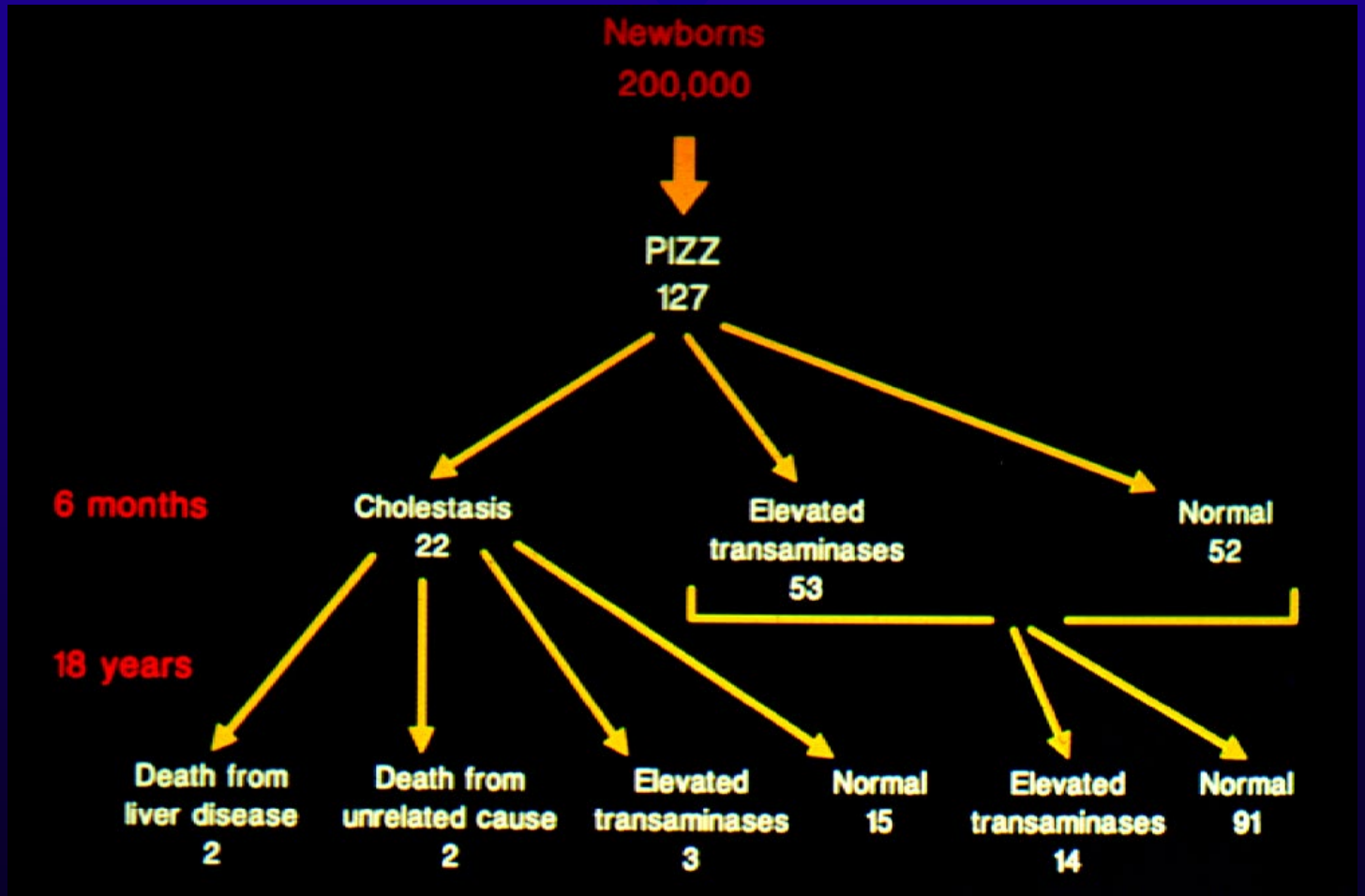
Human ZZ Liver



Build up of alpha1 Z within liver cells

Newborn Screening Study, Sweden



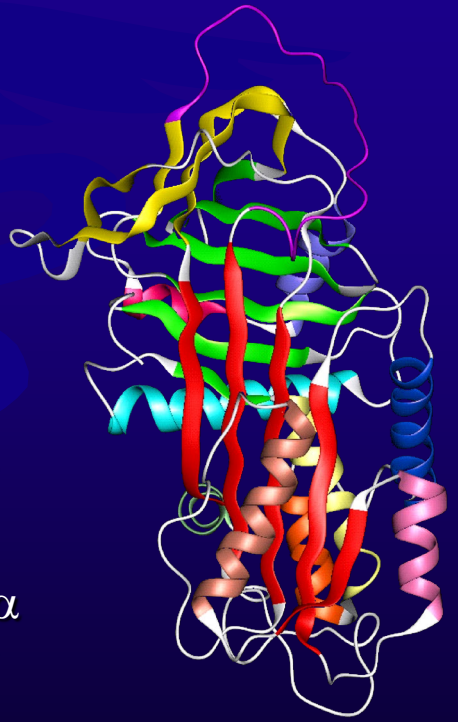
Why do AATD get Lung Disease?

- Uncontrolled proteolytic attack
- The Z mutation favors the spontaneous formation of AAT loop-sheet polymers within the lungs
  - Neutrophil Elastase can co-localize to the lung in areas of Z polymers
- The patient's own AAT that is available to protect the lungs is approximately five times less effective at inhibiting neutrophil elastase than the normal M protein variant

Barbara Lisowska-Myjak in Clinica Chimica Acta 352 (2005) 1 –13
Ravi Mahadeva et. al in American Journal of Pathology, Vol. 166, No. 2, Feb 2005
Mulgrew AT, Taggart CC, Lawless MW, et al. Z alpha1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. *Chest.* 2004;125(5):1952-1957.

More Than an Antiprotease

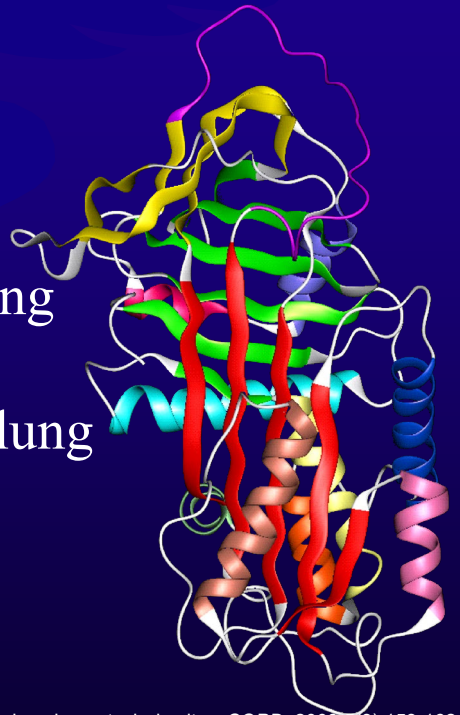
- Acute-phase reactant[1]
- Anti-inflammatory[1,2]
- Broad-spectrum antiprotease[2,3]
- Inhibits α-defensin cytotoxicity and proinflammatory properties[3]
- Antioxidant with 9 methionines[3]
- AAT is a direct inhibitor of Caspase-3[4]
- A1AT blocks cigarette smoke & thrombin-dependent activation of TNFα and MMP-12 in alveolar macrophages[5]



1. American Thoracic Society, European Respiratory Society. *Am J Respir Crit Care Med*. 2003;168:818-900.
2. Köhnlein T, Welte T. *Alpha-1 Antitrypsin Deficiency: Clinical Aspects and Management*. Bremen, Germany: UNI-MED Verlag AG; 2007.
3. Brantly M. *Am J Respir Cell Mol Biol*. 2002;27:652-654.
4. Petrache I, Fijalkowska I, Medler TR, et al. 1-Antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. Am J Pathol 2006; 169:1155≳1166
5. Churg A, Wang X, Wang RD, Meixner SC, Pryzdial ELG, Wright JL. 1-Antitrypsin Suppresses TNFa and MMP-12 Production by Cigarette Smoke Stimulated Macrophages. Am J Respir Cell Mol Biol 2007;37:144-151.

More Than an Antiprotease

- Inhibition of apoptosis of pulmonary vascular endothelial cells[1]
- Direct anti-inflammatory effects on inflammatory cells[2]
- Direct anti-inflammatory effects on lung vascular cells[3]
- Regulation of alveolar and epithelial lung fluid volume.[4]



1. Aldonyte R, Hutchinson ET, Jin B, et al. Endothelial alpha-1-antitrypsin attenuates cigarette smoke induced apoptosis in vitro. *COPD.* 2008;5(3):153-162.
2. Nita I, Hollander C, Westin U, Janciauskiene SM. Prolastin, a pharmaceutical preparation of purified human alpha1-antitrypsin, blocks endotoxin-mediated cytokine release. *Respir Res.* 2005;6:12.
3. Subramaniyam D, Virtala R, Pawlowski K, et al. TNF-alpha-induced self expression in human lung endothelial cells is inhibited by native and oxidized alpha1-antitrypsin. *Int J Biochem Cell Biol.* 2008;40(2):258-271.
4. Lazrak A, Nita I, Subramaniyam D, et al. Alpha1-Antitrypsin Inhibits Epithelial Na+ Transport in vitro and in vivo. *Am J Respir Cell Mol Biol.* 2009.

AAT and Apoptosis

- Induction of pulmonary endothelial apoptosis results in emphysema.[1]
- AAT is internalized in pulmonary endothelial cells and inhibits apoptosis.[2]
  - Uptake into cells is blocked by cigarette smoke
- AAT is a direct inhibitor of activated Caspase-3.[3]

1: Giordano, RJ. et al. J Biol Chem. 2008 Oct 24; 283 (43) 29447-60.
2: Sohrab, S. et al. FASEB J. 2009 Sep 23(9) 3149-58.
3: Petrache I., et al. Am J Pathol 2006; Oct169 (4):1155≳1166

Incidence of A_1AT

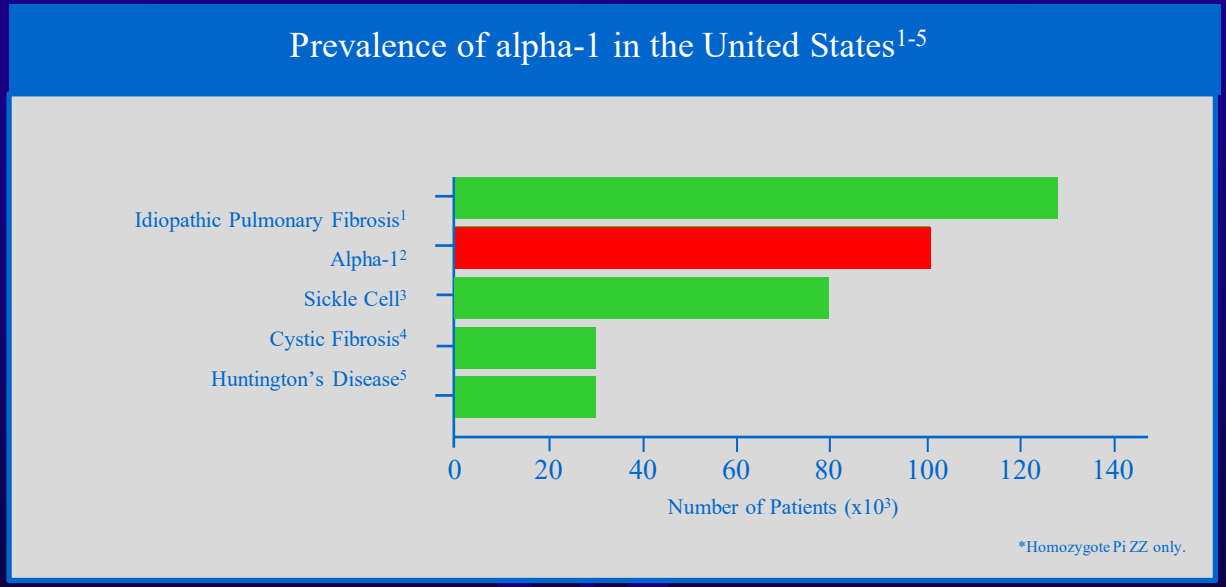
- The most prevalent potentially fatal genetic disorder of adults in the United States.
  - An estimated 19 - 25 million individuals carry one deficient gene
  - At least 100,000 Americans may have Pi*ZZ A_1ATD
    - Less than 10% diagnosed

de Serres FJ, et. al; Clin Genet 2003; 64:382-97
de Serres FJ, et. al; Chest 2002; 122: 1818-29.

Population Screening Studies

- 200,000 neonates screened in Sweden
  - Pi*ZZ was 127, or 1 in 1575.
    - Sveger T. NEJM 1976; 294: 1316-21
    - Sveger T. Acta Paediatr Scsnf 1988; 77:847-51
- 20,000 blood donors tested in St. Louis, MO.
  - Pi*ZZ was 1 in 2857
    - Silverman EK, et.al Am Rev Respir Dis 1989; 140:961-6

Genetic COPD: More Common Than Previously Thought



- Not a rare disorder, JUST RARELY TESTED FOR.[6]
  - Up to 90% of those with alpha-1 are undiagnosed[2]
  - In a registry study of more than 1000 patients, the average interval between onset of symptoms and diagnosis was 8.3 years[2]

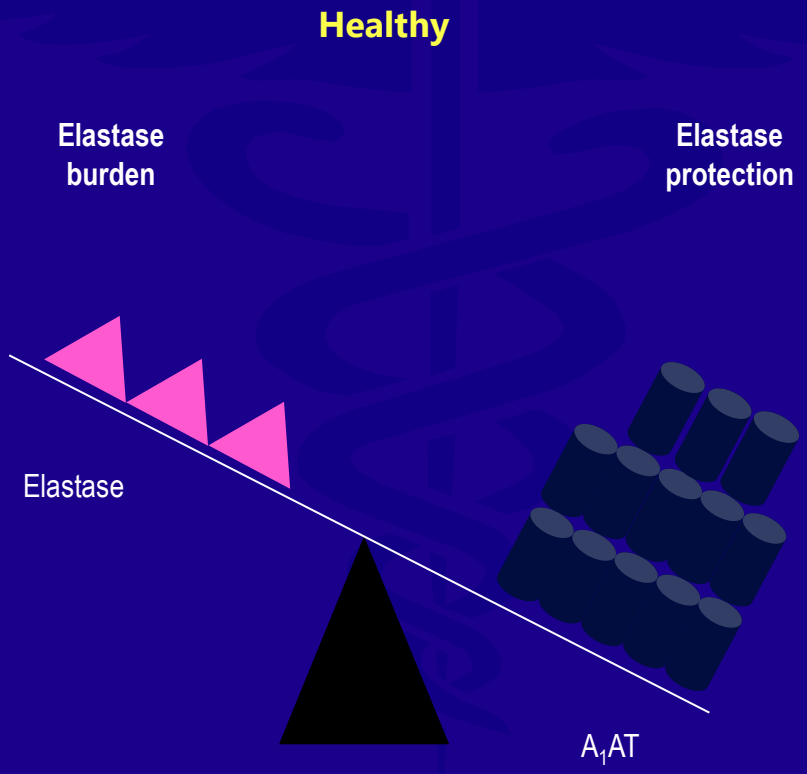
1. http://www.coalitionforpf.org/cpf_faq.php. Accessed October 4, 2011. 2. Campos MA, et al. *Chest.* 2005;128(3):1179-1186. 3. http://sicklecelldisorder.com/index.php/the-disease.html. Accessed October 4, 2011. 4. http://www.cff.org/AboutCF/Faqs/#What_is_cystic_fibrosis? Accessed October 4, 2011. 5. http://www.hdsa.org/about/our-mission/what-is-hd.html. Accessed October 4, 2011. 6. de Serres FJ. *Environ Health Perspect.* 2003;111(16):1851-1854.

Incidence Amongst "Sick" Patients

- 965 consecutive emphysema patients tested for alpha one antitrypsin deficiency.
  - 1.9% of this group were Pi*ZZ
  - 8.0% of this group were Pi*MZ
- Average age of Pi*ZZ patients was 56

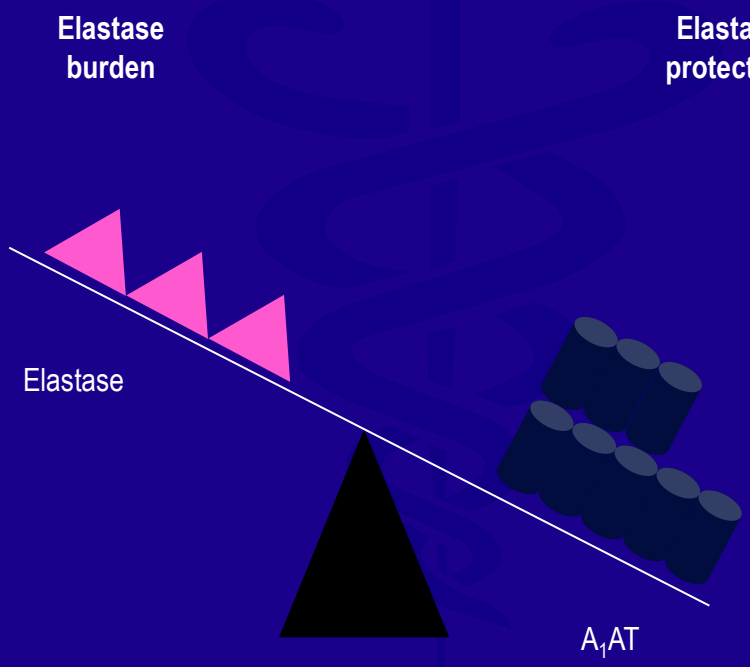
Lieberman J., et al Chest 1986; 89: 370-73.

Balance of Neutrophil Elastase & A_1AT



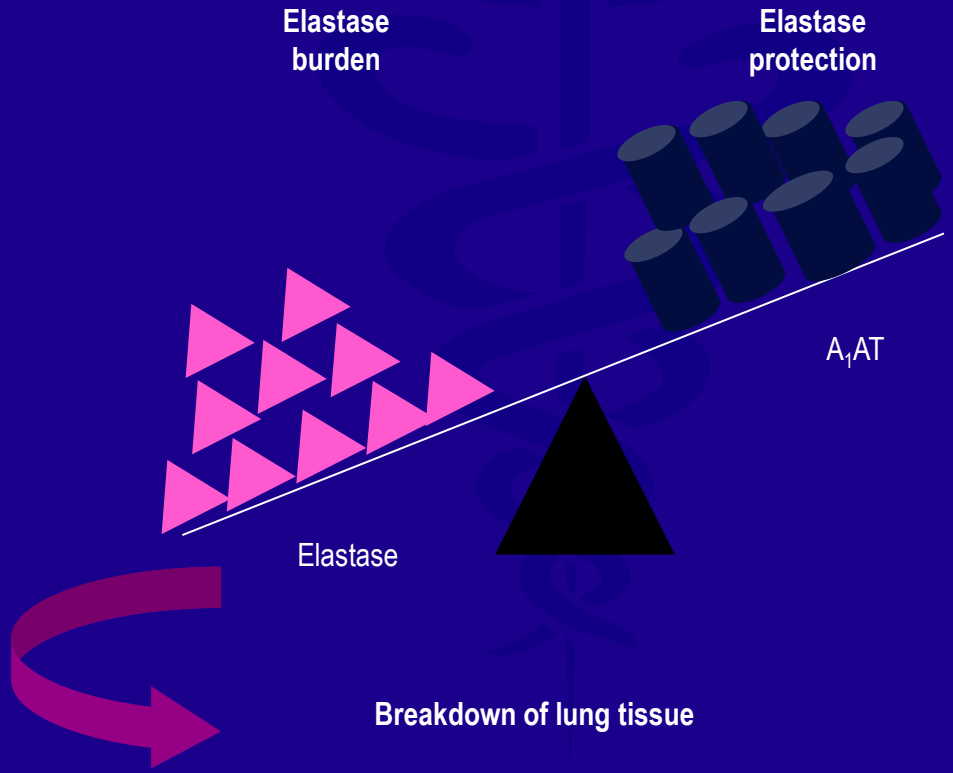
Balance of Neutrophil Elastase & A_1AT

MZ

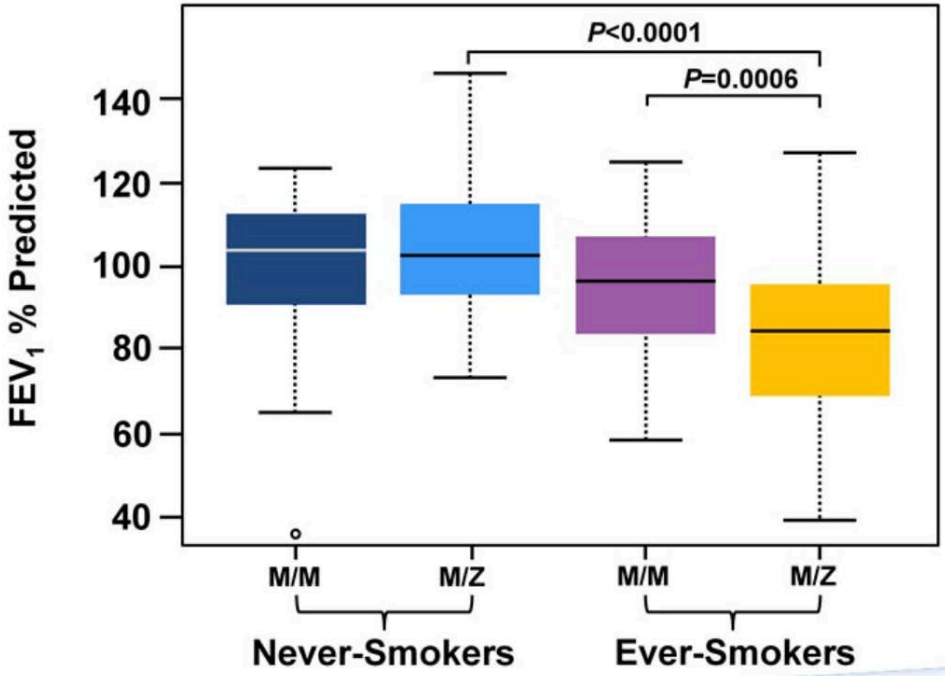


Balance of Neutrophil Elastase & A_1AT

MZ



- In M/Z individuals, FEV_1 % predicted was significantly lower in ever-smokers compared with never-smokers



Molloy K et al. *Am J Respir Crit Care Med.* 2014;189(4):419-427.

Clinical Presentation of AATD

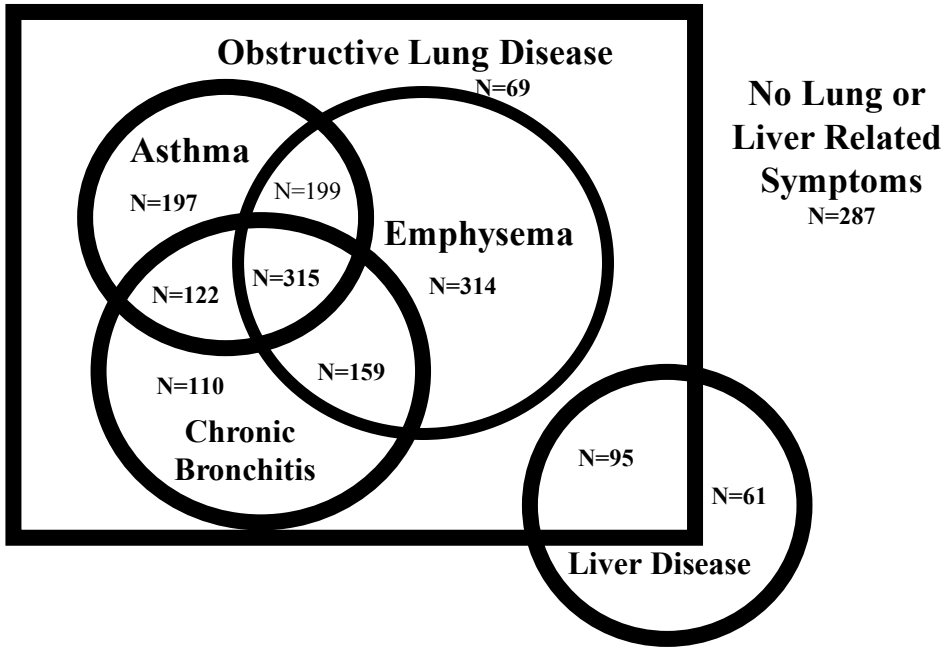
Lung Disease

- Alpha-1-related lung disease presents with common respiratory symptoms:
  - Dyspnea (84%)
  - Decreased exercise tolerance (68%)
  - Wheezing (76%)
  - Cough (42%)
  - Excess sputum production (50%)
  - Frequent lower respiratory tract infections
  - History of suspected allergies and/or asthma

Alpha-1 Association Website. www.alpha1.org/shouldTested/index.asp. Accessed January 2008.
McElvaney NG. *Chest* 1997;111:394-403.
The Alpha-1-Antitrypsin Deficiency Registry Study Group. *Am J Resp Crit Care Med*. 1998;158:49-59

Diagnoses Reported in AATD

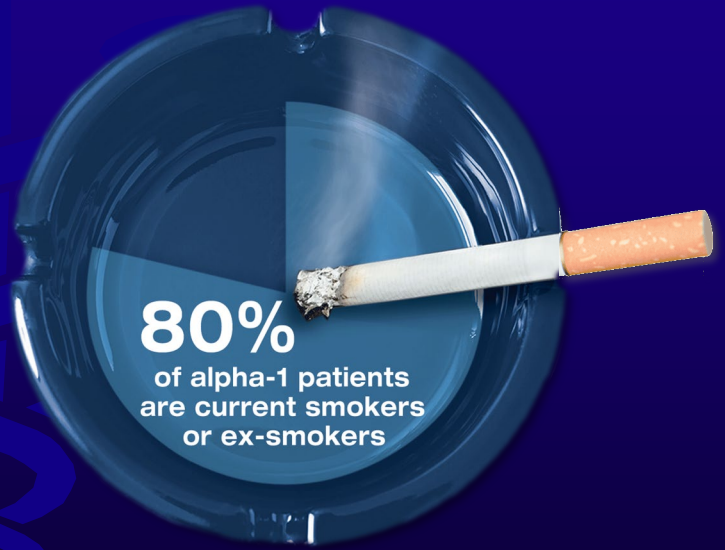
(N=1851)



Strange C, et al. Respiration 2006;73(2):185.

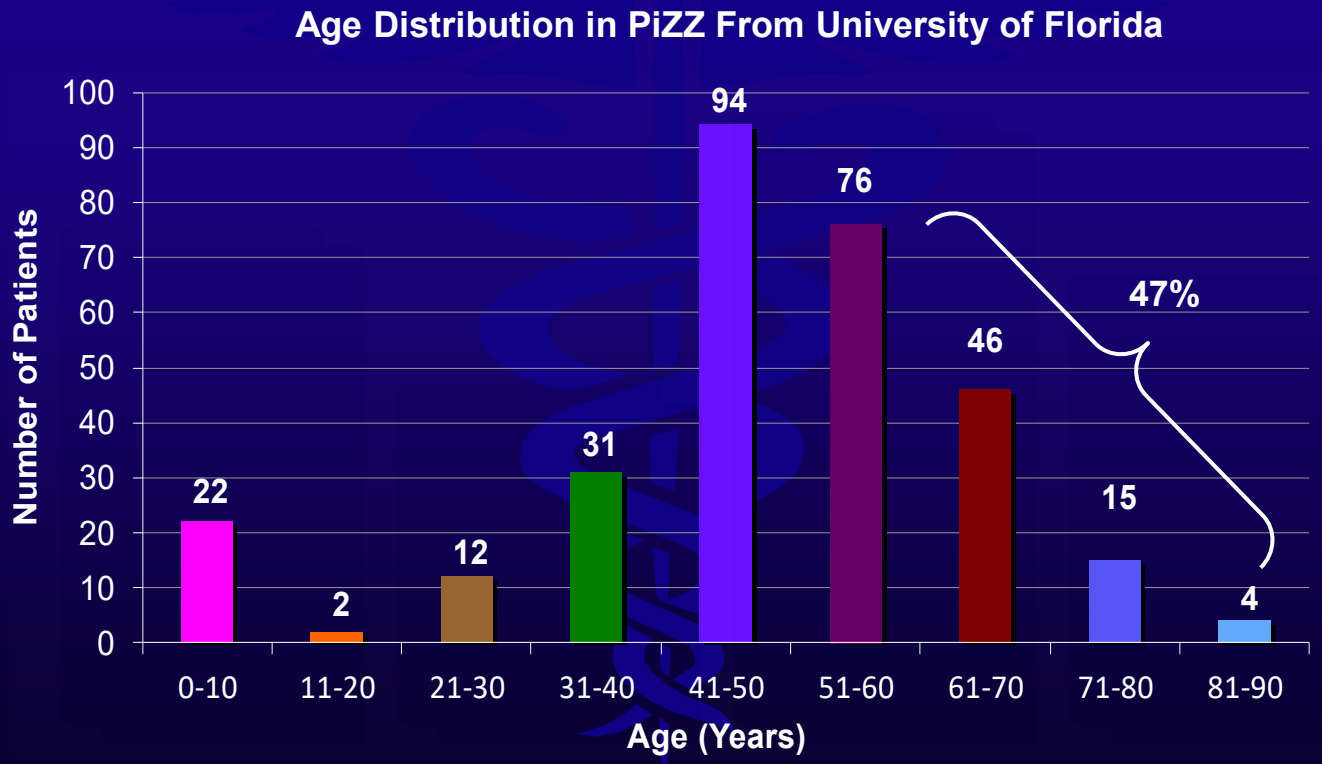
Smoking

- Smokers and ex-smokers make up a large part of the alpha-1 population
  - In a National Registry study of 1129 patients with alpha-1, 80% were either current smokers (8%) or ex-smokers (72%)[1]
  - In a separate study of 878 patients, 82.3% reported tobacco use with a pack-year history of 23.2 ± 14.5 years[2]



1. The Alpha-1-Antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med.* 1998;158(1):49-59. 2. Campos MA, et al. *COPD.* 2009;6(1):31-40.

Average Age at Diagnosis?



Based on 302 patients with PiZZ identified out of 26,520 patients tested

Slide courtesy of Mark Brantly, MD. Division of Pulmonary and Critical Care Medicine. University of Florida College of Medicine.

Treatment Options

- Standard Therapies in COPD Treatment
  - Total Smoking avoidance
  - Pulmonary Rehab
  - Bronchodilators
  - Inhaled steroids
  - Vaccination
  - Oxygen
  - Nutrition
  - Lung transplant and Liver Transplant
  - Augmentation Therapy

http://journal.copdfoundation.org/jcopdf/id/1115/The-Diagnosis-and-Management-of-Alpha-1-Antitrypsin-Deficiency-in-the-Adult

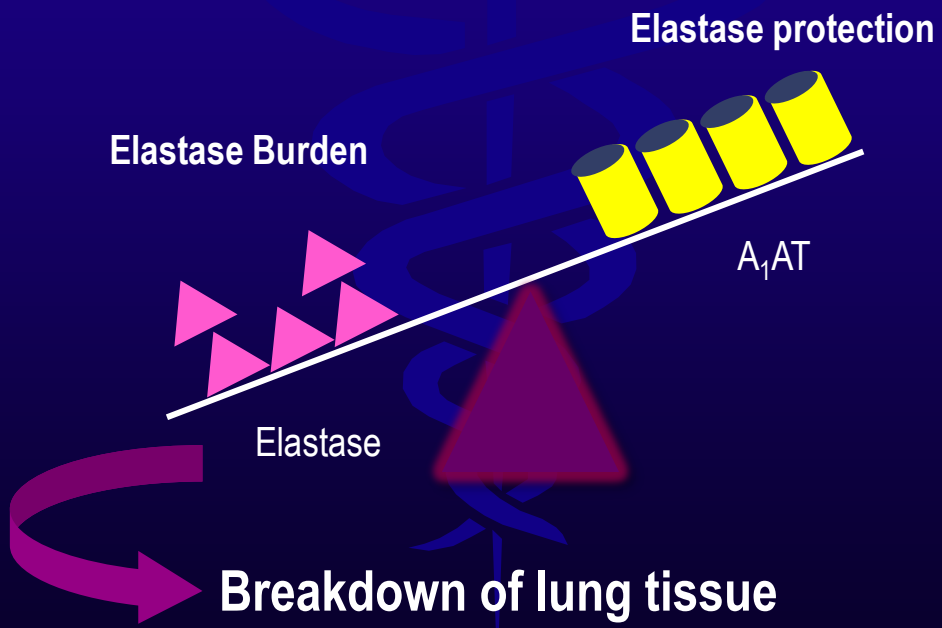
Augmentation Therapy

- Augmentation therapy is used to increase serum and lung epithelial lining fluid (ELF) levels of A_1AT
- It is the recommended treatment for adult patients with A_1ATD and evidence of air flow obstruction


www.shannonburns.com
©2002 Shannon Burns
"You're still alive?"
S.BURNS

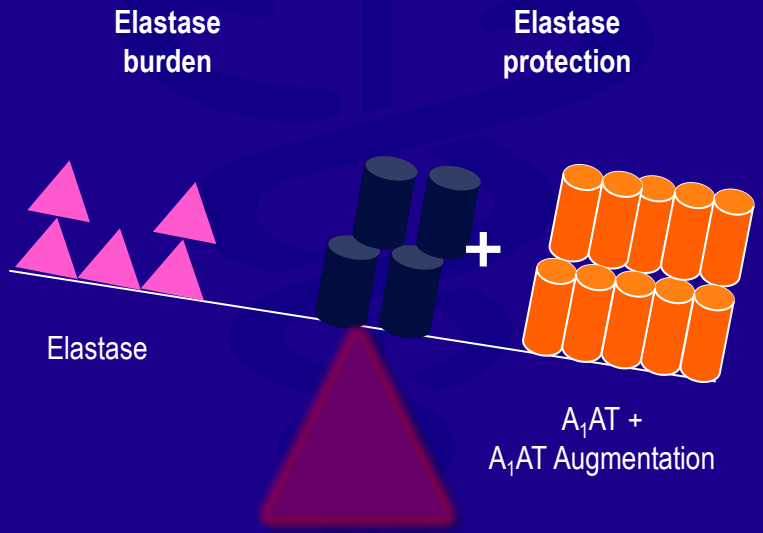
Balance of Neutrophil Elastase & A_1AT

A_1AT Deficient



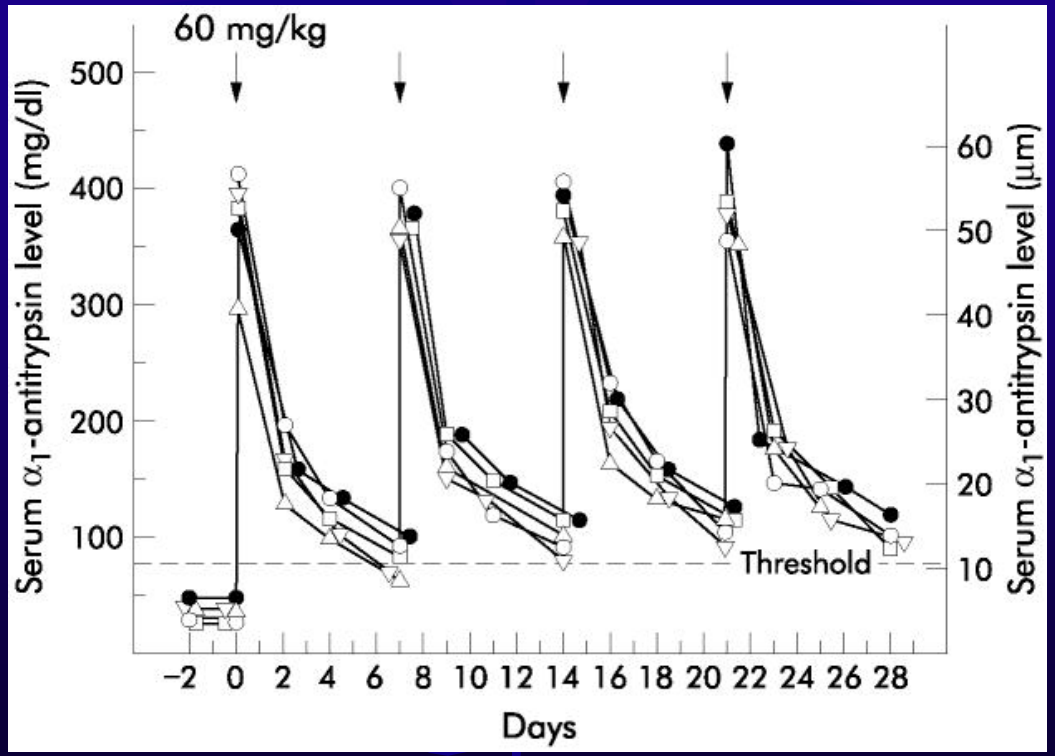
Role of Augmentation Therapy

A_1AT Augmentation Therapy



Breakdown of lung tissue

Why Augmentation Therapy?

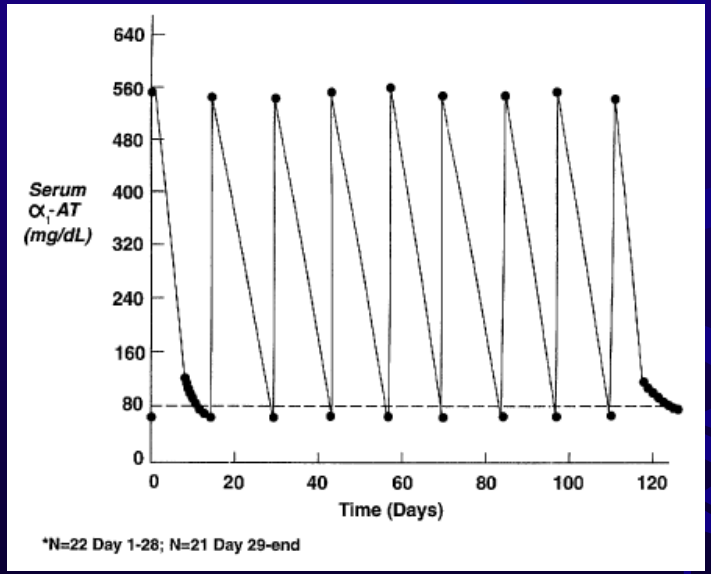


Thorax 2004;59 708–712

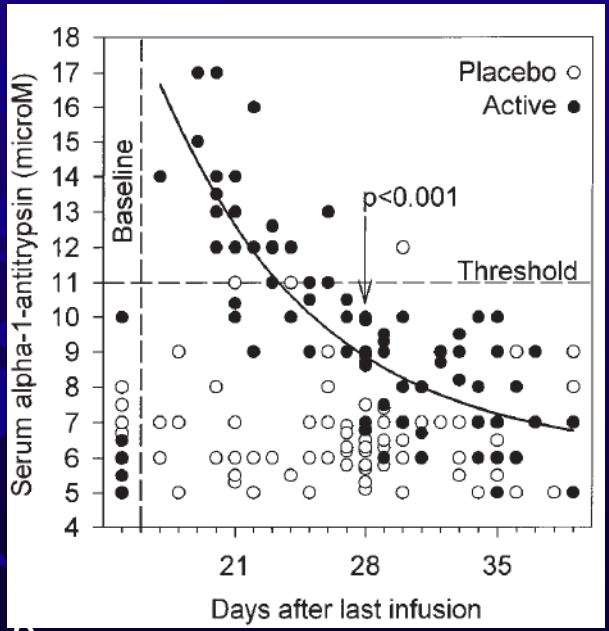
Wewers in NEJM 1987;316:1055

Importance of Weekly Dosing

AAT Serum Levels During Biweekly Infusions



AAT Serum Levels During Monthly Infusions



1. Barker AF, et al. Chest. 1997;112(4):872-874.
2. Dirksen, A et al. *Am J Respir Crit Care Med* 1999;160:1468–1472.

Augmentation Therapy:

Study	N	Design	Results
Seersholm, et al.[1]	N=295	Prospective, controlled, nonrandomized, random effect modeling study	Slower decline in lung function in treated group vs untreated group (*P*=0.02)
AAT Deficiency Registry Study Group[2]	N=927	Prospective, multicenter, nonrandomized study	Decreased mortality in patients receiving therapy (*P*=0.02); slower decline in lung function in treated patients with moderately decreased lung function (*P*=0.03)
Dirksen, et al. Danish/Dutch Study Group[3]	N=56	Double-blind, randomized, prospective study	No significant difference in FEV_1 in patients treated with AAT concentrate vs albumin
Wencker, et al.[4]	N=96	Multicenter, retrospective cohort study	Slower decline in FEV_1 during treatment period vs pretreatment period for entire group (*P*=0.019)
Chapman, et al. Canadian AIR Registry[5]	N=63	Retrospective, observational study	Slower decline in FEV_1 by 33.7 mL/year in patients receiving therapy (*P*=0.019)

1. Seersholm N, et al. *Eur Respir J*. 1997;10:2260-2263. 2. The Alpha-1-Antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med*. 1998;158:49-59. 3. Dirksen A, et al. *Am J Respir Crit Care Med*. 1999;160:1468-1472. 4. Wencker M, et al. *Chest*. 2001;119:737-744. 5. Chapman KR, et al. Poster presented at: 2005 ATS International Conference; May 20-25, 2005; San Diego, CA. Poster 210.

RAPID study

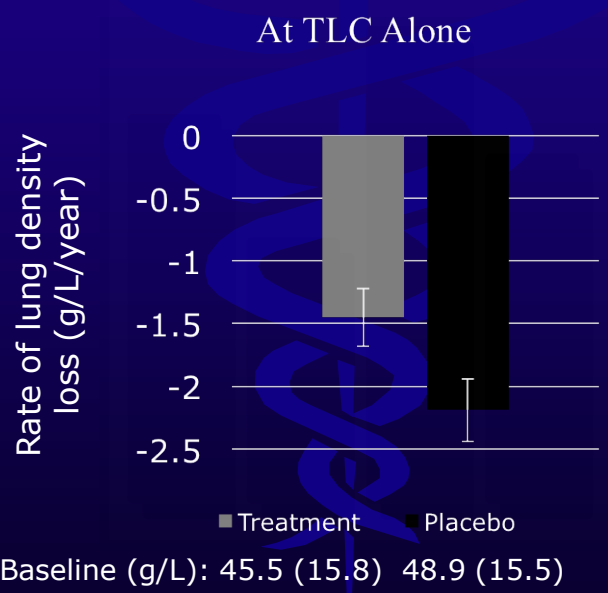
- Randomized double-blinded placebo controlled study following 180 patients over 2 years
- CT densitometry is the primary endpoint.

Chapman, et. al. The Lancet 386 (9991) July 2015. 360-268

RAPID

Randomized, double-blind, placebo-controlled study of the efficacy and safety of AAT augmentation in 180 patients over 2 years (177 patients were analyzed)

Annual Rate of Lung Density Loss



Chapman, et. al. The Lancet 386 (9991) July 2015. 360-268

Results

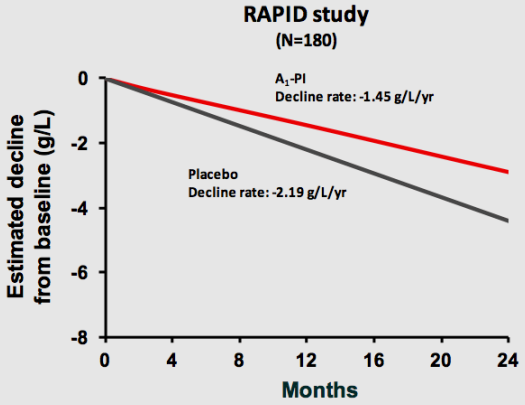
Measured at TLC, the annual rate of lung density loss was significantly less in patients in the replacement group:
(−1.45 g/L per year [SE 0.23])

Than in the placebo group (−2.19 g/L per year [0.25])

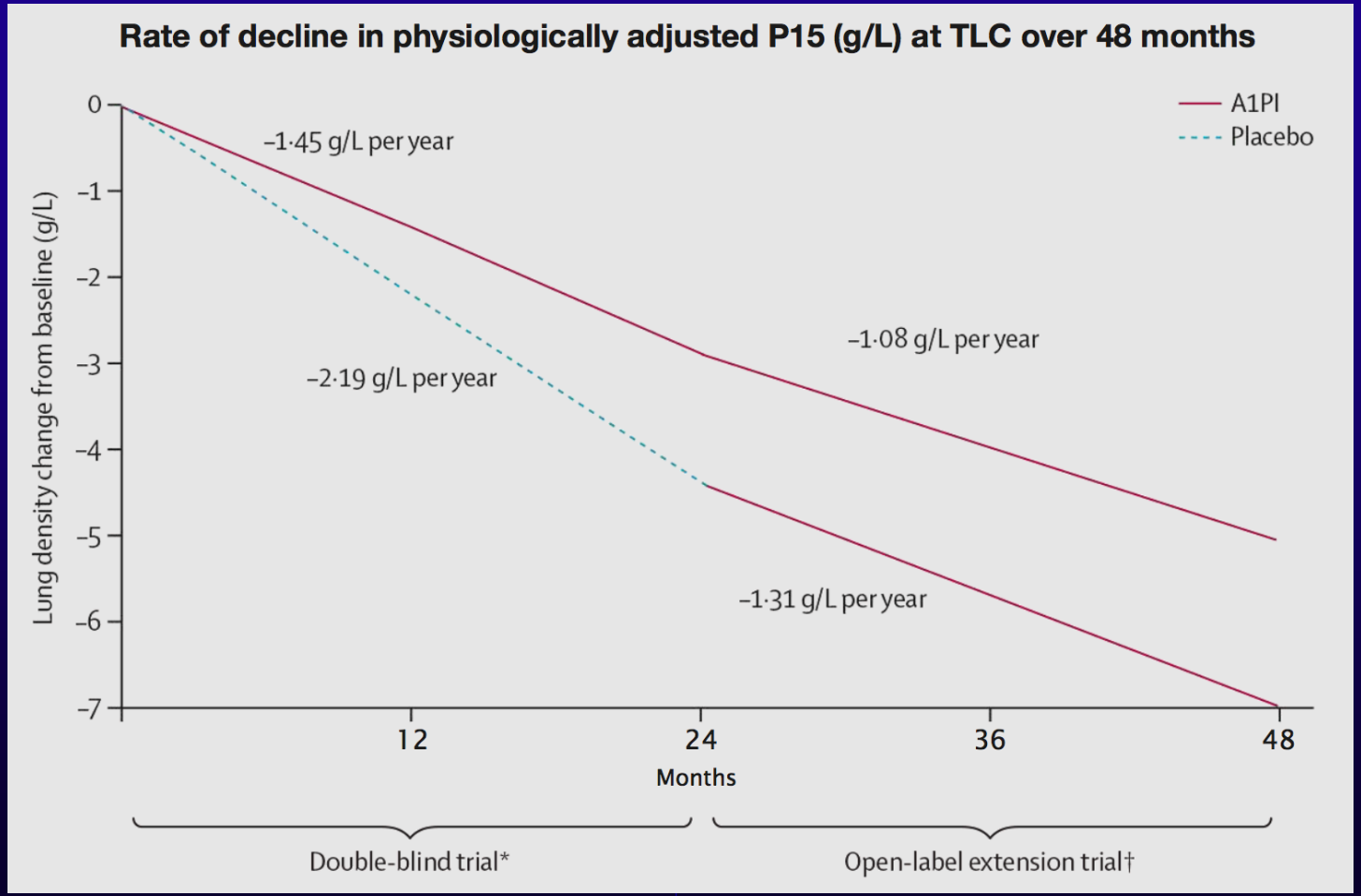
Difference 0.74 g/L per year [95% CI 0.06–1.42], p=0.03)

	Baseline Lung Density g/L (SD)	Annual Change g/L (SEM)
A_1-PI	45.5 (15.8)	-1.45 (0.23)
Placebo	48.9 (15.5)	-2.19 (0.25)
Treatment difference		0.74 g/L/yr, *P*=0.033 (2-sided test)

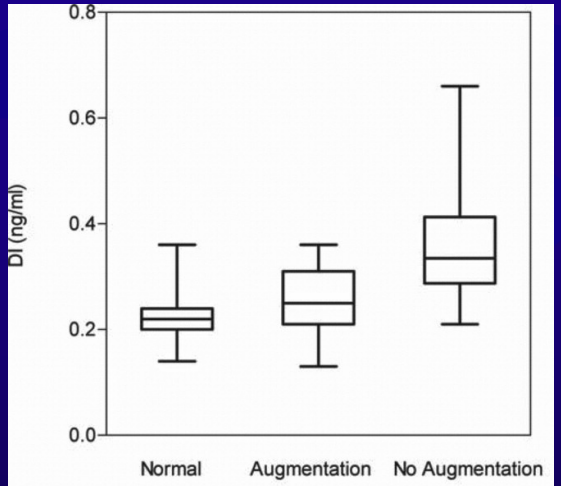
SD=standard deviation; SEM=standard error of the mean.



- A_1-PI treatment produced a statistically significant reduction in the rate of lung density decline in AATD patients compared with placebo at TLC
- 34% reduction in annual rate of lung density decline (ITT population, physiologic adjustment) at TLC


Rate of decline in physiologically adjusted P15 (g/L) at TLC over 48 months
A1PI
Placebo
–1·45 g/L per year
–2·19 g/L per year
–1·08 g/L per year
–1·31 g/L per year
Lung density change from baseline (g/L)
0
-1
-2
-3
-4
-5
-6
-7
12
24
36
48
Months
Double-blind trial*
Open-label extension trial†

Desmosine



The upper boundary of the box indicates 75th percentile and the lower boundary indicates 25th percentile. Whiskers (error bars) above and below the box indicate the maximum and minimum.

Mean ± SD of Normals 0.22 ± 0.04, n=47
Mean ± SD of Augmentation 0.25 ± 0.01, n=50
Mean ± SD of No Augmentation 0.36 ± 0.01, n=50

***t*-test**
Normals vs Augmentation $p=0.0035$
Augmentation vs No Augmentation $p<0.0001$
Normals vs No Augmentation $p<0.0001$

Shuren Ma, Yong Y. Lin, Jiangtao He, Farshid N. Rouhani, Mark Brantly & Gerard M. Turino (2013) Alpha-1 Antitrypsin Augmentation Therapy and Biomarkers of Elastin Degradation, COPD: Journal of Chronic Obstructive Pulmonary Disease, 10:4, 473-481, DOI: 10.3109/15412555.2013.771163

RAPID Study

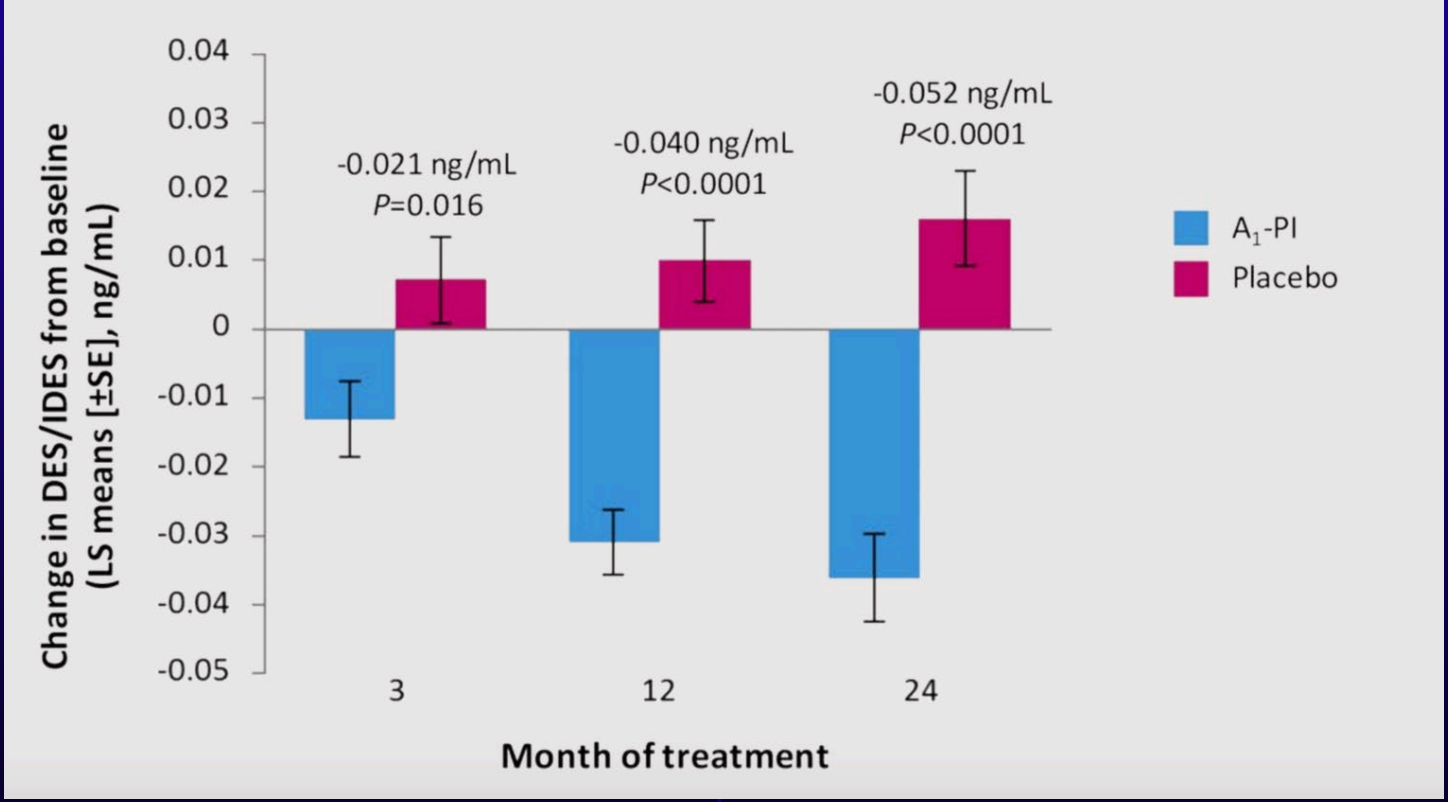
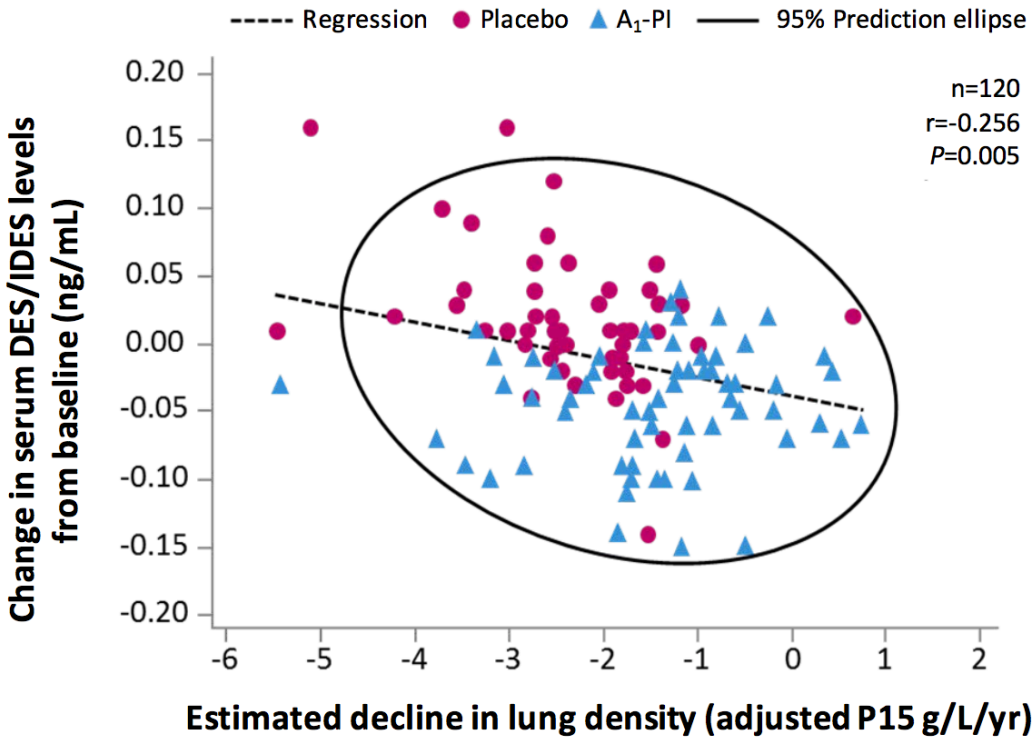

0.04
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
-0.04
-0.05
Change in DES/IDES from baseline (LS means [±SE], ng/mL)
-0.021 ng/mL
P=0.016
-0.040 ng/mL
P<0.0001
-0.052 ng/mL
P<0.0001
3
12
24
Month of treatment
A1-PI
Placebo

Figure 3.2 Scatter Plot of DES/IDES Plasma Levels Change From Baseline vs Adjusted P15 Slope (TLC) at Month 24[27]



Overview of Emerging Treatment Options

- Lung Only:
  - InhibRx recombinant AATD
  - Kamada's inhaled AATD
  - Mereo's inhibitor of neutrophil elastase

Overview of Emerging Treatment Options

- Liver Only:
  - Silencing/inhibiting RNA

Overview of Emerging Treatment Options

- Lung and Liver:
  - Vertex (814 and 864 stopped)
  - Gene therapy studies (all pre-clinical)
  - Wave Life Sciences

Paul Bolno, MD, MBA
President and Chief Executive Officer
Wave Life Sciences

Summary of AATD Presentations

WAVE®
LIFE SCIENCES

WVE-006 is a potential first- and best-in-class candidate for AATD

- AATD represents a large and growing market with significant unmet medical need
- WVE-006 is a first-in-class RNA editing candidate and the most advanced program currently in development using an oligonucleotide to harness an endogenous enzyme for editing
- Comprehensive preclinical data with WVE-006 supports potential best-in-class profile
- Advancing WVE-006 toward CTA submissions in 2023
- Editing data in AATD unlocks additional future applications of ADAR

WAVE LIFE SCIENCES

Chandra Vargeese, PhD
Chief Technology Officer,
Head of Platform Discovery Sciences
Wave Life Sciences

Future Applications of AIMers

WAVE® LIFE SCIENCES

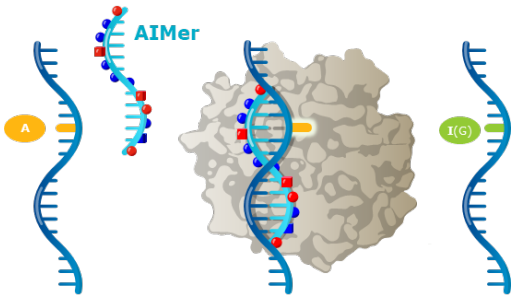
Expanding addressable disease target space using AIMers to activate pathways and upregulate expression

Correct G-to-A driver mutations with AIMers

Restore or correct protein function ✓

WVE-006
(GalNAc AIMer)
AATD

Modulate protein interactions with AIMers



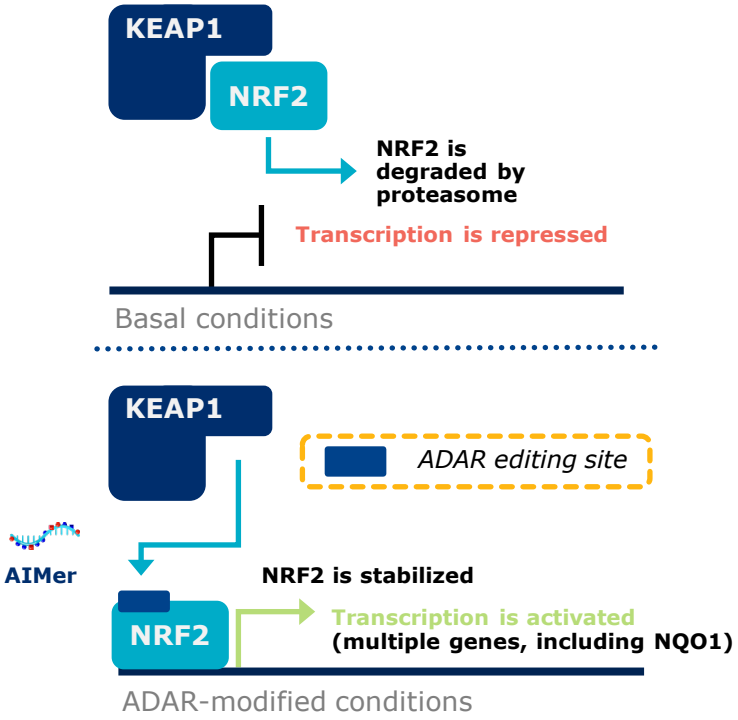
- ☑ **Modulate protein-protein interaction** — *Achieved POC*
- ☑ **Upregulate expression** — *Achieved POC*
- ☐ Modify function
- ☐ Post-translational modification
- ☐ Alter folding or processing

AIMers provide dexterity, with applications beyond precise correction of genetic mutations, including upregulation of expression, modification of protein function, or alter protein stability

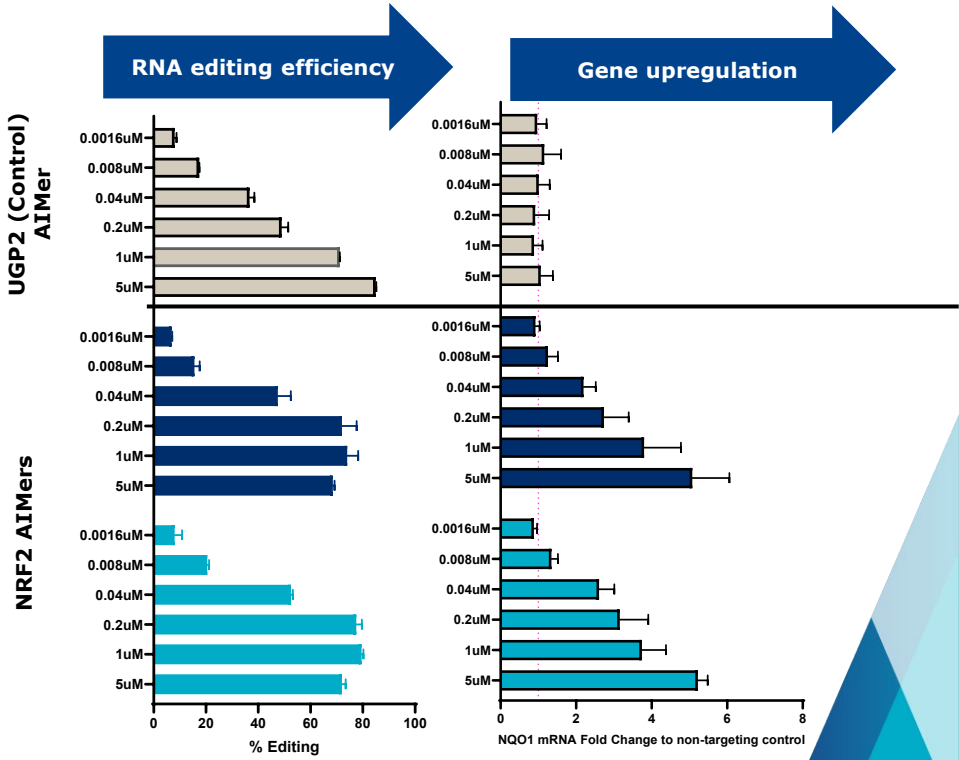
WAVE LIFE SCIENCES

POC: proof of concept

Dose dependent modulation of protein/protein interactions

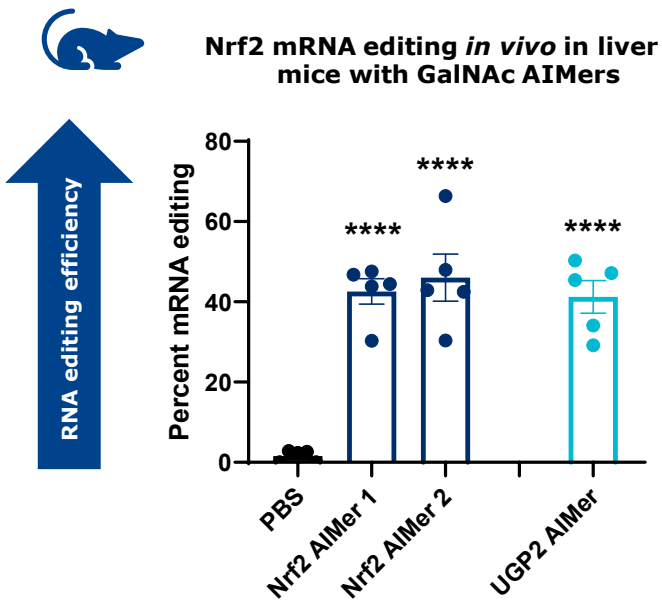


Dose-dependent gene upregulation (NQO1) *in vitro* following Nrf2 editing to disrupt protein/protein interaction

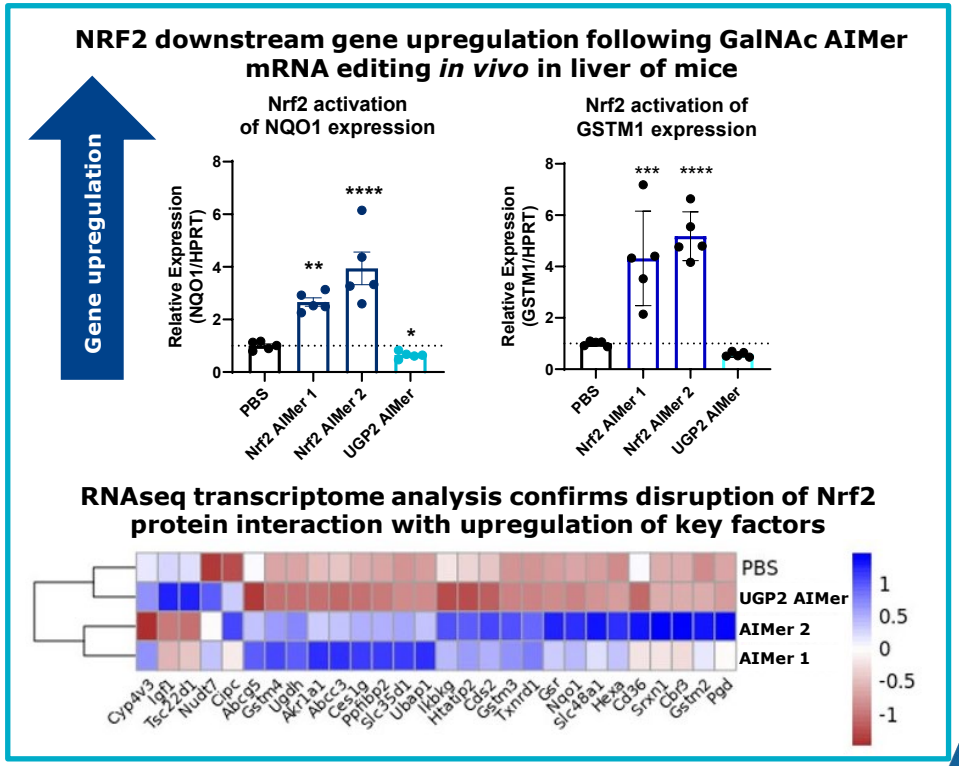


n=2; Primary hepatocytes 48h of treatment with the indicated dose concentration of AIMers

AIMers enable activation of gene pathway *in vivo* with single edit



Note: Editing percentage for UGP2 control AIMer indicates editing of UGP2 mRNA

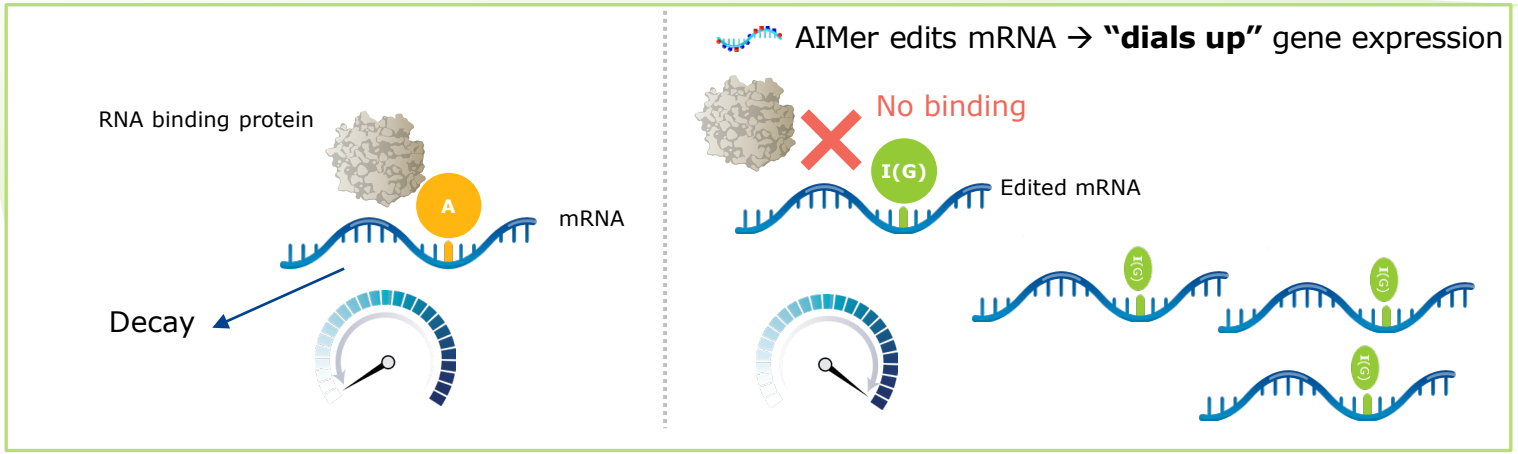


Methods: hADAR C57BL/6 mice dosed subQ (days 0, 2, 4) at 10mg/kg GalNAc-conjugated AIMers. Livers harvested (day 7), analyzed for editing and NQO1 expression via Sanger sequencing or qPCR, respectively. Data analyzed via One-way ANOVA with Tukey's multiple comparison test. Asterisks indicate statistical significance to PBS-treated animals as follows: * = p<0.05; ** = p<0.01; *** = p<0.001; **** = p<0.0001

Upregulation: AIMers can edit RNA motifs to restore or upregulate gene expression

RNA binding proteins recognize sequence motifs to regulate various mRNA properties

Stability	Transport	Processing	Protein production
• Enhance or inhibit mRNA decay	• Intracellular localization	• Splicing • Capping • PolyA usage	• Translational efficiency



WAVE LIFE SCIENCES

Large range of addressable diseases

Applicable disease

- Diseases that are expected to benefit from specific increases in gene expression
- Examples include haplo-insufficient diseases and recessive / loss-of-function diseases
  - ~2-fold upregulation would address haplo-insufficient diseases

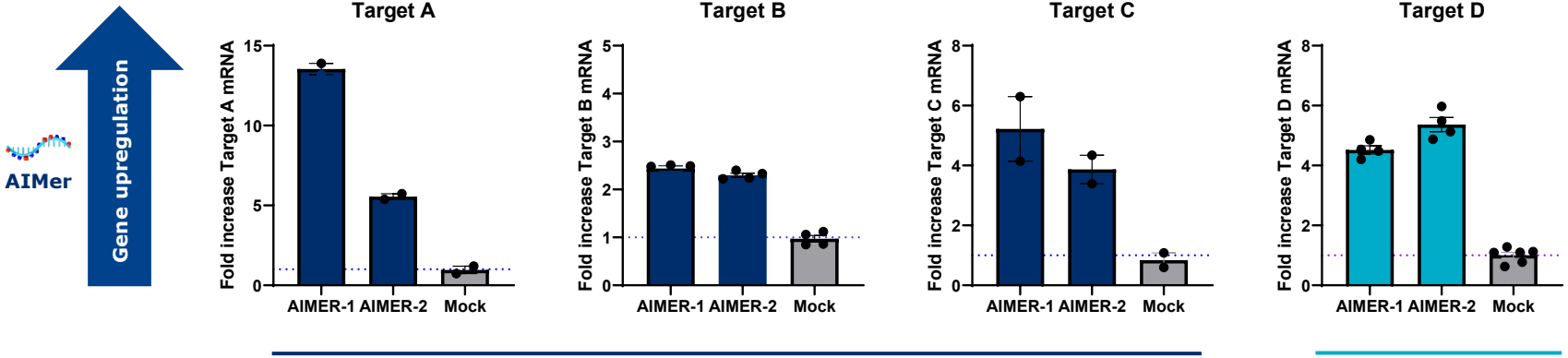
Benefit of AIMer approach

- Delivery to multiple tissue and cell types without need for complex vehicles
- Compatible with GalNAc and other potential ligands
- Titratable dosing
- Preservation of endogenous expression / regulation

WAVE LIFE SCIENCES

AIMers can edit RNA motifs to upregulate gene expression in hepatocytes and T-cells *in vitro*

Editing RNA Motifs to regulate RNA half-life to upregulate RNA expression is possible for clinically-relevant targets, including both metabolic and immune targets



Primary human hepatocytes (*in vitro*)

Primary human T-cells (*in vitro*)

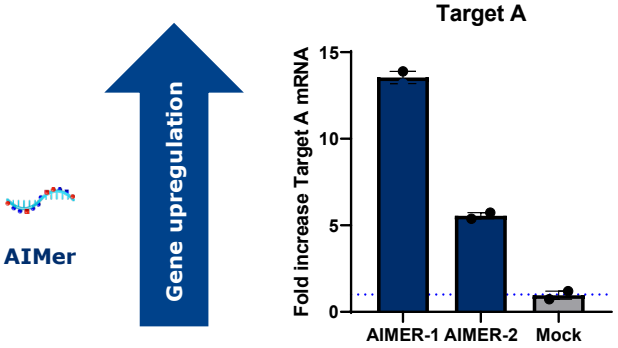
Achieving >2-fold mRNA upregulation *in vitro* across multiple different targets with AIMer editing

WAVE LIFE SCIENCES

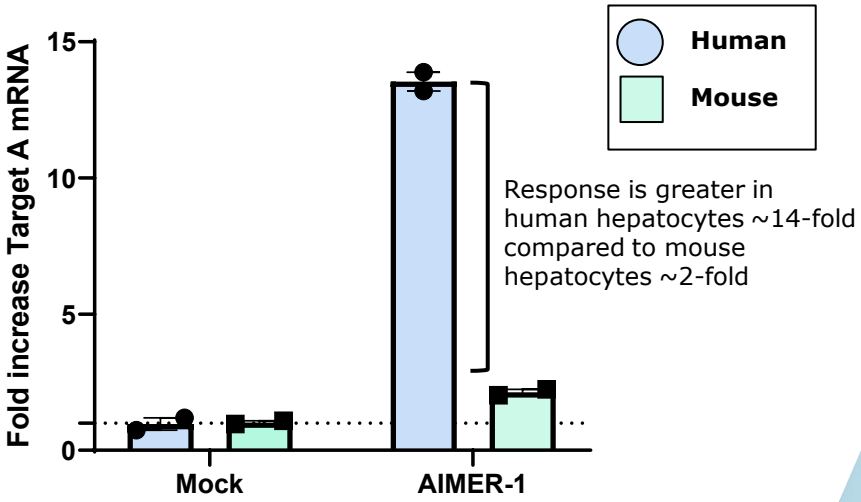
Proof-of-concept: Considerations to translate Target A upregulation results *in vivo*

Target A (undisclosed liver target)

- High unmet need with potential for multiple large indications
- Preserves endogenous protein function
- Serum protein with biomarkers of pathway activation
- Potential benefit 3-fold+ upregulation in mouse models



Mouse model may underpredict potential translation of Target A upregulation



In vitro Target A mRNA fold upregulation (gymnotic 10uM, ~48h)

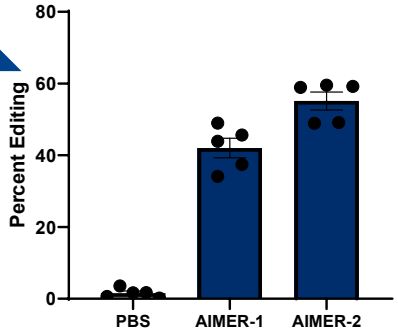
AIMers upregulate mRNA and downstream serum protein *in vivo* above anticipated threshold

Target A (undisclosed liver target)

- High unmet need with potential for multiple large indications
- Preserves endogenous protein function
- Serum protein with biomarkers of pathway activation
- Potential benefit 3-fold+ upregulation in mouse

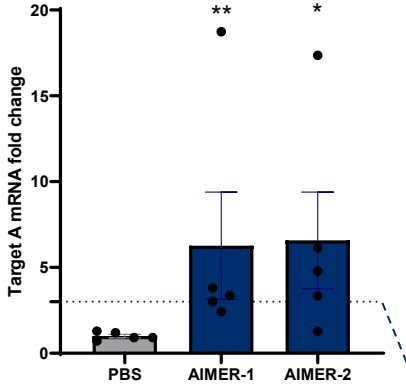
RNA editing

mRNA editing
7 days post-initial dose
GalNAc AIMer

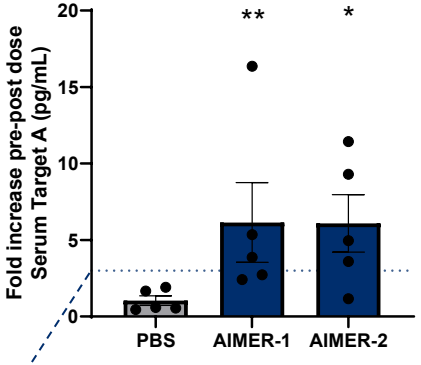


Upregulation

mRNA upregulation
7 days post-initial dose
GalNAc AIMer



Protein upregulation
7 days post-initial dose
GalNAc AIMer



Potential threshold for benefit

- ✓ *In vitro* to *in vivo* translation of mouse Target A mRNA upregulation
- ✓ *In vivo* mRNA upregulation corresponds to an upregulation of Target A protein in serum at Day 7 demonstrating proof-of-concept

hADAR mouse dosed subcutaneously 3 x 10 mg/kg GalNAc-conjugated AIMer or PBS days (0, 2, 4), taken down at day 7

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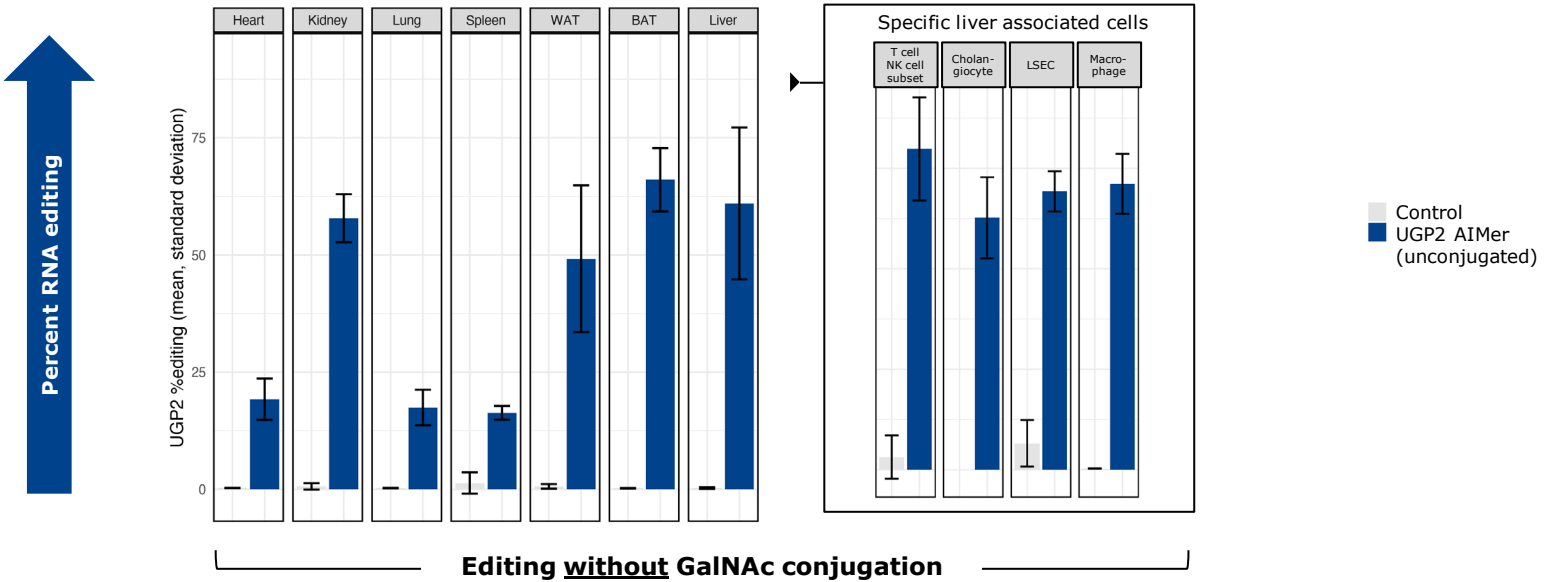
Systemic *in vivo* editing without delivery vehicles

Substantial RNA editing across multiple tissues following single subcutaneous dose of UGP2 AIMer



Full potential of addressable therapeutic areas continues to be explored, including through leveraging collaborations

Right: Single dose of 100mg/kg unconjugated UGP2 AIMer, seven days post dose; WAT: White adipose tissue; BAT: Brown adipose tissue; CD3+: T-cells and subset of NK cells; EpCAM+(Epithelial cell adhesion molecule): mainly cholangiocytes within liver; LSEC cells (Liver Sinusoidal Endothelial Cells); M0 cells: macrophages

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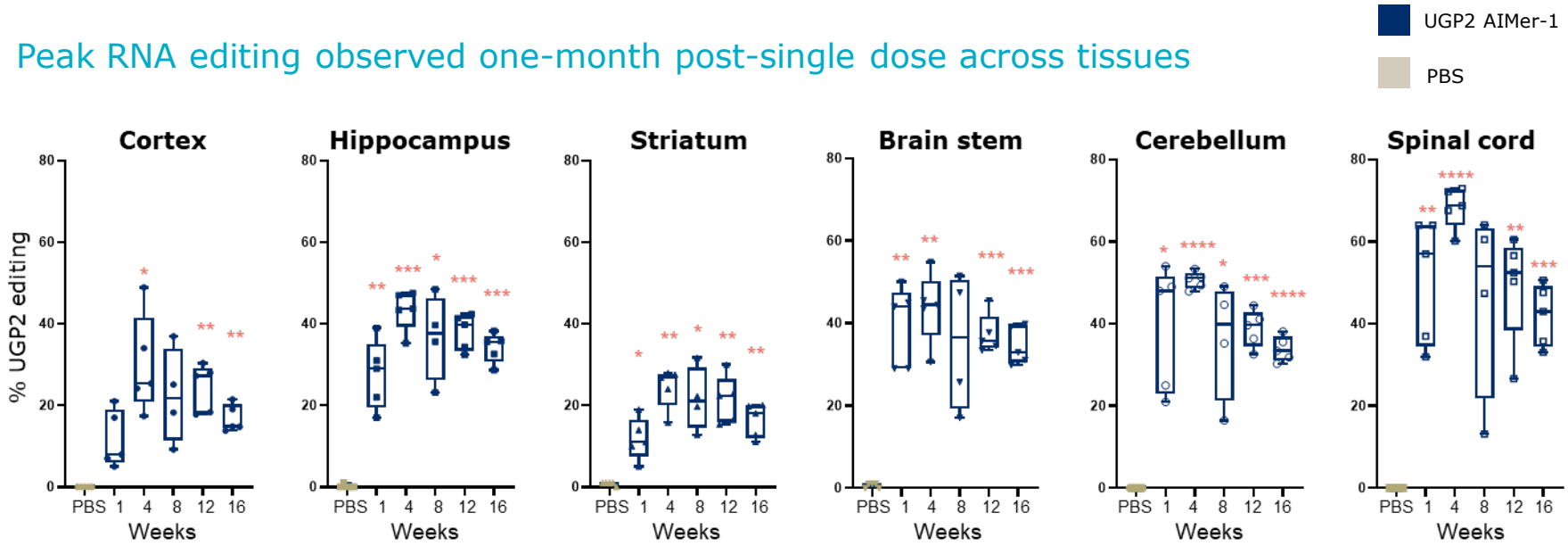
Substantial *in vivo* editing without delivery vehicles in CNS tissues

Peak RNA editing observed one-month post-single dose across tissues



Peak editing	30%	>40%	25%	>40%	50%	>65%

Potential CNS editing targets to benefit from learnings taken from clinical CNS silencing programs

Transgenic huADAR mice administered 100 μg AIMer or PBS on day 0 and evaluated for UGP2 editing across CNS tissues at 1, 4, 8, 12, and 16-weeks post dose. Percentage UGP2 editing determined by Sanger sequencing. Stats: 2-way ANOVA compared to PBS (n=5 per time point per treatment) *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ICV intracerebroventricular; PBS phosphate buffered saline

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Wave is advancing AIMers for different applications and different tissue types

- AIMers have the potential for many different applications to treat diseases beyond restoring or correcting protein function
- AIMers enable activation of gene pathway *in vivo* with single edit
- AIMers can upregulate or "dial-up" mRNA / protein production by altering stability of RNA binding proteins
- Beyond liver, preclinical *in vivo* data supports ability to edit with systemic delivery in multiple tissues and with local (IVT, ICV) delivery in CNS tissues

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Paul Bolno, MD, MBA
President and Chief Executive Officer
Wave Life Sciences

Closing remarks

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Rapidly building a best-in-class RNA editing capability led by WVE-006 for AATD

- WVE-006 is a potential first- and best-in-class RNA editing candidate for the treatment of AATD
- Planning for clinical development of WVE-006 is underway, with CTA submissions expected in 2023
- AATD is a large market opportunity with high unmet need, especially for a therapy to address both lung and liver manifestations, such as WVE-006
- Proof-of-concept with WVE-006 in clinic is expected to unlock value for future editing applications, such as upregulation of protein expression
- Actively evaluating AIMers for potential disease targets across a range of tissue types, including CNS

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Q&A

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Realizing a brighter future for people affected by genetic diseases

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