Wave Life Sciences R&D Day

September 28, 2023

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Forward-looking statements

This document contains forward-looking statements. All statements other than statements of historical facts contained in this document, including statements regarding possible or assumed future results of operations, preclinical and clinical studies, business strategies, research and development plans, collaborations and partnerships, regulatory activities and timing thereof, competitive position, potential growth opportunities, use of proceeds and the effects of competition are forward-looking statements. These statements involve known and unknown risks, uncertainties and other important factors that may cause the actual results, performance or achievements of Wave Life Sciences Ltd. (the "Company") to be materially different from any future results, performance or achievements expressed or implied by the forward-looking statements. In some cases, you can identify forward-looking statements by terms such as "may," "will," "should," "expect," "plan," "aim," "anticipate," "could," "intend," "target," "project," "contemplate," "believe," "estimate," "predict," "potential" or "continue" or the negative of these terms or other similar expressions. The forward-looking statements in this presentation are only predictions. The Company has based these forward-looking statements largely on its current expectations and projections about future events and financial trends that it believes may affect the Company's business, financial condition and results of operations. These forward-looking statements speak only as of the date of this presentation and are subject to a number of risks, uncertainties and assumptions, including those listed under Risk Factors in the Company's Form 10-K and other filings with the SEC, some of which cannot be predicted or quantified and some of which are beyond the Company's control. The events and circumstances reflected in the Company's forward-looking statements may not be achieved or occur, and actual results could differ materially from those projected in the forward-looking statements. Moreover, the Company operates in a dynamic industry and economy. New risk factors and uncertainties may emerge from time to time, and it is not possible for management to predict all risk factors and uncertainties that the Company may face. Except as required by applicable law, the Company does not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise.

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Agenda

PRESENTATION	SPEAKER
Welcome & Introduction	**Kate Rausch** Vice President, Investor Relations & Corporate Affairs
Wave Evolution and Growth Drivers	**Paul Bolno, MD, MBA** President and Chief Executive Officer
GSK Perspectives: An Inflection Point with RNA Medicines	**Tony Wood, PhD** Chief Scientific Officer, GSK
	Carolyn Buser-Doepner, PhD Vice President, Novel Human Genetics Research Unit, GSK
RNAi: INHBE and Beyond	**Chandra Vargeese, PhD** Chief Technology Officer
AIMers: Editing to Upregulate	**Chandra Vargeese, PhD** Chief Technology Officer
Mapping the "Edit-verse"	**Kenneth Longo, PhD** Vice President, Data Science
Mining the "Edit-verse"	**Ginnie Yang, PhD** Senior Vice President, Translational Medicine
Closing Remarks	**Paul Bolno, MD, MBA** President and Chief Executive Officer
Q&A	**Wave Leadership Team**

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Wave Evolution and Growth Drivers

Paul Bolno, MD, MBA
President and CEO

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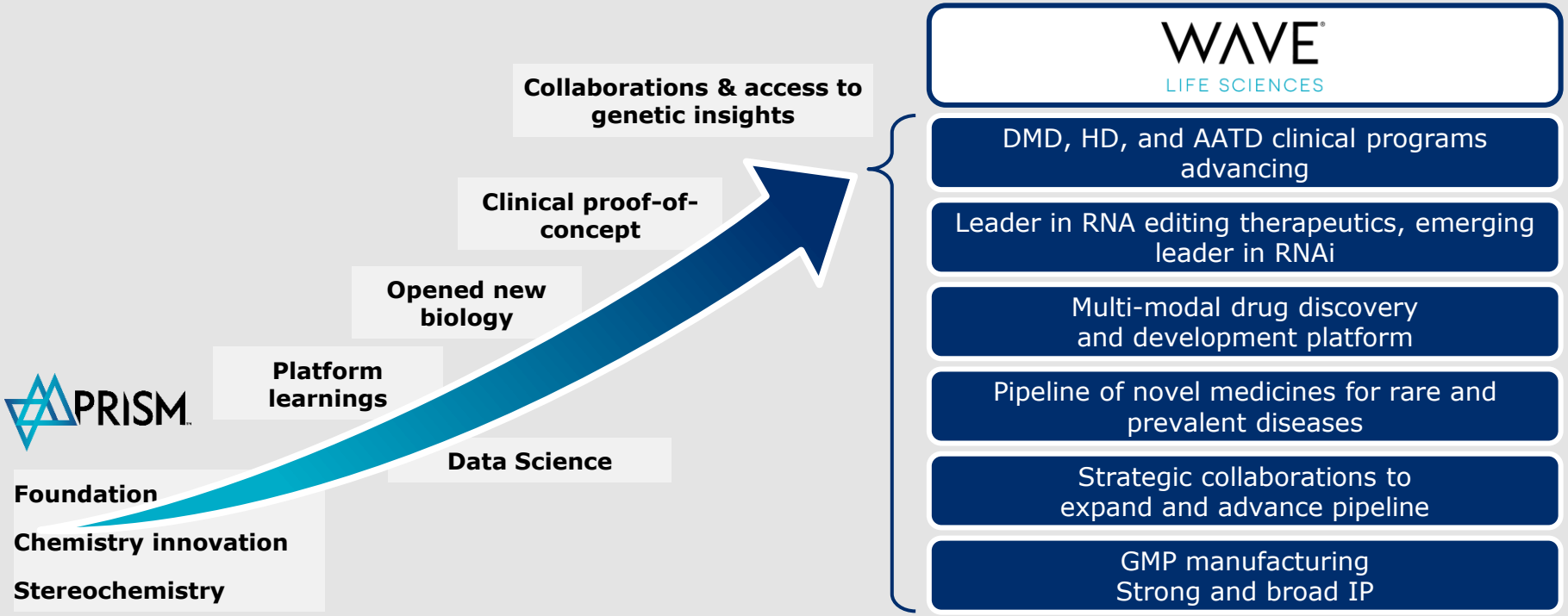
Wave today is well positioned for significant and sustained growth



Data from DMD and HD programs demonstrate clinical translation of Wave's platform chemistry

DMD – WVE-N531	High tissue concentrations and highest reported exon-skipping with three biweekly doses
HD – WVE-003	Most advanced allele-selective approach, with reductions in mHTT, preservation of wtHTT with single dose
AATD – WVE-006	Clinical development initiated on the first RNA editing medicine

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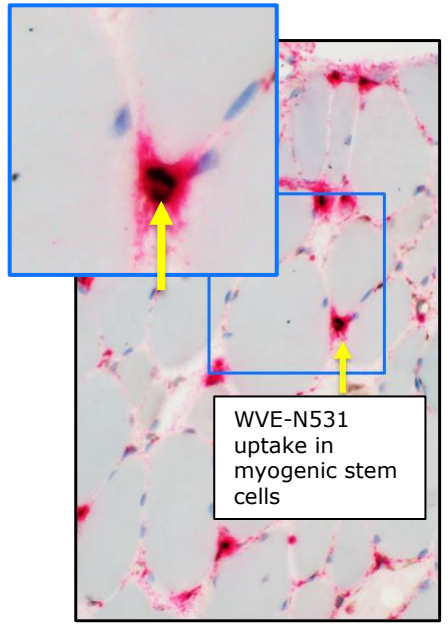
WVE-N531 Part A data in DMD: High exon-skipping & muscle concentrations after three bi-weekly doses

	suvodirsen	WVE-N531
Mean muscle concentration	0.7 µg/g	42 µg/g
Mean exon skipping	Not detectable	53%
Half-life in plasma	18 hours	25 days
Dose	22 weekly doses of 5 mg/kg	3 biweekly doses of 10 mg/kg

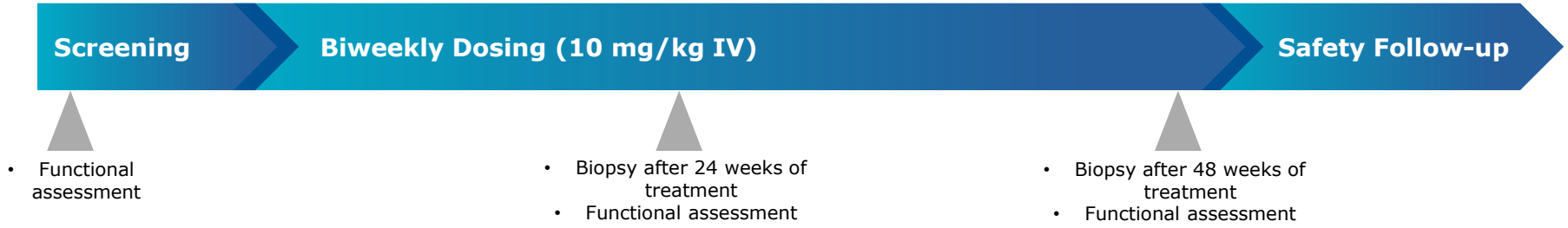
WVE-N531 uptake in myocyte stem cells



Important for potential muscle regeneration

WVE-N531 data presented March 22, 2023 at Muscular Dystrophy Association Clinical and Scientific Conference; WVE-N531 biopsies collected ~2 weeks post-last dose (3 biweekly doses of 10 mg/kg) 42 µg/g = 6.1 µM; Suvodirsen biopsies collected post-last dose (weekly doses of 5 mg/kg) on week 22; Half-life as indicated by PK analysis; suvodirsen: discontinued first-generation non-PN chemistry compound; Right: Dual staining utilizing in-situ hybridization for WVE-N531 and PAX7 immunohistochemistry for stem cells

FORWARD-53, a potentially registrational Phase 2 clinical trial of WVE-N531 in DMD (Exon 53)



- **Design of FORWARD-53**: Phase 2, open-label, 10 mg/kg every other week, up to 10 patients
- **Endpoints**: Dystrophin (powered for >5% of normal), safety/tolerability, pharmacokinetics, functional assessments (incl. NSAA and others)
- **Biopsies**:
  - After 24 weeks of treatment
  - After 48 weeks of treatment

FORWARD-53

Data from FORWARD-53 expected in 2024

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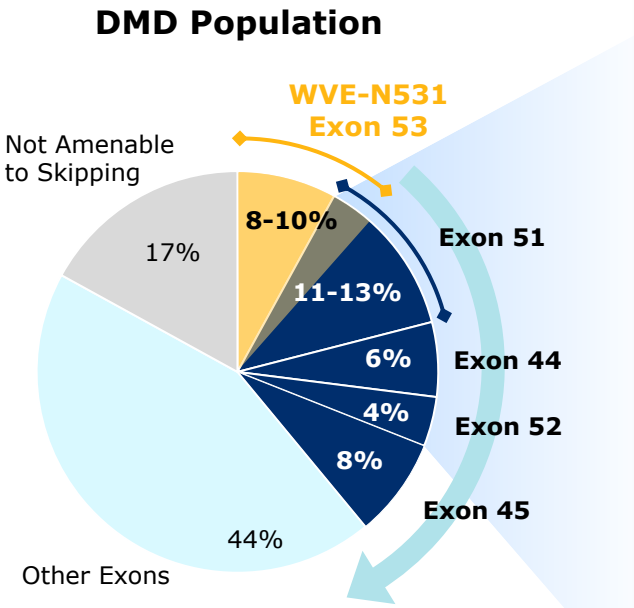
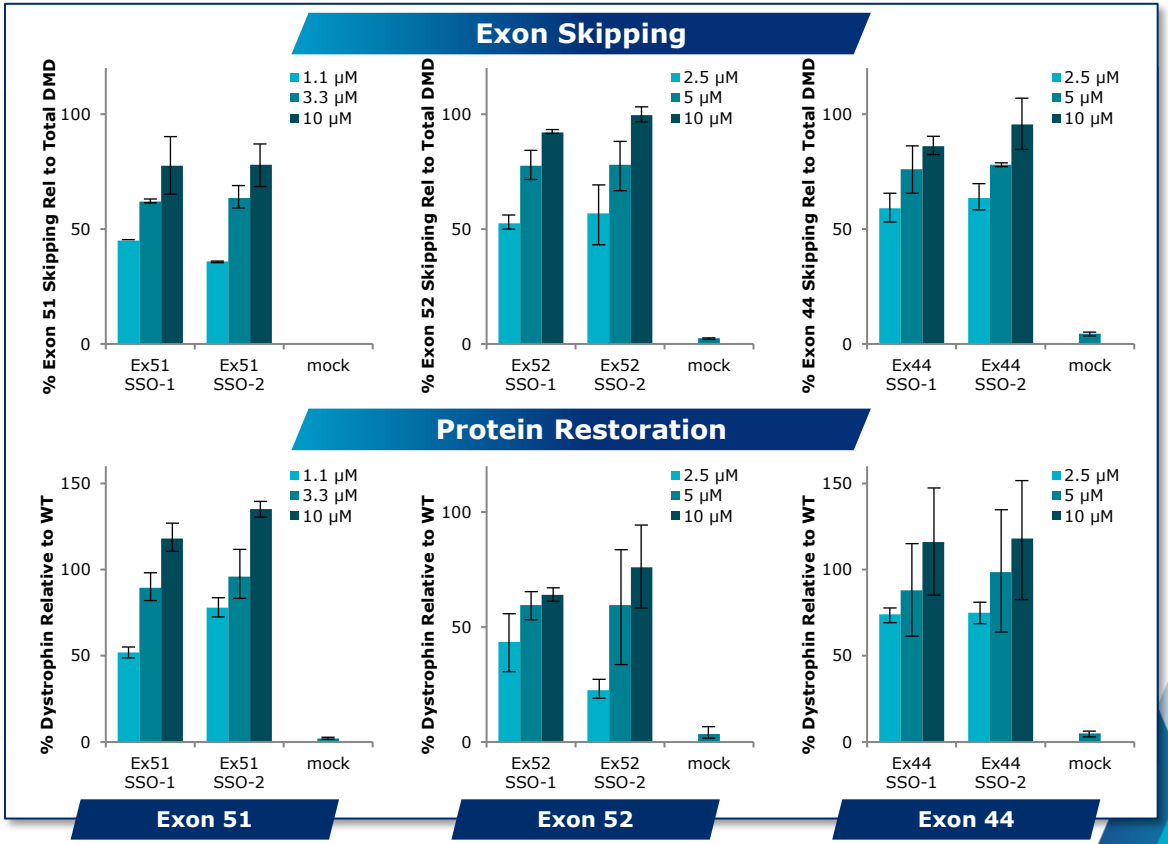
IV: intravenous; NSAA: North star ambulatory assessment

Potential for Wave to address up to ~40% of DMD population



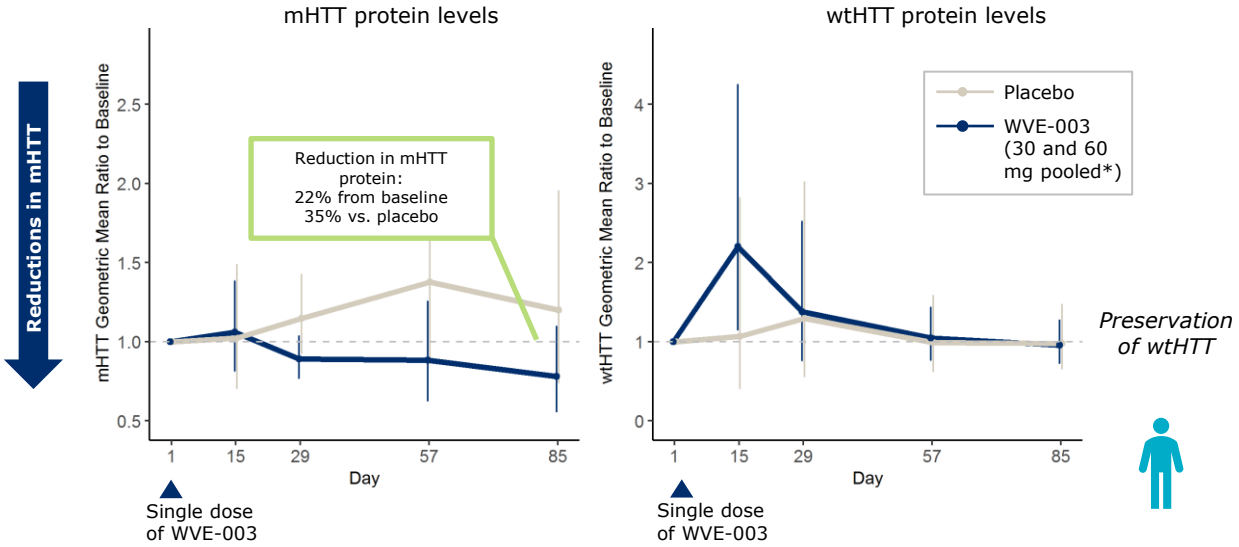
Exon skipping and dystrophin restoration demonstrated *in vitro*



Left: Aartsma-Rus, *et al.* 2009 *Hum Mutat* 30, 293.

WVE-003 in HD: Reductions in mHTT and preservation of wtHTT in single dose cohorts; multidose underway

Reductions in mean CSF mHTT and preservation of wtHTT observed in pooled analysis of single dose cohorts in SELECT-HD clinical study



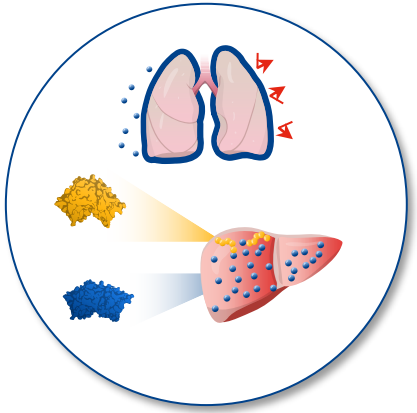
Additional single-dose and available multi-dose data expected in 2H 2023
Complete multi-dose data from first cohort with extended follow-up expected 2Q 2024

mHTT: mutant huntingtin protein; wtHTT: wild-type huntingtin protein
*Pooled considering no apparent dose response between 2 cohorts; Data cut-off: August 29, 2022

Preclinical data support WVE-006 as comprehensive approach to address AATD-related lung and liver disease

Achieved key treatment goals with preclinical *in vitro* and *in vivo* datasets:

- ✓ **Significant increase in serum AAT of up to 30 uM in NSG-PiZ mice**
  - ~50% editing supports restoration to MZ phenotype
- ✓ **Restored wild-type M-AAT protein**
  - ~50% of AAT protein in serum is wild-type M-AAT
- ✓ **Editing is highly specific**
  - No bystander edits
- ✓ **Functionality of M-AAT protein**
  - >3-fold improvement in neutrophil elastase inhibition activity
- ✓ **Improvement in liver phenotype**
  - Decreased lobular inflammation and PAS-D globule size, prevents increase in hepatocyte turnover



200,000 Pi*ZZ patients in US and Europe

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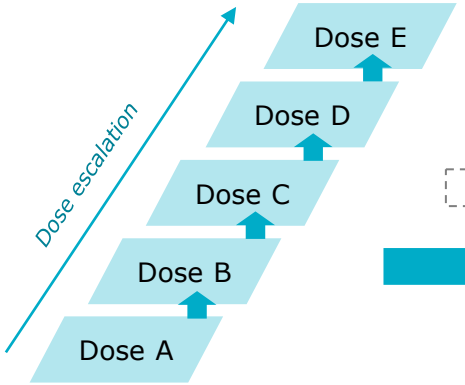
Proof of mechanism data in patients with AATD expected in 2024

RestorAATion-1: Healthy Volunteers → *Informs dose & dose frequency* → **RestorAATion-2: AATD Patients**

SAD → MAD cohorts



Expect to initiate dosing in 4Q 2023

Up to 7 doses

- **High dose**
- **Medium dose**
- **Low dose**

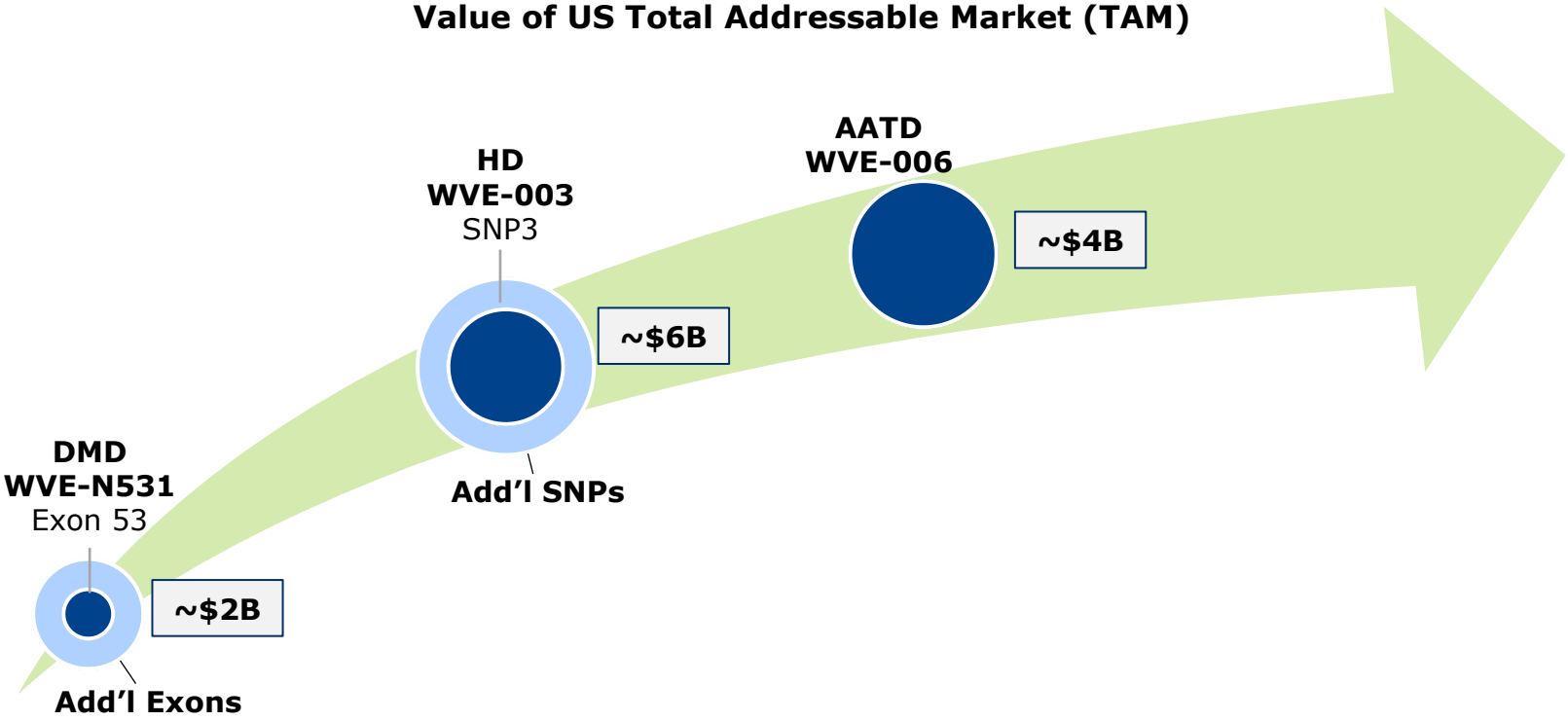
Multiple assessments of serum AAT throughout cohort

Study key objectives

- Safety and tolerability
- Pharmacokinetics
- Serum M-AAT levels

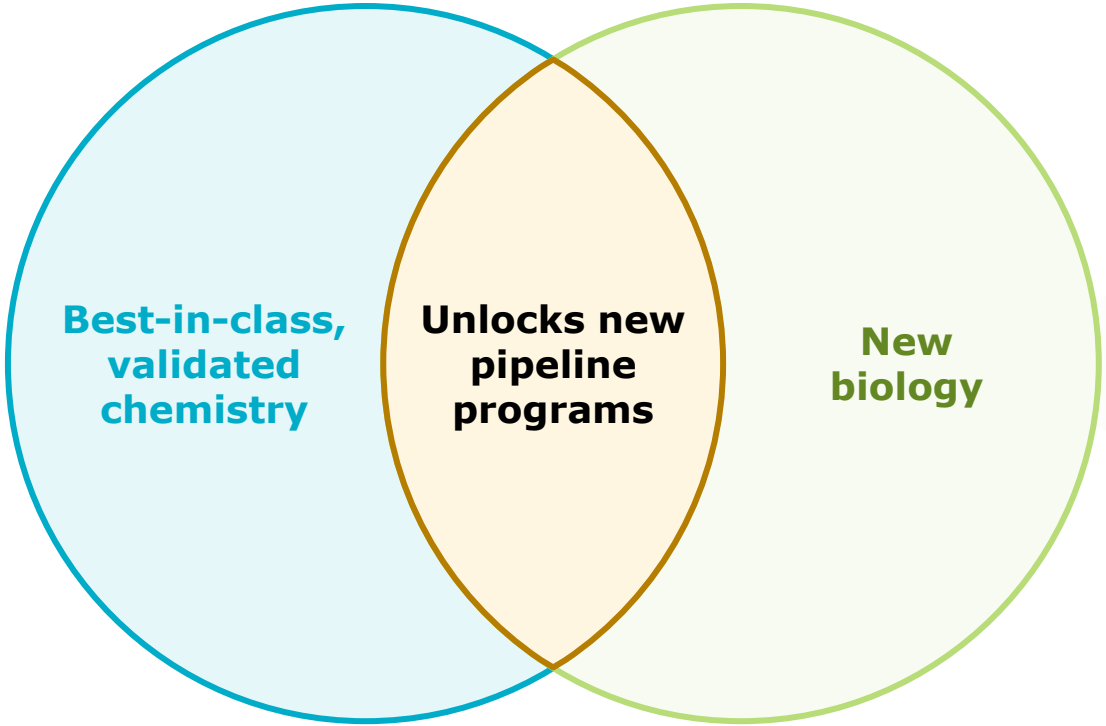
HV: healthy volunteer; SAD: single-ascending dose; MAD: multi-ascending dose

Wave's current clinical programs represent significant market opportunity



Note: Bubble size illustrative of size of total addressable US market (assuming 100% share of addressable patients)

Combining novel biology with validated, best-in-class chemistry to open opportunities for first-in-class medicines



- Accessing new endogenous enzymes for novel modalities (RNA editing)
- Opening up new targets, including prevalent diseases

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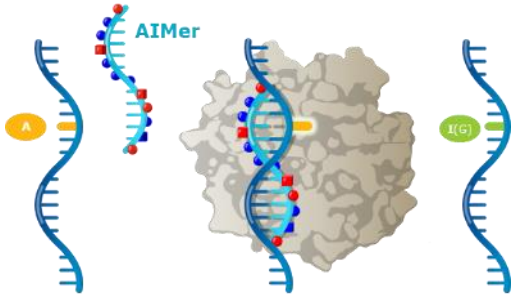
RNA editing enables correction of driver mutations as well as modulation of RNA and downstream proteins

Correct G-to-A driver mutations with AIMers

Restore or correct protein function ☑

WVE-006
(GalNAc AIMer)
AATD



Modulate protein expression with AIMers

☑ **Upregulate expression to increase endogenous protein activity**

Applications to address rare and common diseases

- Mutation-agnostic approach to increase protein levels in diseases resulting from loss of protein function
- Increase endogenous production of therapeutic proteins

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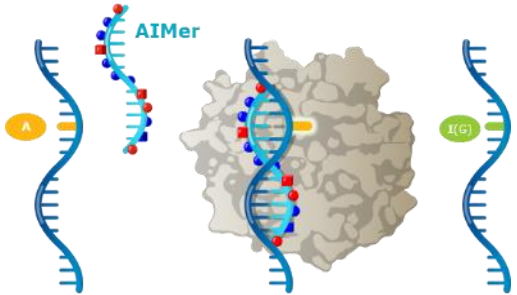
Potential to address prevalent diseases with RNA editing upregulation

Correct G-to-A driver mutations with AIMers

Restore or correct protein function ☑

WVE-006
(GalNAc AIMer)
AATD

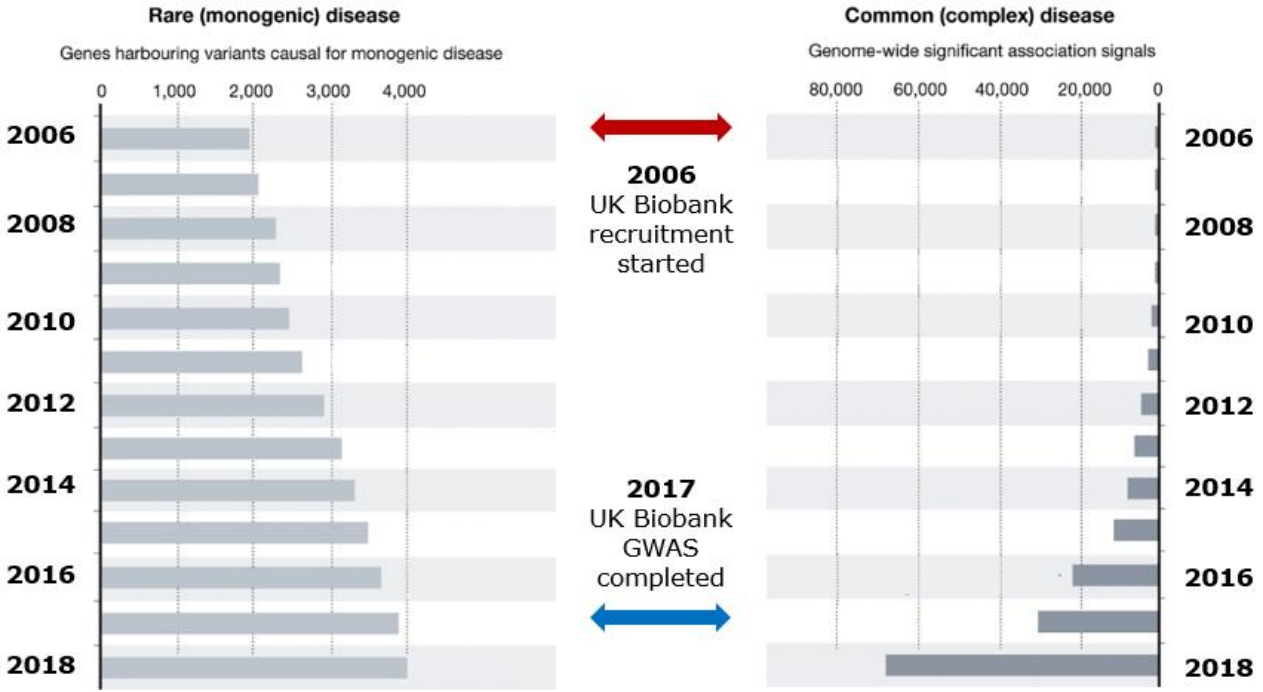


Modulate protein expression with AIMers

☑ **Upregulate expression to increase endogenous protein activity**

Potential to address prevalent diseases

Increasing genetic insights for rare and common diseases is unlocking new target opportunities



Accessing UK Biobank and building proprietary machine learning models to generate unique genetic insights

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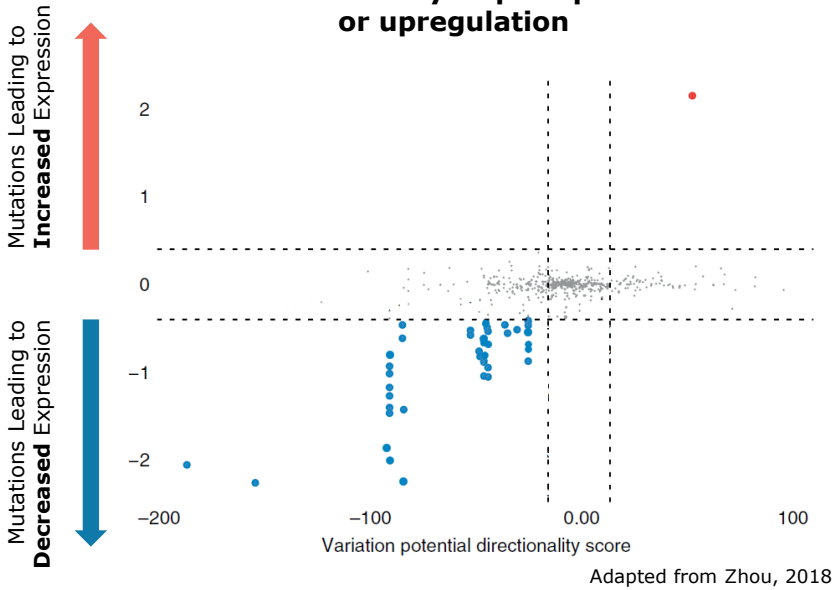
Claussnitzer, et al. Nature (2020) 577, 179; King et al. PLoS Genet (2019) 15, e1008489

Majority of disease associated mutations are predicted to decrease protein expression

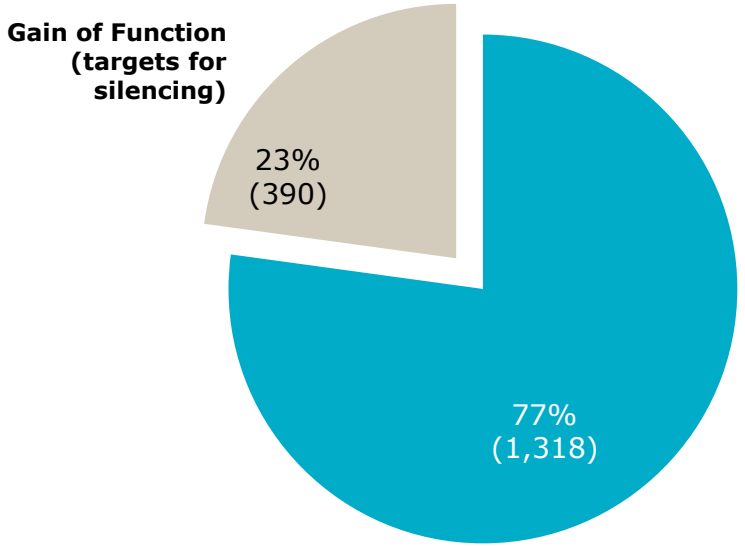
Mutations most commonly require protein restoration or upregulation



Adapted from Zhou, 2018

- *Potential disease targets requiring **upregulation***
- *Potential disease targets requiring **silencing***

Diseases in OMIM with gain of function or loss of function description



Wave is uniquely positioned to address these targets with editing for correction / upregulation and splicing modalities

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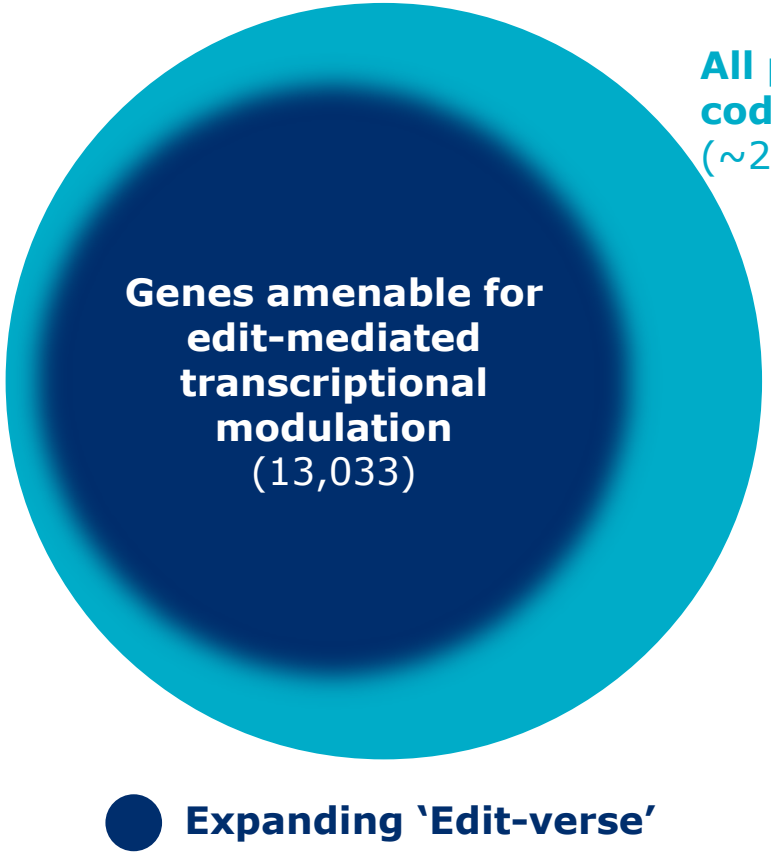
1. Zhou, et al. 2018 *Nat Genet* **50**, 1171–1179; https://doi.org/10.1038/s41588-018-0160-6; 2. OMIM

The AIMer-targetable ‘Edit-Verse’ is substantial

- **The Edit-verse is the editable gene-disease universe, including upregulation**
- **>13,000 genes** with a high-probability[1] of being amenable to transcriptional regulation with A-to-G editing
- Model development ongoing to expand access to **more protein-coding genes** and expand the Edit-verse



[1](score >95th p-tile)

Robust RNA medicines pipeline with five new clinical candidates by year-end 2025

Program	Discovery	Preclinical	Clinical	Rights	Patient population (US & Europe)
RNA EDITING					
WVE-006 SERPINA1 (AATD)				GSK exclusive global license	200K
Multiple undisclosed Correction				100% global	>20K (multiple)
Multiple undisclosed Upregulation				100% global	>3M (multiple)
SPLICING					
WVE-N531 Exon 53 (DMD)			Phase 1/2	100% global	2.3K
Other exons (DMD)				100% global	Up to 18K
SILENCING: ANTISENSE					
WVE-003 mHTT (HD)			Phase 1/2	Takeda 50:50 Option	25K Manifest (SNP3) 60K Pre-Manifest (SNP3)
SILENCING: RNAi					
INHBE* (Metabolic disorders)				100% global	47M

*Through GSK collaboration, Wave can advance up to three collaboration programs (the first of which is INHBE) and GSK can advance up to eight collaboration programs.

AATD: Alpha-1 antitrypsin deficiency; DMD: Duchenne muscular dystrophy; HD: Huntington's disease

Editing for correction

Editing for upregulation

GSK Perspectives: An Inflection Point with RNA Medicines

Tony Wood, PhD
Chief Scientific Officer, GSK

Carolyn Buser-Doepner, PhD
Vice President, Novel Human Genetics Research Unit, GSK

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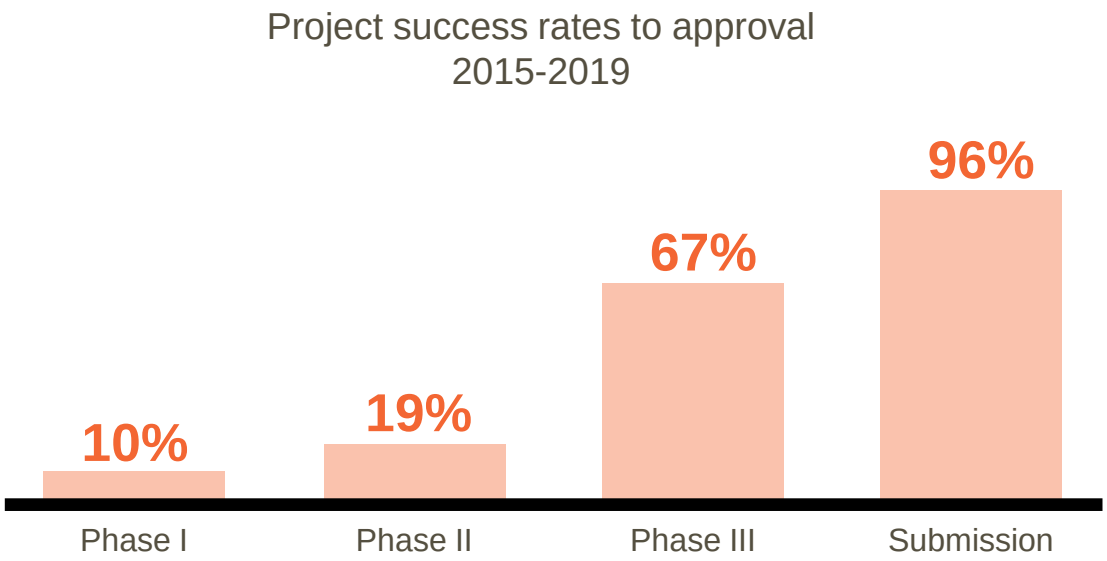
Biggest challenge of drug development:

90% of potential medicines that enter the clinic FAIL!



Why?

Traditional target selection has been flawed

- Animal models not predictive
- Correlation vs. causation

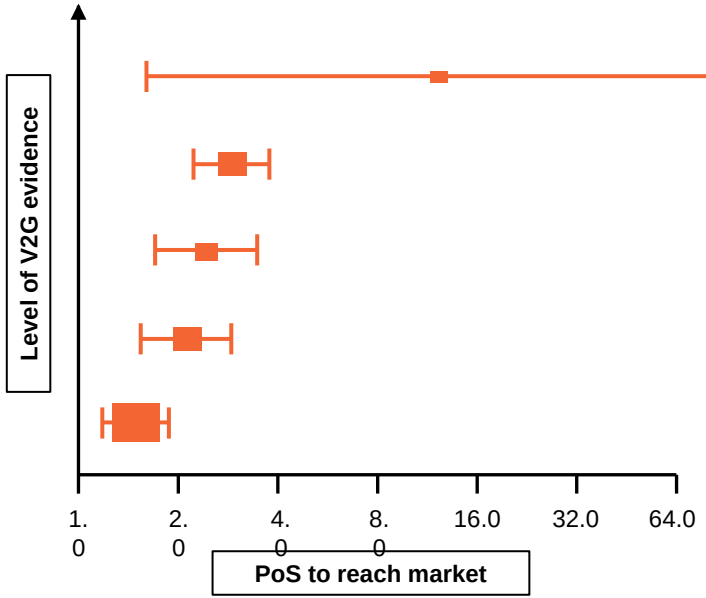
GSK

2020 Global R&D Performance Metrics Programme; CMR International, a Clarivate Analytics Business

Genetic evidence supports drug development

Drugs with human genetic evidence are >2x more likely to succeed

V2G method	pValue	Odds ratio
pLoF	4.8e-03	9.95
OMIM	2.0e-30	3.14
missense	2.1e-11	2.77
eQTL/pQTL	4.4e-11	2.31
nearest gene	5.1e-08	1.55



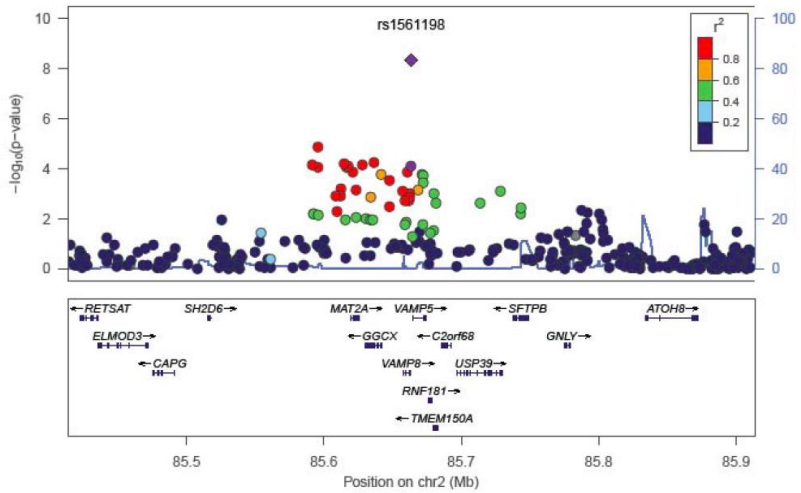
Drugs with human genetic evidence are >2x more likely to succeed

PoS proportional to strength of evidence for causality

Ref: Nelson *et al.* Nat Genet 2015; 47:846-60 & GSK internal analysis

Genetic association is just the first step

More challenging to identify the causal gene and its function (V2G2F)



Multiple candidate genes in region

>95% of GWAS variants outside of exons

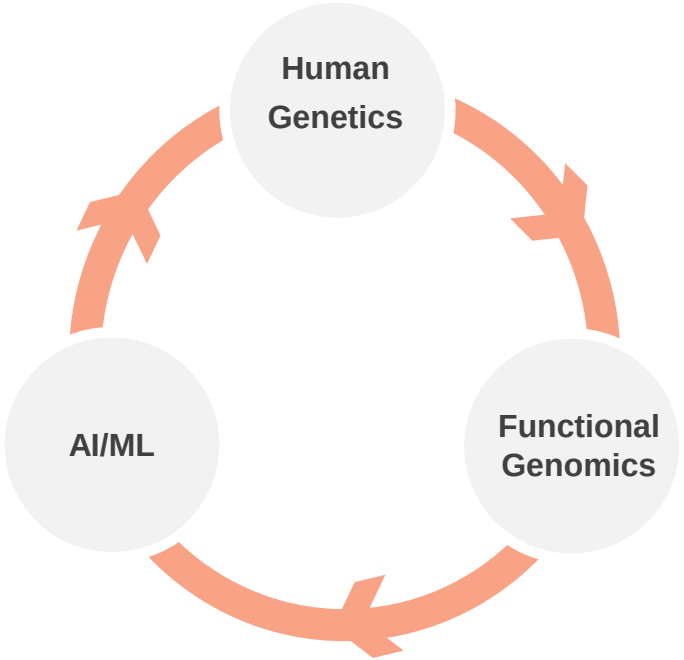
Need for experimental "wet" work to validate *in silico* associations

- Fine mapping (sequencing)
- Expression (eQTL / pQTL)
- Other molecular features (chromatin structure & interactions)
- Gene perturbation (CRISPR, TALE)

GSK

GSK has built competitive advantage for target identification

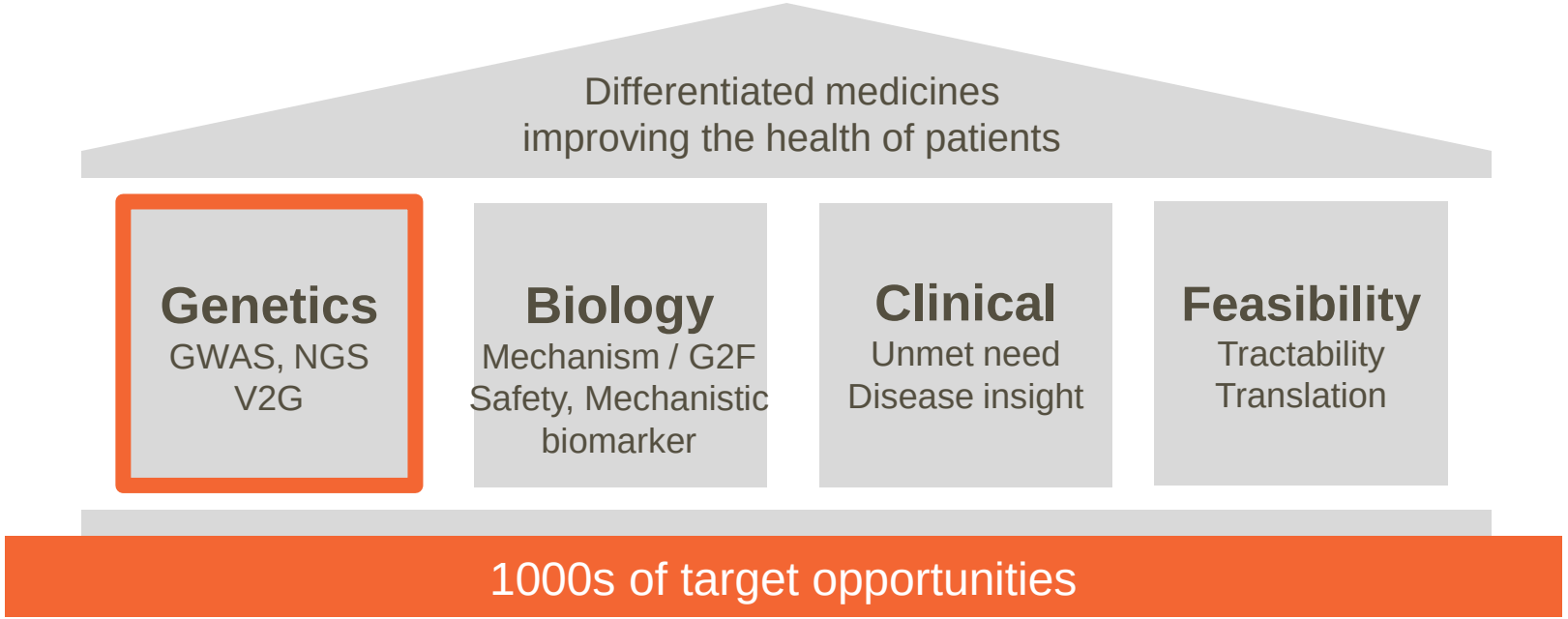
Mapping of genetic signals points towards modality to regulate RNA



Rationale for RNA oligo therapy

- >90% of genetic signals from GWAS map to non-coding elements
- Modulation of proteome
- Modulation of regulome

Pillars underlying target identification and prioritization



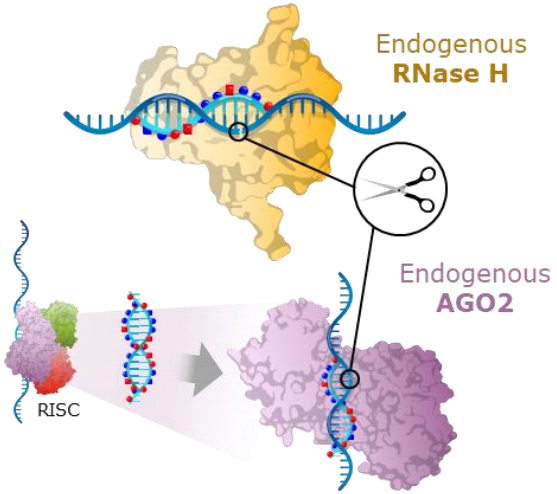
Greater than 70% of GSK targets have human genetic validation

Across multiple indications, enriched for immunology targets

Wave's best-in-class multi-modal platform enables silencing, splicing and editing modalities

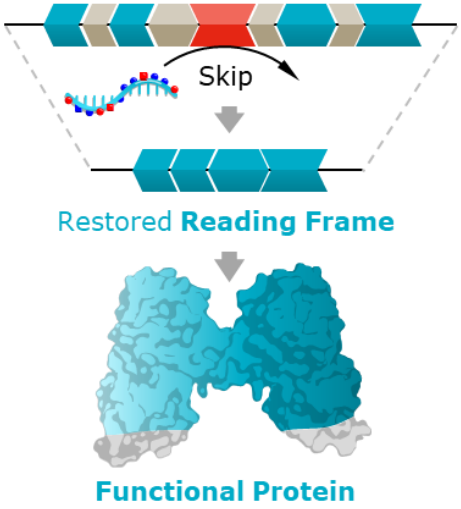
Silencing

- Degrade RNA transcripts to **turn off** protein production



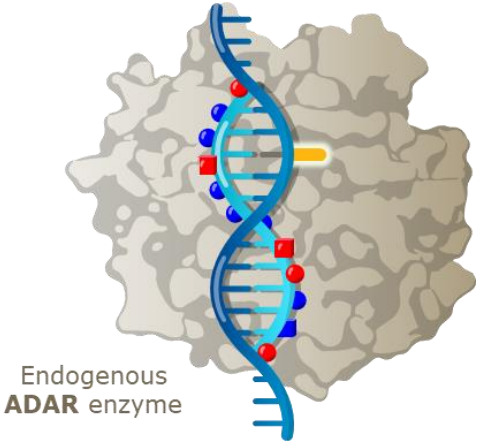
Splicing

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



RNA Base Editing

- Edit RNA bases to **restore** or **modulate** protein production



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RNAi: INHBE and Beyond

Chandra Vargeese, PhD
Chief Technology Officer

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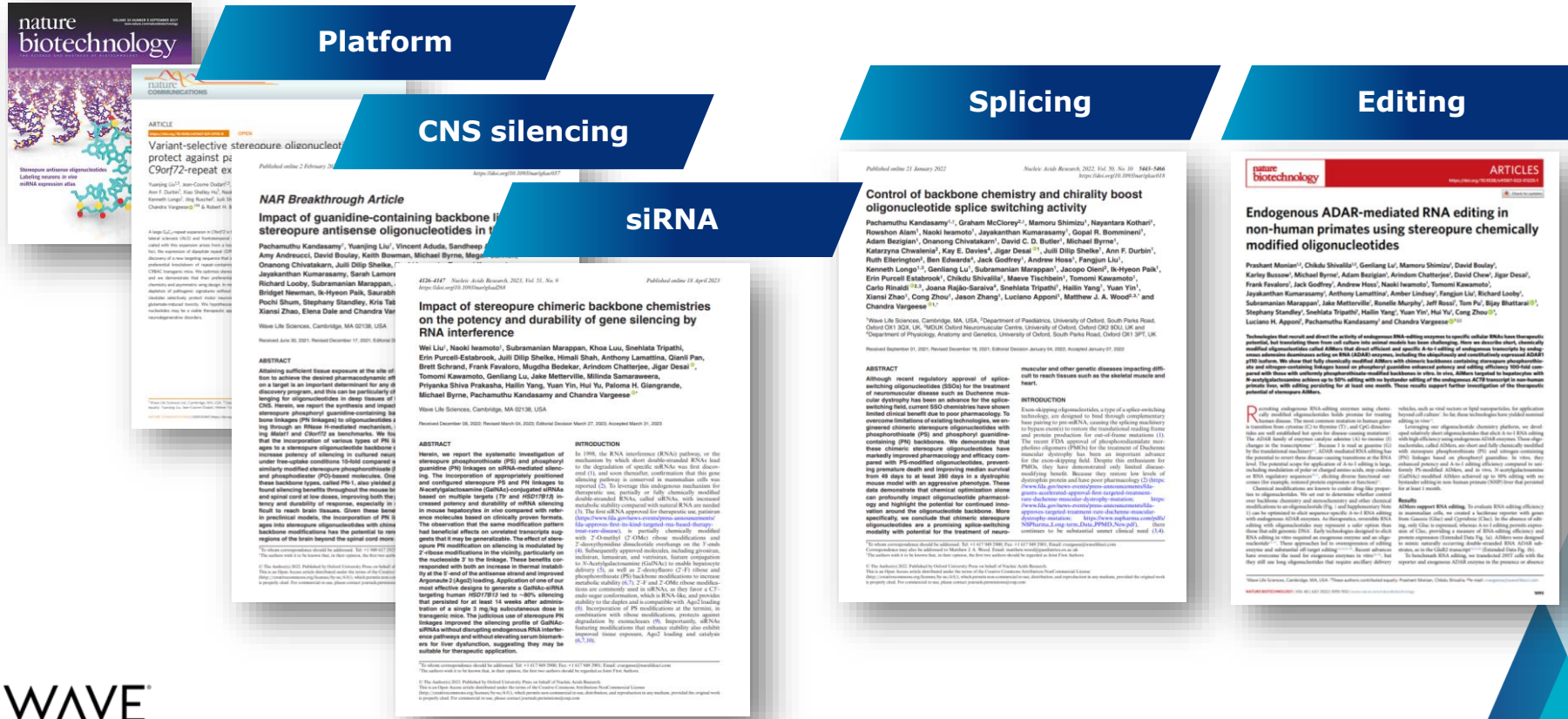
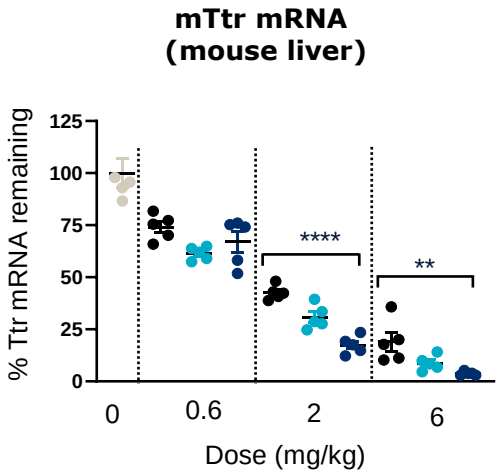
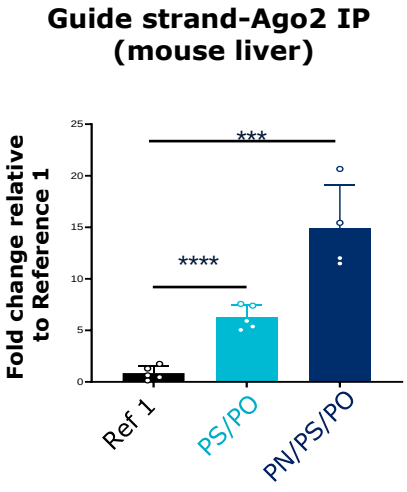
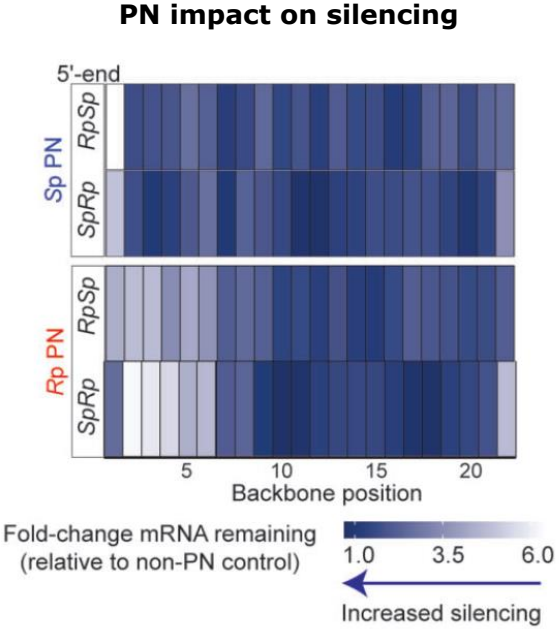
Multiple recent publications on Wave leadership in RNA therapeutics



Incorporation of PN modification improves *Ttr* GalNAc-siRNA in mice







PS/PO/PN backbone
PS/PO backbone
Reference compound
PBS

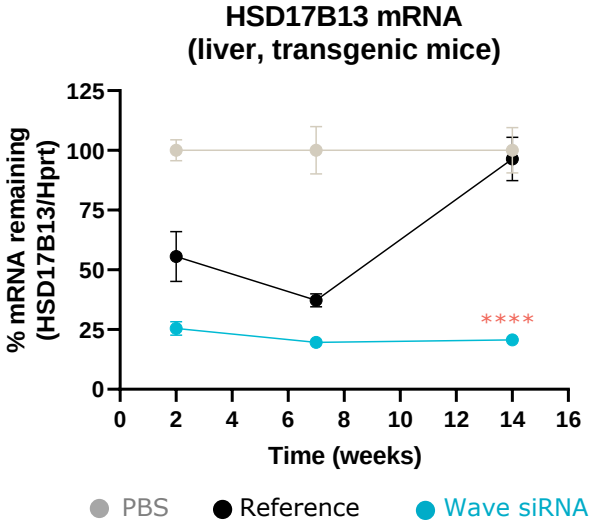
Liu et al., 2023 Nuc Acids Res

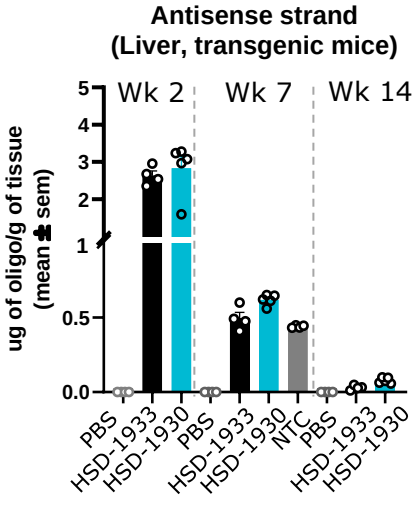
Potential for best-in-class RNAi enabled by Wave's PRISM platform

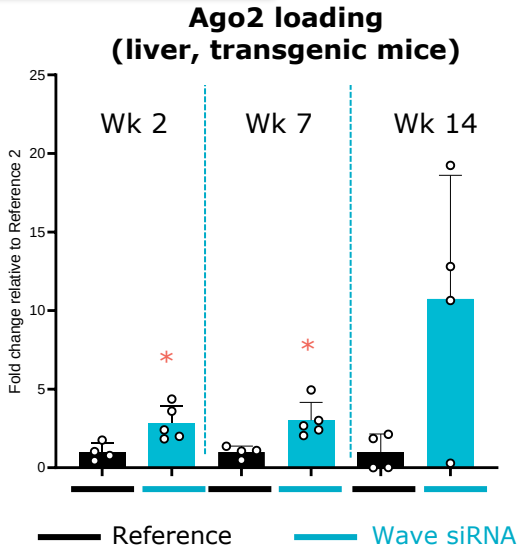
Nucleic Acids Research

Impact of stereopure chimeric backbone chemistries on the potency and durability of gene silencing by RNA interference

- Unprecedented Ago2 loading increases potency and durability of silencing following administration of single subcutaneous dose







RNAi is one of multiple Wave modalities being advanced in strategic research collaboration with GSK

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Left, Middle, and right: Mice expressing human *HSD17B13* transgene treated with siRNA (3 mg/kg) or PBS, liver mRNA, guide strand concentration, Ago2 loading quantified. Stats: Two-way ANOVA with post-hoc test * P<0.05, ****P<0.0001. Liu et al., 2023 *Nuc Acids Res* doi: 10.1093/nar/gkad268;

Driven by clinical genetics, Wave's first RNAi program addresses high unmet need in metabolic disorders, including obesity

INHBE program is Wave's first wholly owned program emerging from GSK collaboration

- Leverages novel genetic insights accessed through GSK collaboration
- INHBE loss-of-function heterozygous carriers exhibit healthy metabolic profile[1,2,3]:
  - ✓ Reduced waist-to-hip circumference
  - ✓ Reduced odds ratio of Type 2 diabetes by 28%, and coronary artery disease
  - ✓ Reduced serum triglycerides
  - ✓ Elevated HDL-c
  - ✓ Reduced HbA1c
  - ✓ Lowered ApoB
- INHBE expressed primarily in liver and gene product (subunit of activin E) acts on its receptor in adipose tissue[4]
- GalNAc-siRNA for targeted delivery to hepatocytes

≥50% reduction of INHBE with siRNA expected to restore a healthy metabolic profile

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1. *Nat Commun 2022. https://doi.org/10.1038/s41467-022-32398-7; 2. Nat Commun 2022. https://doi.org/10.1038/s41467-022-31757-8; 3. PLOS ONE 2018. https://doi.org/10.1371/journal.pone.0194798; 4. Adam, RC. et.al. Proc Natl Acad Sci USA. 2023, 120(32): e2309967120.*

INHBE GalNAc-siRNA represents an evolution in treatment for metabolic diseases, including obesity

- Metabolic syndrome* is associated with type 2 diabetes, cardiovascular disease, hypertension, stroke, cancer, and increased mortality[1,2]
- Estimate ~47M people in US and Europe with metabolic disorders, including obesity
- Therapeutic options beyond GLP1s are needed
  - GLP1 receptor agonists lead to weight loss at the expense of muscle[4]
  - GLP1 receptor agonists suppress general reward system[7]
  - GLP1 receptor agonists associated with poor tolerability profile[5] with 68% drop-off after 1 year[6]
- Preferred approach would improve metabolism and increase fat loss while maintaining muscle mass
- Restoration of metabolic health via INHBE silencing can simultaneously address obesity and other drivers of metabolic syndrome

*Patients diagnosed with metabolic syndrome based on having 3 of the following: abdominal obesity, high bp, high blood glucose, high TG, or low HDL

1. Liang, et al. 2023 Postgraduate Medical Journal 99(1175):985; 2. Lakka, et al. 2002 JAMA 288(21):2709; 3. Ryan and Yockey 2017 Curr Obes Rep 6(2):187; 4. Sargeant, et al. 2019 Endocrinol Metab (Seoul) 34(3):247-262; 5. Liu, et al. 2022 Front. Endocrinol. 13:1043789; 6. Prime Therapeutics Claims Analysis, July 2023; 7. Müller, et al. 2019 Molecular Metabolism 30: 72-130.

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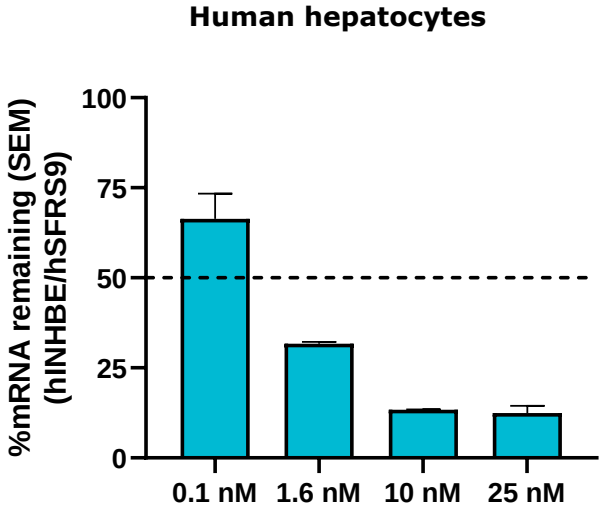
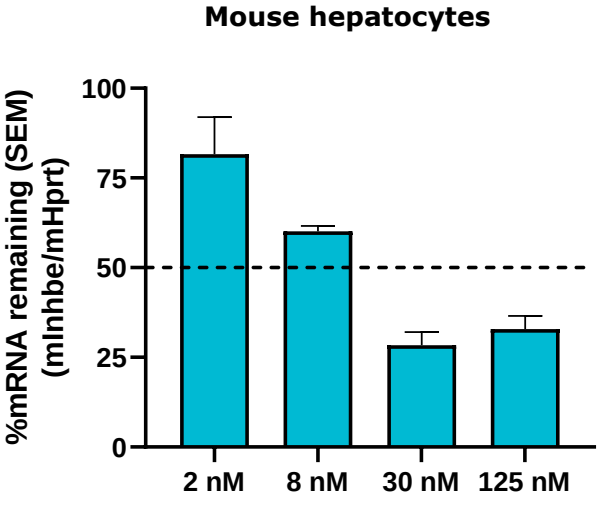
INHBE knockdown of 90% demonstrated in human hepatocytes with GalNAc-siRNA

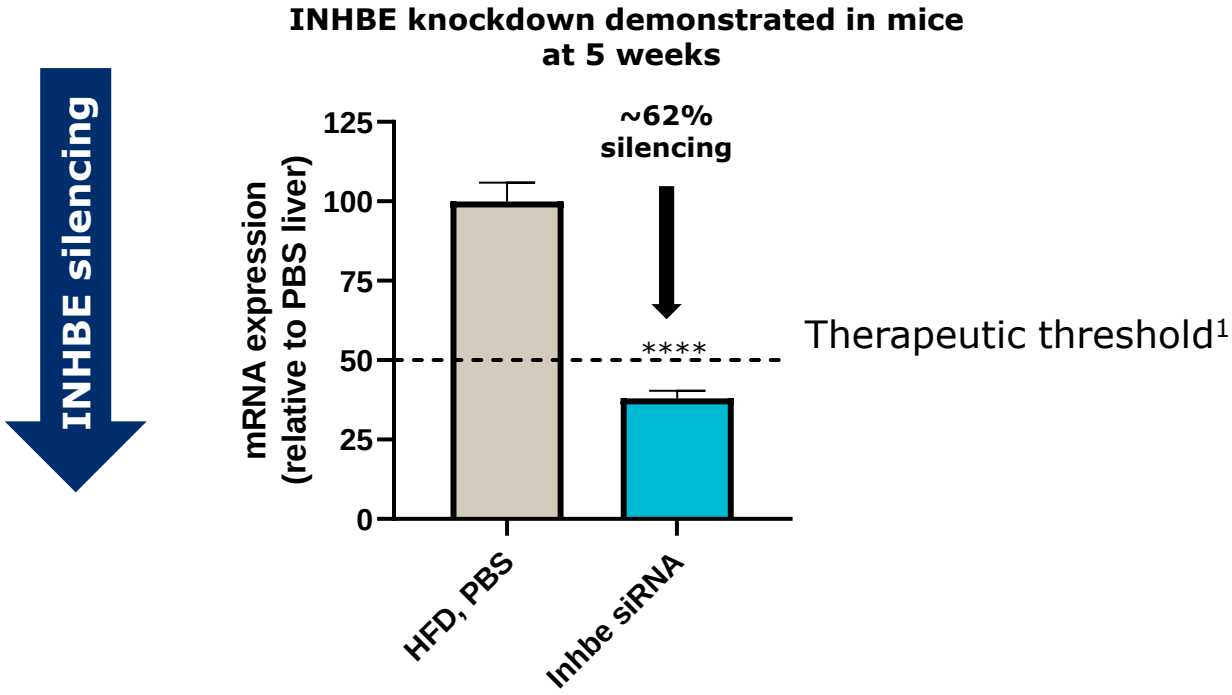




- This cross-reactive sequence demonstrates ~90% maximal knock-down in human hepatocytes and ~65% in mouse hepatocytes
- Additional human selective sequences are in development

Primary hepatocytes were treated with a cross-reactive siRNA via free uptake. INHBE mRNA was quantified by RT-qPCR.

INHBE silencing achieved *in vivo* with GalNAc-siRNA exceeds therapeutic threshold

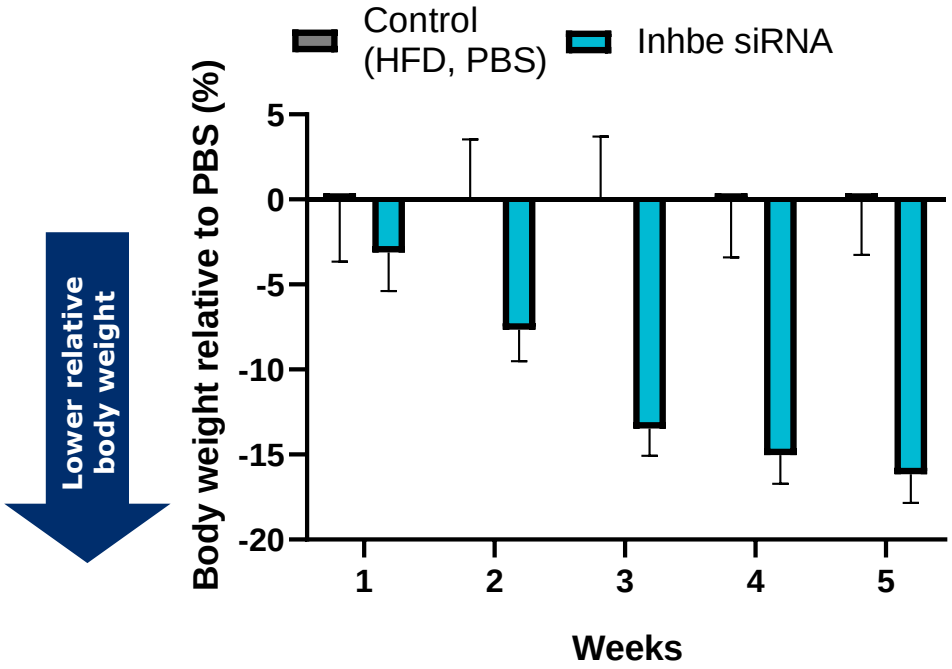


HFD: high-fat diet. Stats: two-sided Welch's T Test **** P < 0.0001

1. Adam, RC. et.al. Proc Natl Acad Sci USA. 2023, 120(32): e2309967120.

INHBE knockdown led to 16% lower body weight

Similar effect seen in semaglutide preclinical studies

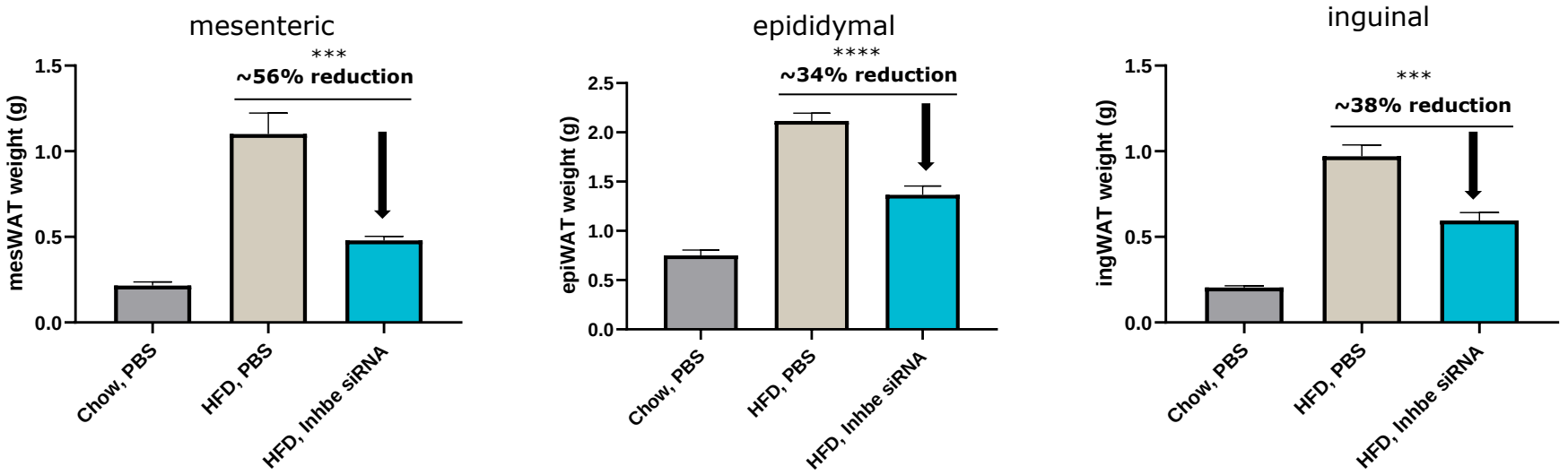


Data plotted by body weight difference as a percentage of PBS treated young DIO mice; *Coskun, T. et. al. Mol. Metab. 2018, 18, 3.* Stats: Repeated Measures ANOVA; Inhbe siRNA vs. Control significantly different at $P < 0.05$ level weeks 2 through 5

INHBE reduction leads to significant decrease in visceral fat at 5 weeks

- INHBE knockdown in young DIO mice resulted in less fat mass across multiple types of white adipose tissue, without loss of brown fat

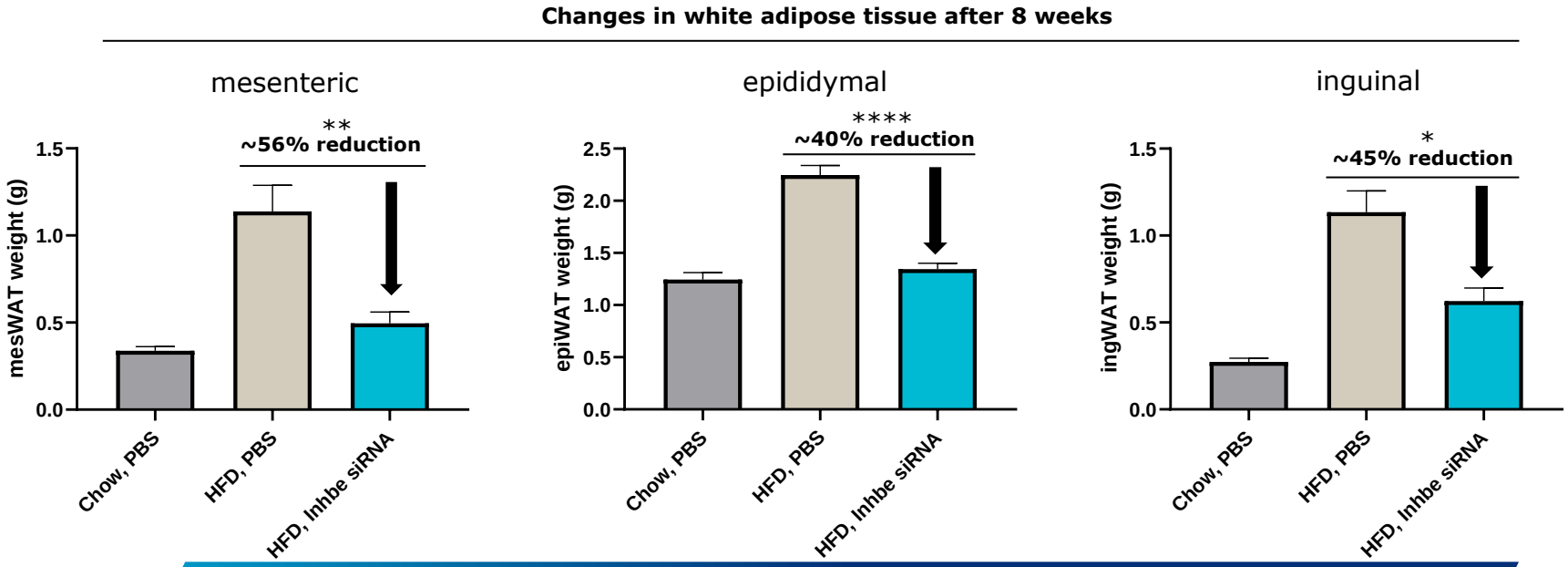
Changes in white adipose tissue after 5 weeks



Stats: white-adjusted Two-way ANOVA with Bonferroni-adjusted post hoc comparisons per tissue type allowing heteroscedasticity (only HFD, Inhbe siRNA vs. HFD, PBS shown) *** P < 0.001, **** P < 0.0001

>50% reduction of INHBE mRNA recapitulates phenotype of heterozygous LoF carriers

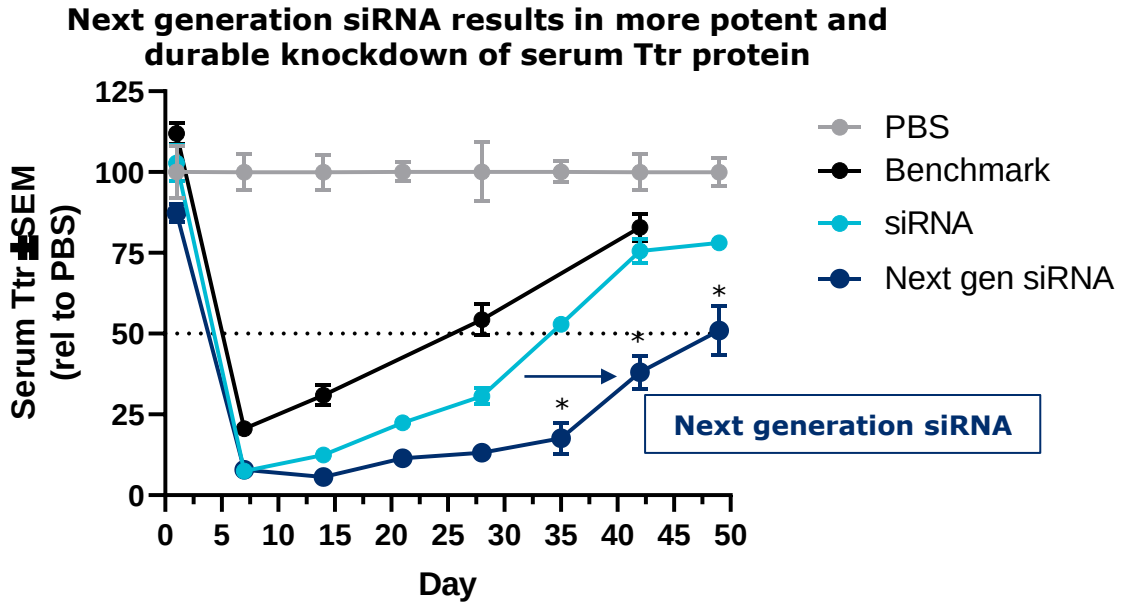
- Subsequent 8-week study demonstrates further reduction in excess visceral fat



First demonstration of siRNA treatment to restore healthy phenotype

Adam, RC. et.al. Proc Natl Acad Sci USA. 2023, 120(32): e2309967120. HFD: high-fat diet. Stats: white-adjusted Two-way ANOVA with Bonferroni-adjusted post hoc comparisons per tissue type allowing heteroscedasticity (only HFD, INHBE siRNA vs. HFD, PBS shown) * P < 0.05, ** P < 0.01, **** P < 0.0001

Wave's next generation GalNAc-siRNA demonstrates best-in-class potential



- Applying next-generation siRNA chemistry to INHBE program
- Potential for infrequent administration

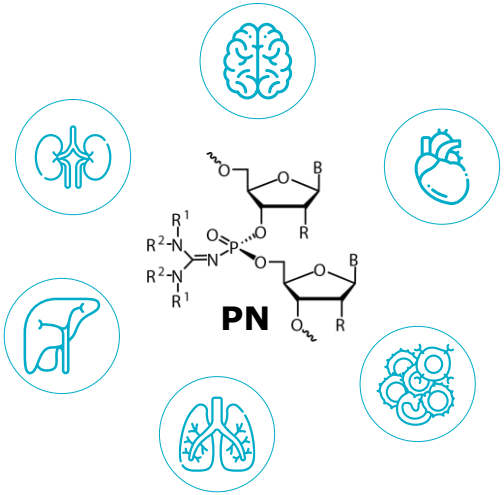
INHBE candidate for metabolic disorders, including obesity, expected in 4Q 2024

Foster, DJ. et.al. Mol Ther. 2018, 26(3), 708. B6 mice administered PBS or 0.5 mg/kg of siRNA (subcutaneous). Benchmark: Stats: Mixed Two-way ANOVA followed by post hoc test comparing siRNA vs. Next gen siRNA per day derived from linear mixed effects model * $P < 0.0001$

Wave's platform chemistry enables siRNA extra-hepatic delivery

- Chemical impact
  - Introduction of neutral backbone
  - Unique structural feature of PN, specifically guanidine
  - Increased lipophilicity
  - Stereochemistry

- Extra-hepatic delivery
  - Titrating siRNA lipophilicity tunable PNs (PN variants)
  - Maintaining high Ago2 loading and intracellular trafficking
  - Titrating plasma protein binding
  - Altered delivery, enhanced potency and durability in various tissues



PN can tune extra-hepatic delivery of siRNA using rational design, including placement, number of modifications and PN variants

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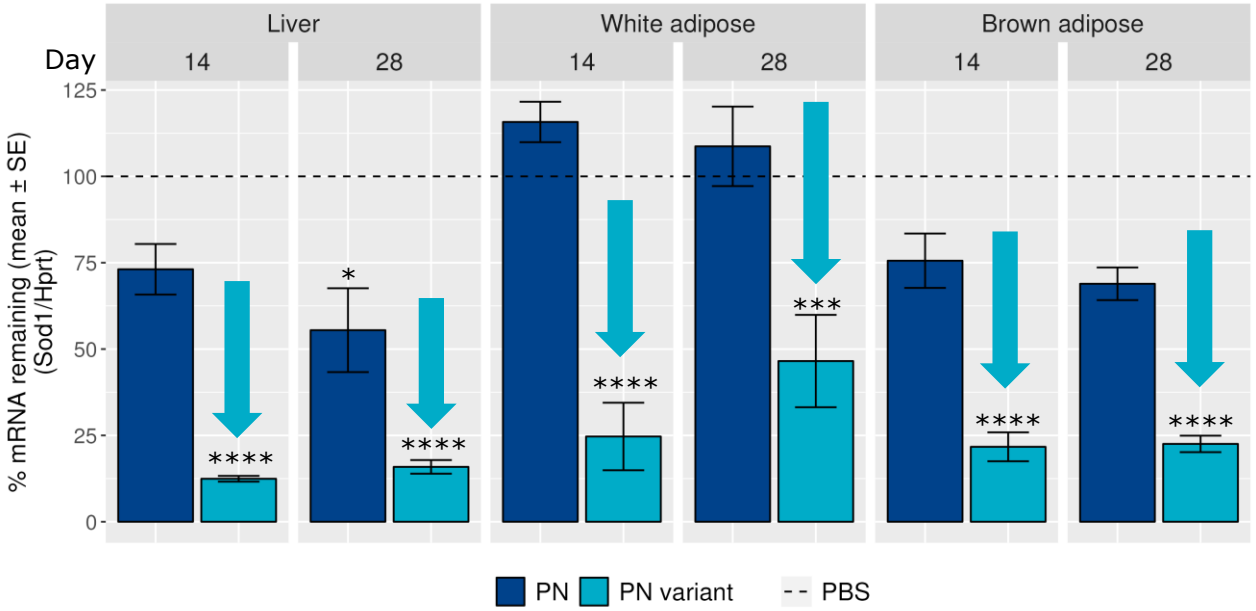
Tunable PN variants enhance potency and alter extra-hepatic delivery of non-GalNAc siRNAs

Non-GalNAc siRNA with PN variants improve silencing in liver and adipose tissue 14 and 28 days post single dose

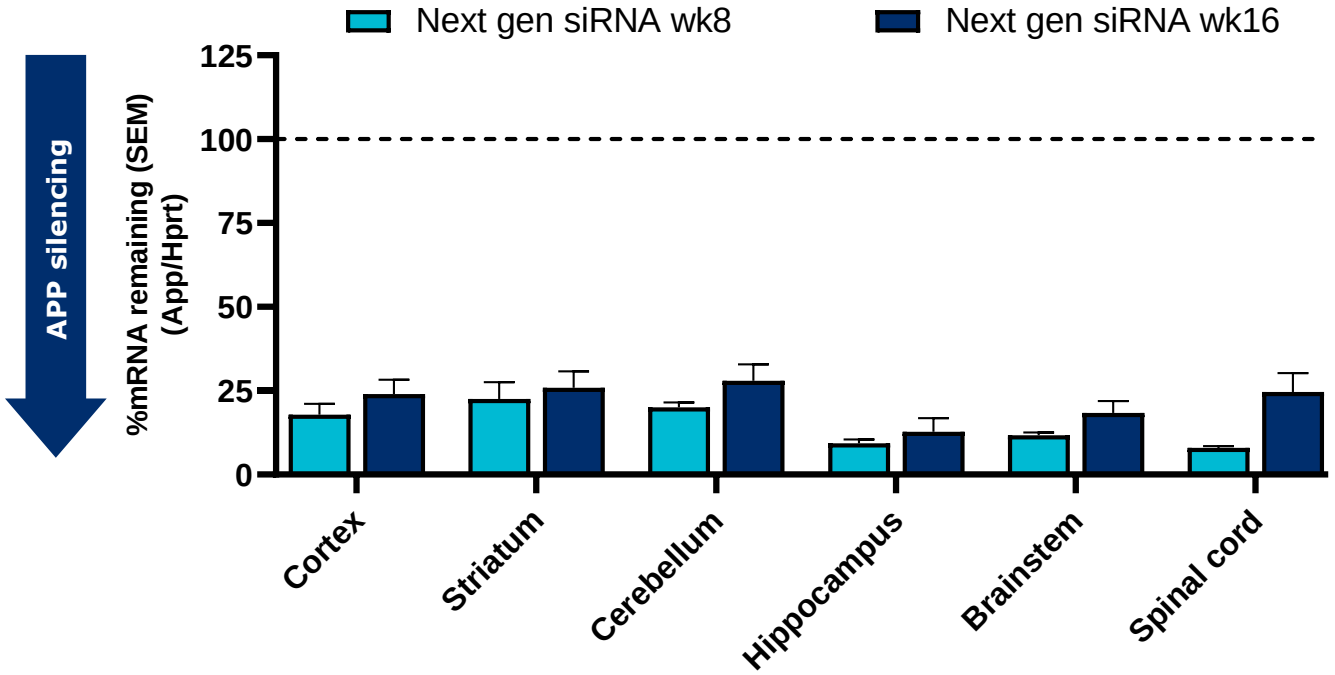


- Reaching adipose tissue in addition to liver with siRNA is important for certain metabolic disorders
- PN variants also enhanced siRNA silencing in muscle tissue, including heart and diaphragm

Stats: Three-way ANOVA followed by Bonferroni-adjusted post hoc test comparing condition to PBS (data not shown) * P < 0.05, *** P < 0.001, **** P < 0.0001; B6 mice administered PBS or 5 mg/kg of Sod1 siRNA (no GalNAc conjugate) subcutaneous injection (n=7). Taqman qPCR assays used for RNA PD, relative fold changes of *Sod1* to *Hprt* mRNA normalized to % of PBS group.

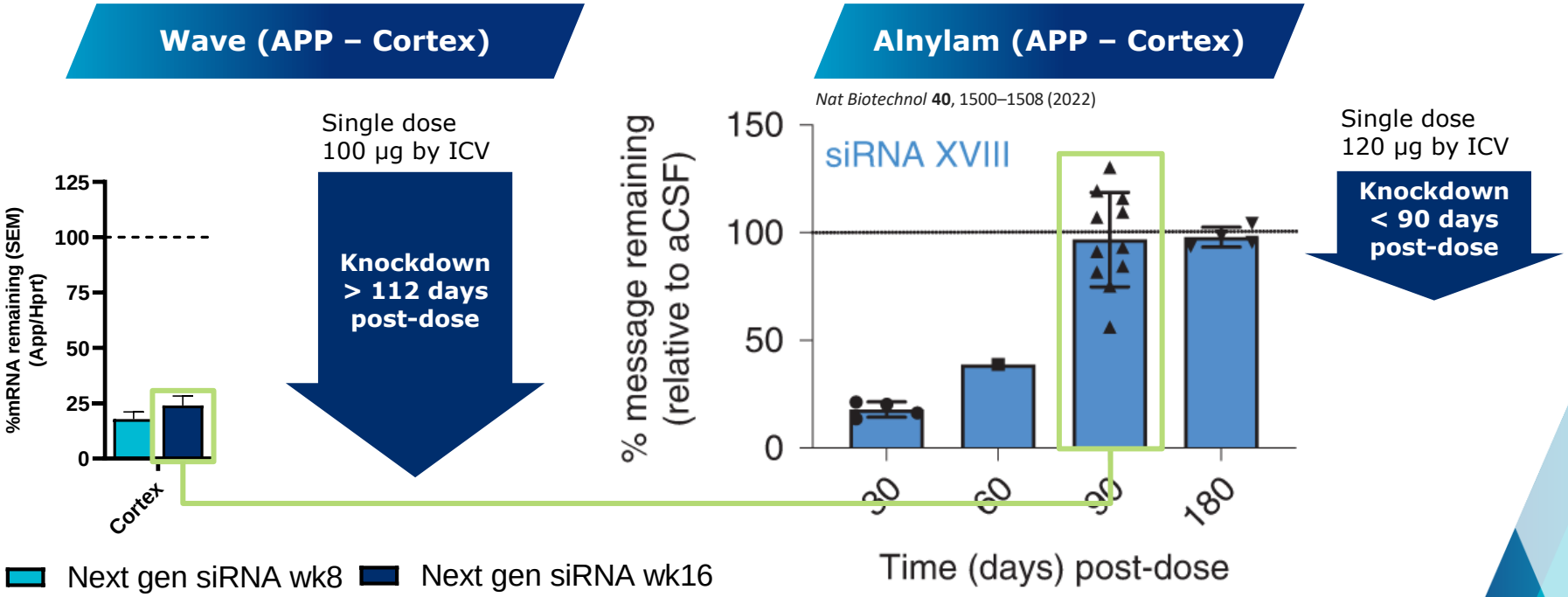
Single dose of next generation siRNA delivers broad, potent and durable CNS target engagement

Sustained APP knockdown of at least 75% throughout the 16-week study *in vivo* in mice



PBS (dotted line) or 100 µg of App siRNA administered ICV (n=7). PCR assays for RNA PD, relative fold changes of *App* to *Hprt* mRNA normalized to % of PBS; Stats: Three-way ANOVA followed by Bonferroni-adjusted post hoc test comparing condition to PBS (data not shown), Next gen siRNA significantly lower than PBS at both time points for all tissues at P < 0.0001 level

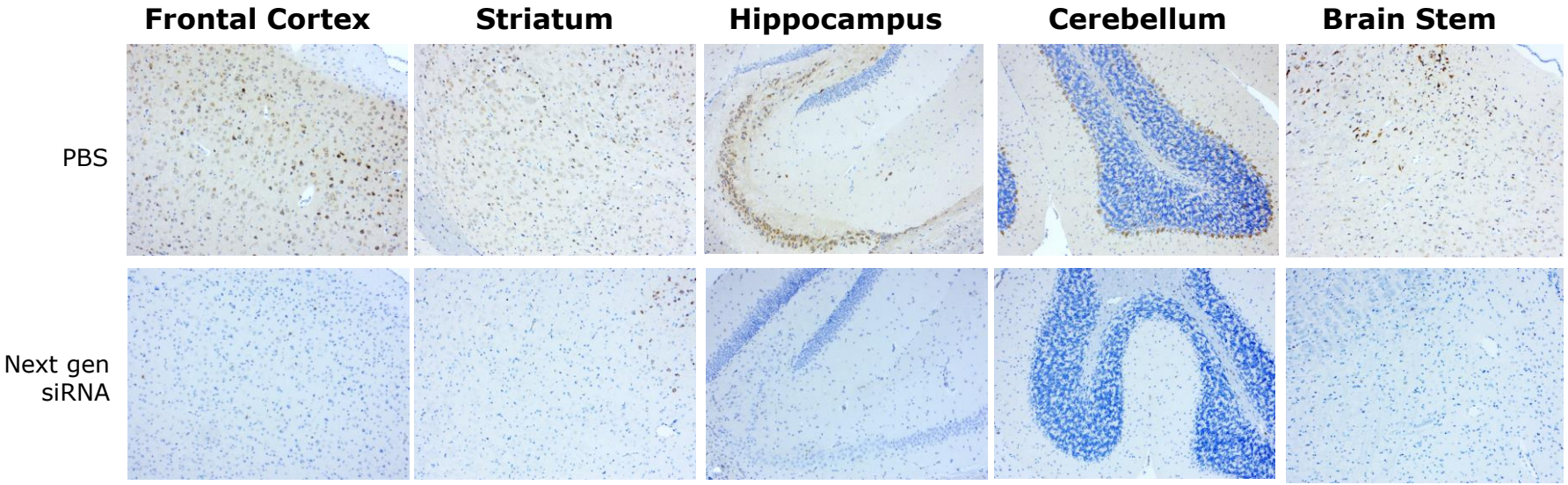
Wave siRNA demonstrates more potent and durable silencing as compared to published state-of-the-art



PBS (dotted line) or 100 µg of App siRNA administered ICV (n=7). PCR assays for RNA PD, relative fold changes of *App* to *Hprt* mRNA normalized to % of PBS; Stats: Three-way ANOVA followed by Bonferroni-adjusted post hoc test comparing condition to PBS (data not shown), Next gen siRNA significantly lower than PBS at both time points for all tissues at P < 0.0001 level. Source: Brown, K.M., Nair, J.K., Janas, M.M. *et al.* Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates. *Nat Biotechnol* **40**, 1500–1508 (2022).

Robust target engagement translates to substantial App protein reduction across brain regions

Reductions observed 8-weeks post single-dose



Immunohistochemical analysis of FFPE Mouse Brain tissue labeling App protein (**Color Brown**) with CS#19389 followed by a ready to use Polymer-HRP 2nd Detection antibody. Nuclei were counterstained with Hematoxylin (**Color Blue**). Single 100 ug ICV injection

First siRNA clinical candidate (INHBE) with proprietary chemistry expected in 4Q 2024

- **INHBE GalNAc-siRNA program is driven by clinical genetics, with potential to be next-generation therapeutic for obesity**
  - ≥50% silencing of INHBE is expected to improve metabolic health
  - INHBE siRNA silencing above therapeutic threshold restores healthy phenotype, with 16% lower body weight, as well as reduction of visceral fat to the level of lean-animals
- **Next generation GalNAc-siRNA formats are best-in-class and being applied to INHBE program**
- **Wave's platform chemistry enables extra-hepatic delivery for other non-hepatic targets**
  - PN-variants on non-GalNAc siRNA enhance silencing in multiple tissues, including liver, adipose tissue and muscle.
  - Single dose of next generation siRNA delivers broad, potent (>75%) and durable CNS target engagement

AIMers: Editing to Upregulate

Chandra Vargeese, PhD
Chief Technology Officer

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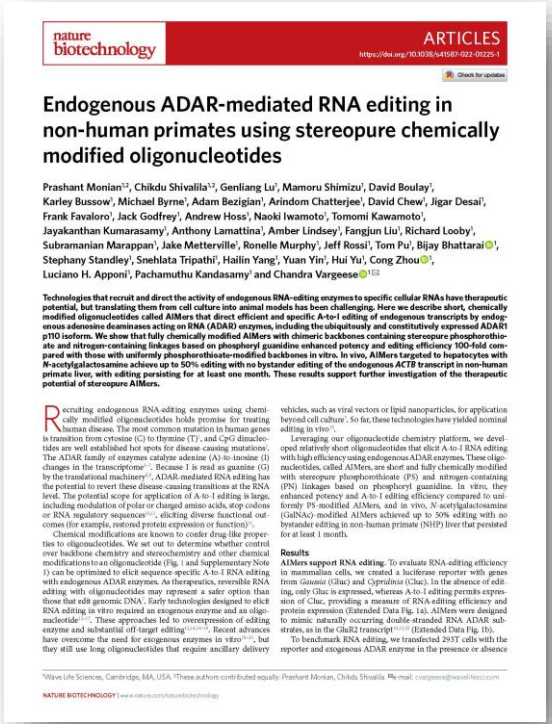
First-generation AIMer designs published in *Nature Biotechnology*



nature biotechnology

ARTICLES

https://doi.org/10.1038/s41587-022-01225-1

Check for updates

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]✉

Technologies that recruit and direct the activity of endogenous RNA-editing enzymes to specific cellular RNAs have therapeutic potential, but translating them from cell culture into animal models has been challenging. Here we describe short, chemically modified oligonucleotides called AIMers that direct efficient and specific A-to-I editing of endogenous transcripts by endogenous adenosine deaminases acting on RNA (ADAR) enzymes, including the ubiquitously and constitutively expressed ADAR1 p110 isoform. We show that fully chemically modified AIMers with chimeric backbones containing stereopure phosphorothioate and nitrogen-containing linkages based on phosphoryl guanidine enhanced potency and editing efficiency 100-fold compared with those with uniformly phosphorothioate-modified backbones in vitro. In vivo, AIMers targeted to hepatocytes with *N*-acetylgalactosamine achieve up to 50% editing with no bystander editing of the endogenous *ACTB* transcript in non-human primate liver, with editing persisting for at least one month. These results support further investigation of the therapeutic potential of stereopure AIMers.

Recruiting endogenous RNA-editing enzymes using chemically modified oligonucleotides holds promise for treating human disease. The most common mutation in human genes is transition from cytosine (C) to thymine (T)[1], and CpG dinucleotides are well established hot spots for disease-causing mutations[2]. The ADAR family of enzymes catalyze adenine (A)-to-inosine (I) changes in the transcriptome[3–5]. Because I is read as guanine (G) by the translational machinery[6,7], ADAR-mediated RNA editing has the potential to revert these disease-causing transitions at the RNA level. The potential scope for application of A-to-I editing is large, including modulation of polar or charged amino acids, stop codons or RNA regulatory sequences[8–11], eliciting diverse functional outcomes (for example, restored protein expression or function)[12–15].

Chemical modifications are known to confer drug-like properties to oligonucleotides. We set out to determine whether control over backbone chemistry and stereochemistry and other chemical modifications to an oligonucleotide (Fig. 1 and Supplementary Note 1) can be optimized to elicit sequence-specific A-to-I RNA editing with endogenous ADAR enzymes. As therapeutics, reversible RNA editing with oligonucleotides may represent a safer option than those that edit genomic DNA[7]. Early technologies designed to elicit RNA editing in vitro required an exogenous enzyme and an oligonucleotide[16–22]. These approaches led to overexpression of editing enzyme and substantial off-target editing[12,19,20,23]. Recent advances have overcome the need for exogenous enzymes in vitro[24–26], but they still use long oligonucleotides that require ancillary delivery vehicles, such as viral vectors or lipid nanoparticles, for application beyond cell culture[7]. So far, these technologies have yielded nominal editing in vivo[27].

Leveraging our oligonucleotide chemistry platform, we developed relatively short oligonucleotides that elicit A-to-I RNA editing with high efficiency using endogenous ADAR enzymes. These oligonucleotides, called AIMers, are short and fully chemically modified with stereopure phosphorothioate (PS) and nitrogen-containing (PN) linkages based on phosphoryl guanidine. In vitro, they enhanced potency and A-to-I editing efficiency compared to uniformly PS-modified AIMers, and in vivo, *N*-acetylgalactosamine (GalNAc)-modified AIMers achieved up to 50% editing with no bystander editing in non-human primate (NHP) liver that persisted for at least 1 month.

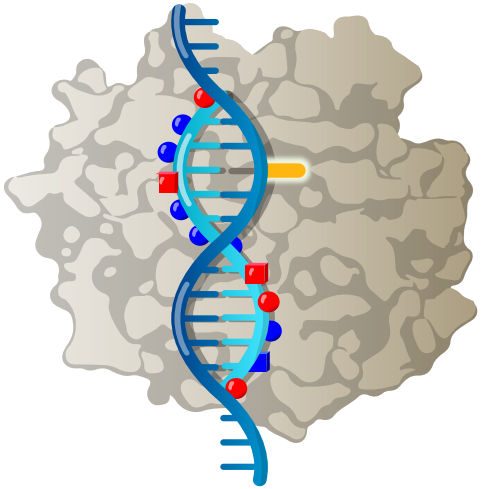
Results

AIMers support RNA editing. To evaluate RNA-editing efficiency in mammalian cells, we created a luciferase reporter with genes from *Gaussia* (Gluc) and *Cypridina* (Cluc). In the absence of editing, only Gluc is expressed, whereas A-to-I editing permits expression of Cluc, providing a measure of RNA-editing efficiency and protein expression (Extended Data Fig. 1a). AIMers were designed to mimic naturally occurring double-stranded RNA ADAR substrates, as in the GluR2 transcript[12,13,23] (Extended Data Fig. 1b).

To benchmark RNA editing, we transfected 293T cells with the reporter and exogenous ADAR enzyme in the presence or absence

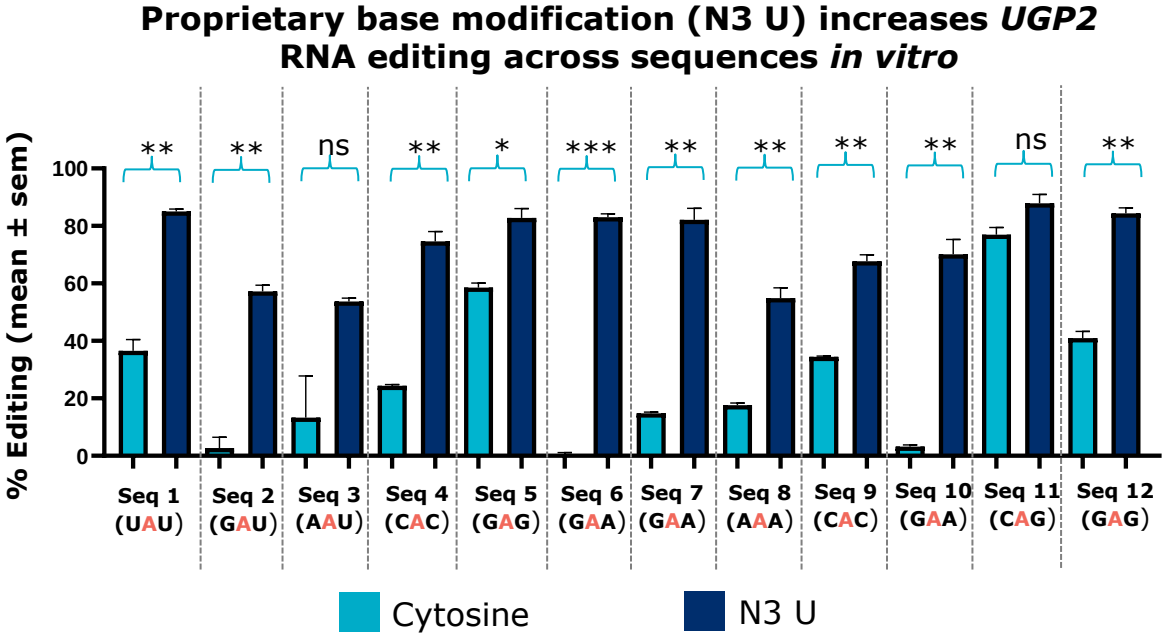
[1]Wave Life Sciences, Cambridge, MA, USA. [2]These authors contributed equally: Prashant Monian, Chikdu Shivalila. ✉e-mail: cvargeese@wavelifesci.com

- Foundational AIMer structure-activity-relationships
- *In vitro-in vivo* translation (NHPs)
- Specificity *in vitro* & *in vivo* (NHPs)
- GalNAc conjugation

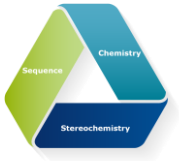


Monian *et al.*, 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1
SAR structure-activity relationship

Proprietary base modifications increase editing across edit region sequences



- N3 U: example of proprietary base modifications
- N3 U consistently improves RNA editing levels, including across sequences



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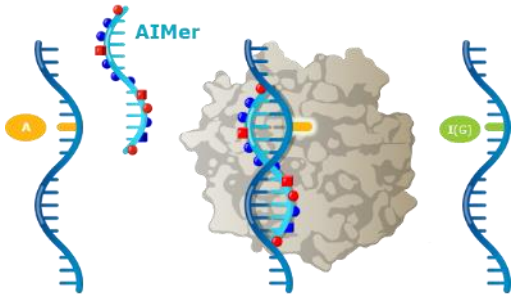
Innovating on applications of ADAR editing

Correct G-to-A driver mutations with AIMers

Restore or correct protein function ✓

WVE-006 (GalNAc AIMer)
AATD

Modulate protein interactions with AIMers



Achieved POC

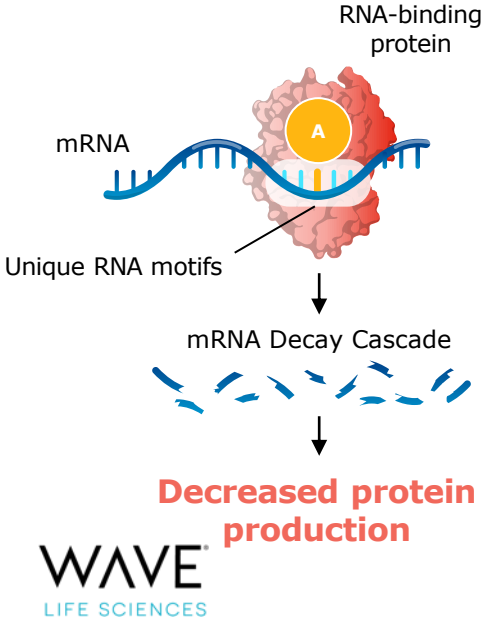
- ✓ **Modulate protein-protein interaction**
- ✓ **Upregulate expression**
- ☐ Modify function
- ☐ Post-translational modification
- ☐ Alter folding or processing

POC: proof of concept

RNA is highly regulated, creating ample opportunity to intervene with AIMers to alter protein expression

RNA binding proteins recognize sequence motifs to regulate mRNA stability

Attenuated Gene Expression



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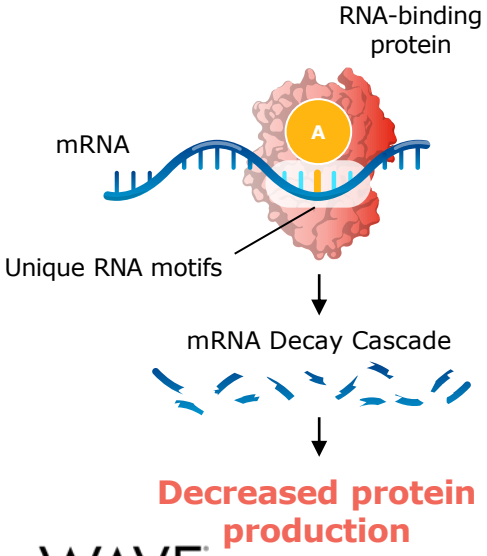
Using catalytically efficient AIMers, mRNA can be edited and durably stabilized

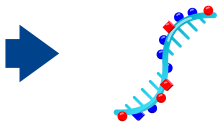
RNA binding proteins recognize sequence motifs to regulate mRNA stability

Attenuated Gene Expression



Decreased protein production

"Dialed up" Gene Expression



Catalytically Efficient AIMer

AIMer **edits** and **durably stabilizes** mRNA

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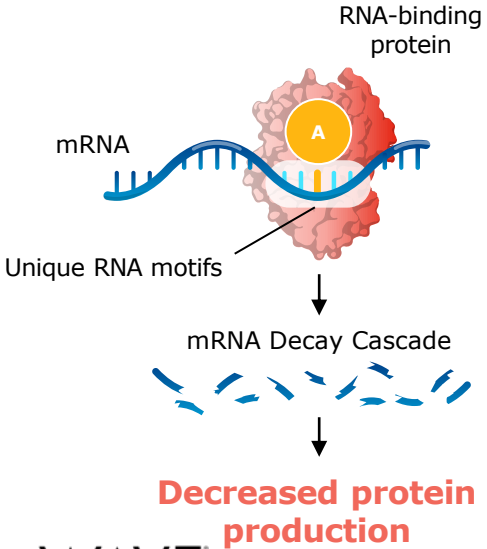
Upregulation: AIMers can edit RNA motifs to restore or upregulate gene expression

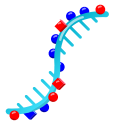
RNA binding proteins recognize sequence motifs to regulate mRNA stability

Attenuated Gene Expression



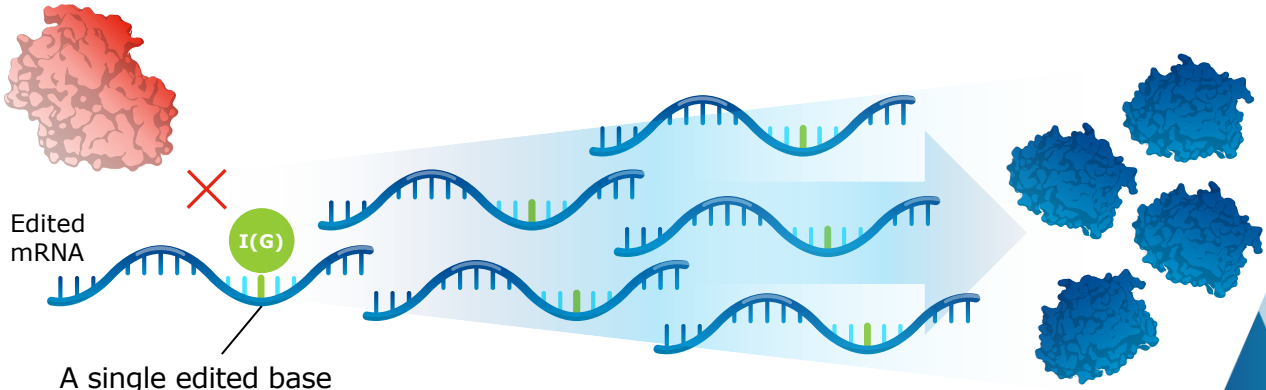
Decreased protein production

"Dialed up" Gene Expression



Catalytically Efficient AIMer

AIMer **edits** and **durably stabilizes** mRNA



A single edited base permanently disrupts the motif

Stable mRNA yields **increased protein production**

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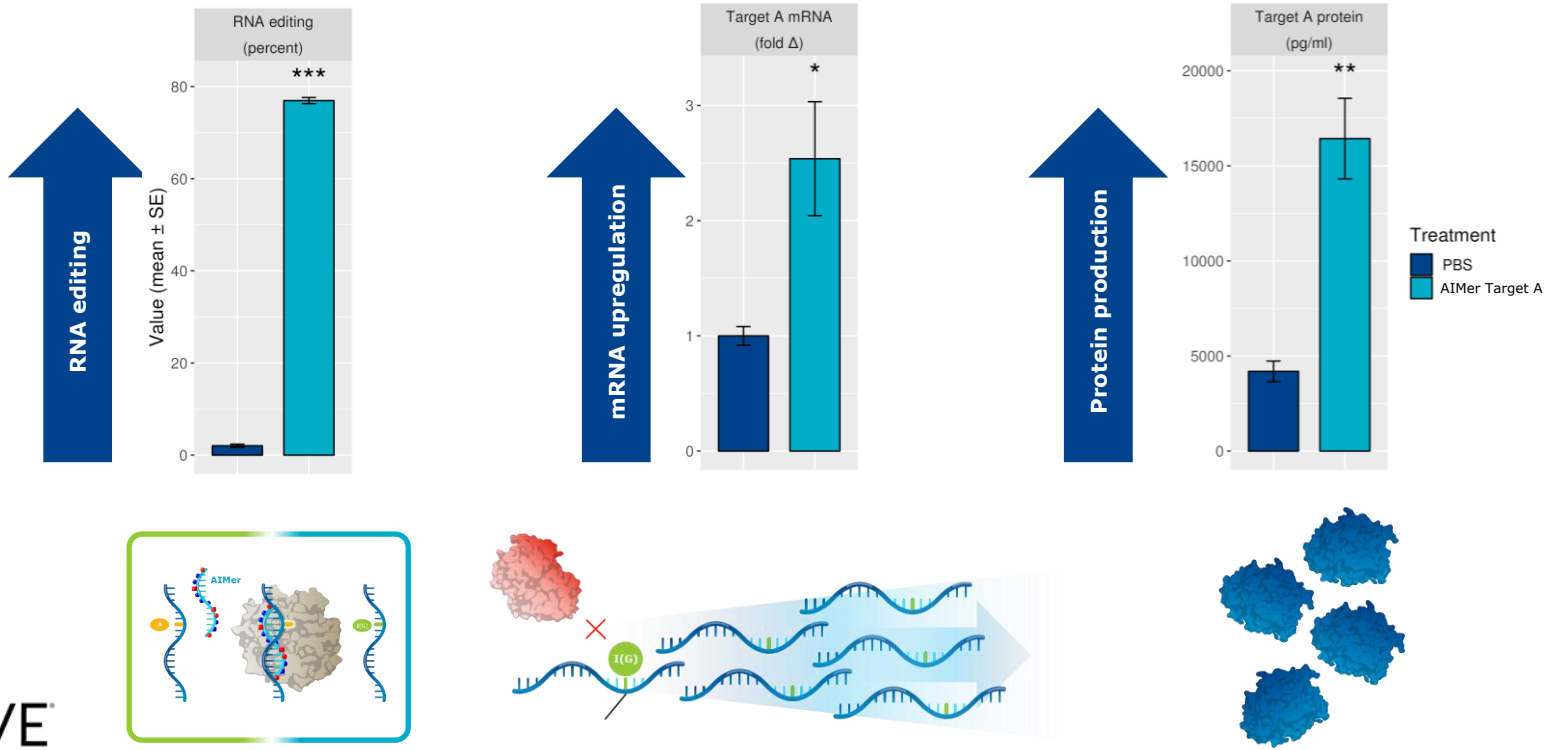
Proof-of-concept: Editing RNA motifs to upregulate mRNA and increase protein

RNA editing, mRNA upregulation and protein expression *in vivo* for an undisclosed Target A



Mapping the "Edit-verse"

Kenneth Longo, PhD
Vice President, Data Science

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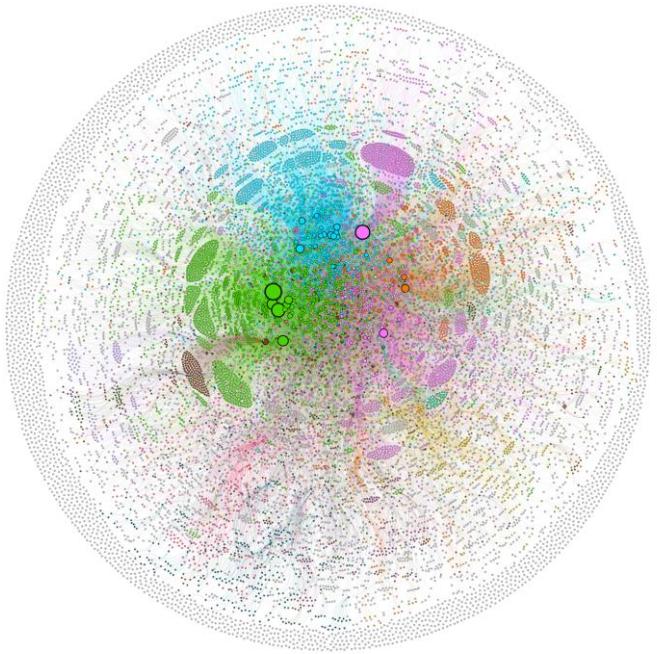
Mapping the RNA editing target universe

- The editable gene-disease network, **"The Edit-Verse",** is enormous and includes coding and non-coding regions of transcripts
- The **upregulation target universe** is particularly interesting because many diseases are associated with reduced protein expression:
  - Haploinsufficient and hypomorphic variants
  - Regulatory variants
- Upregulation offers the potential to address multiple pathogenic mutations **with a single therapy**

Gene-Disease Network



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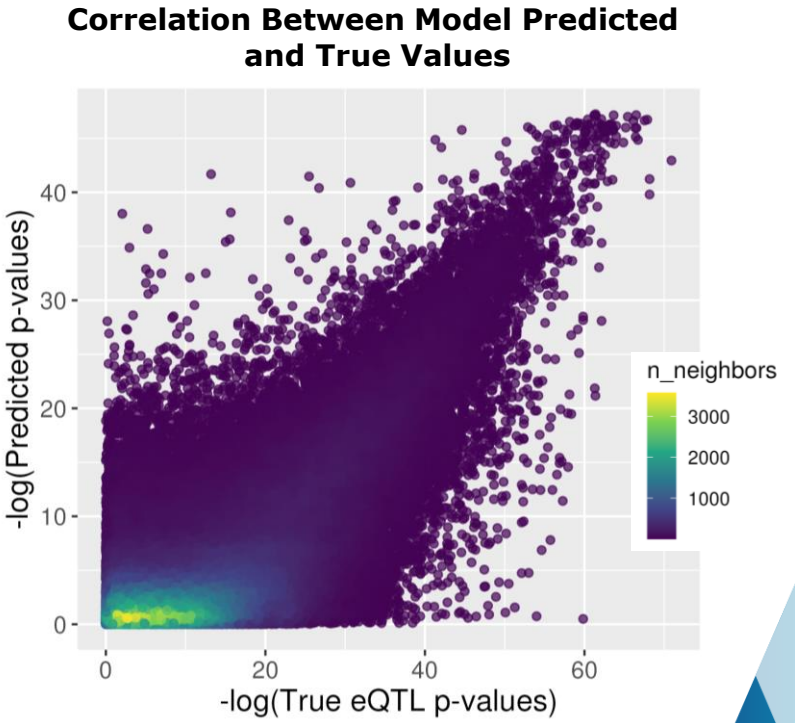
Wave's deep learning model predicts novel edit sites that impact transcriptional regulation

- Proprietary model constructed using large expression quantitative trait loci (eQTL) databases that can predict the impact of editing on gene expression
- Model achieved good predictive accuracy on known eQTLs
- Results include long list of *novel* eQTL sites where an A-to-G edit, never-before observed in nature, confidently predicts changes in transcript levels for >50% of proteome
- Ongoing model development is expected to expand Edit-verse further



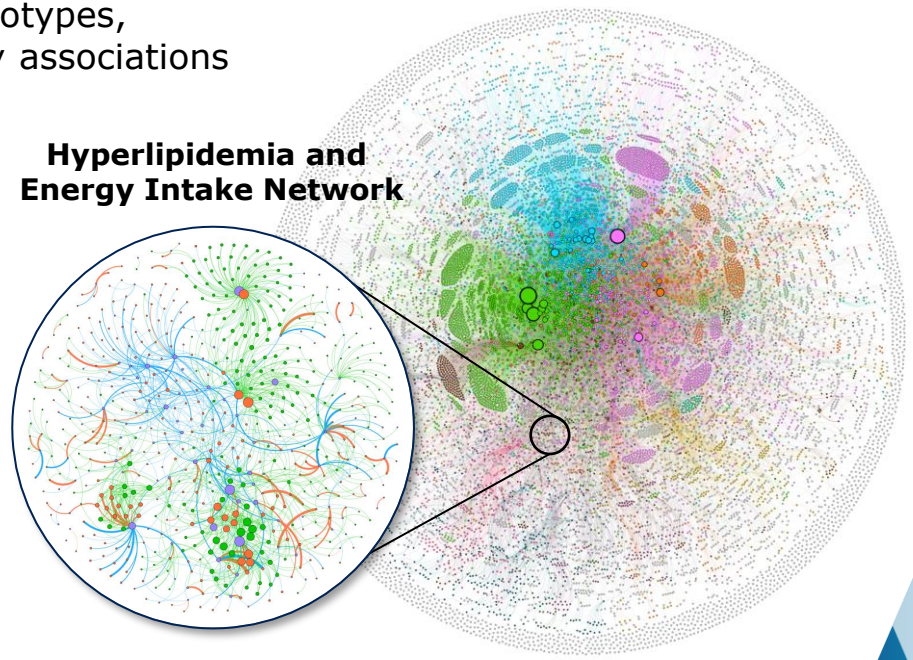
Chen et al, 2022: doi.org/10.1038/s41588-022-01102-2; Zhou et al, 2018: doi.org/10.1038/s41588-018-0160-6

Identify AIMer-mediated upregulation opportunities in disease sub-networks

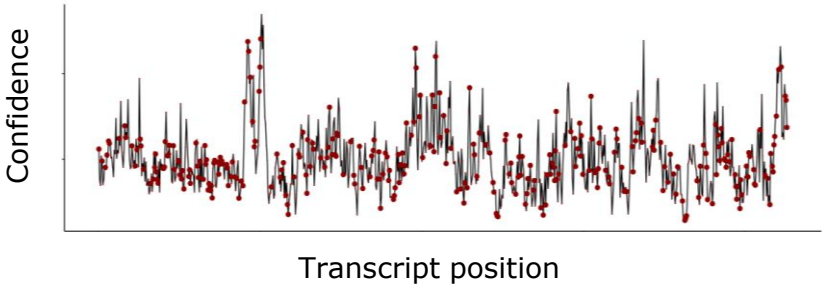
- For instance, we can zoom into network for the hyperlipidemia and energy intake GWAS phenotypes, which contains 96 genes and disease-pathway associations
- We can then make editing predictions for any in-network gene of interest

Gene-Disease Network

Hyperlipidemia and Energy Intake Network



ADAR Amenable Sites Predicted to Impact Half-Life



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Mining the "Edit-verse"

Ginnie Yang, PhD
Senior Vice President, Translational Medicine

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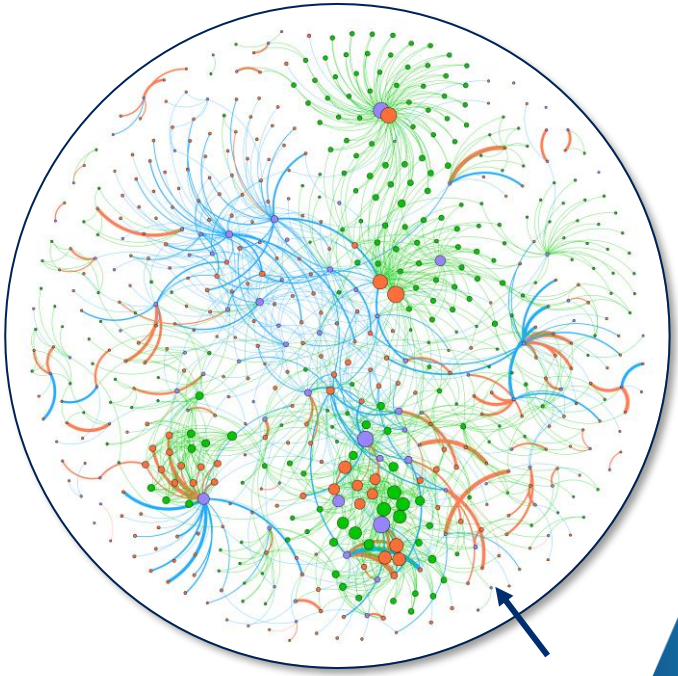
Edit-verse subnetwork reveals “Target A”: Metabolic syndrome target uniquely suited for AIMer upregulation

Target A

- Liver target for upregulation, non-incretin therapy
- Strongly implicated in metabolic disease, with indirect causation in familial disorders
- Few therapies today provide weight loss in this specific patient population
- Estimate 90 million potential patients in the US and Europe with metabolic syndrome and obesity
- Serum protein levels and biomarkers available to assess target engagement

Hyperlipidemia and Energy Intake Network



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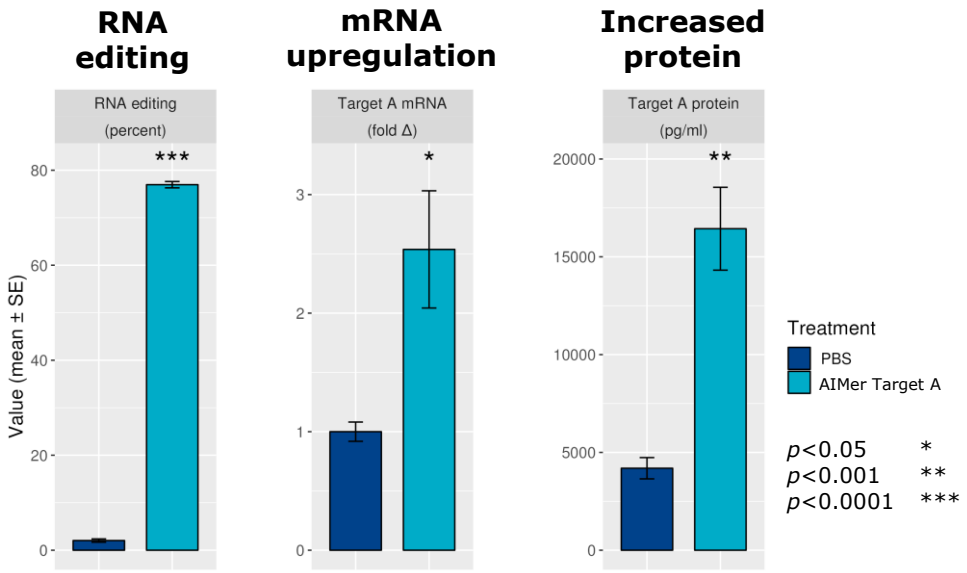
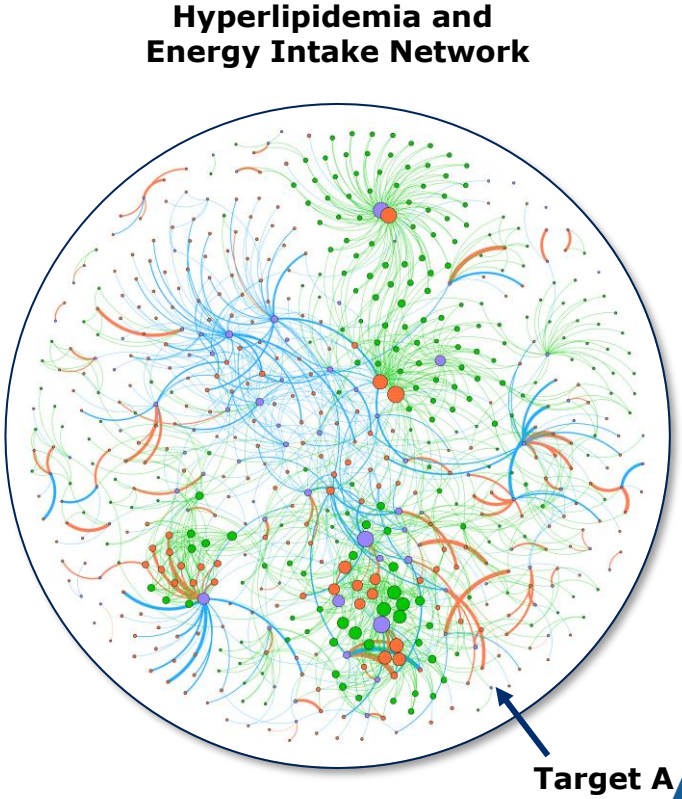
First preclinical *in vivo* PoC: upregulating endogenous protein to restore healthy metabolic phenotype

>75% RNA editing led to >2-fold increase of mRNA, and similar degree of protein upregulation *in vivo* with GalNAc-AIMer in young DIO mice



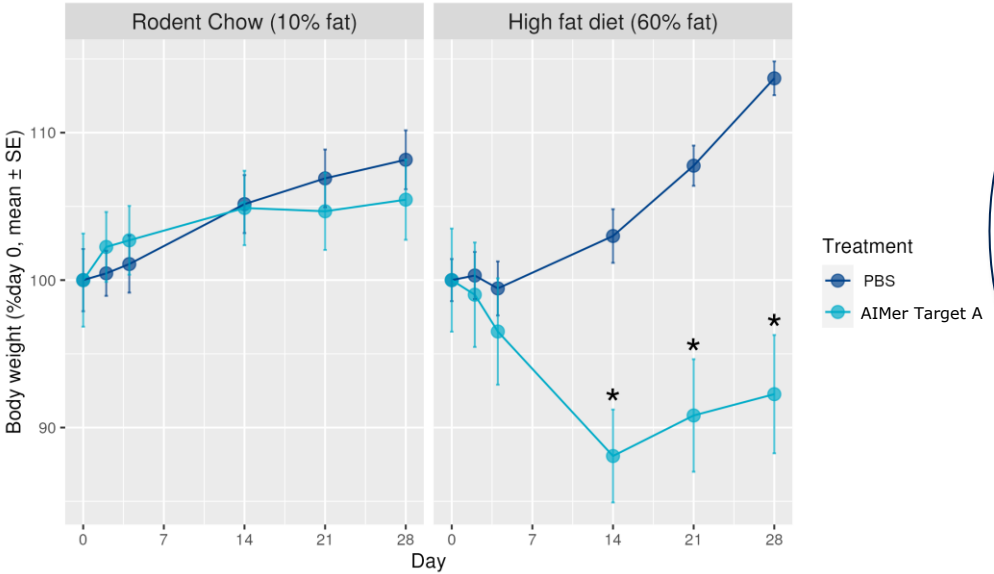


PoC: proof-of-concept Analysis of terminal endpoints (day 31) is shown. Each variable was analyzed using Welch's two-sided t-test. Significance was evaluated a $p<0.05$.

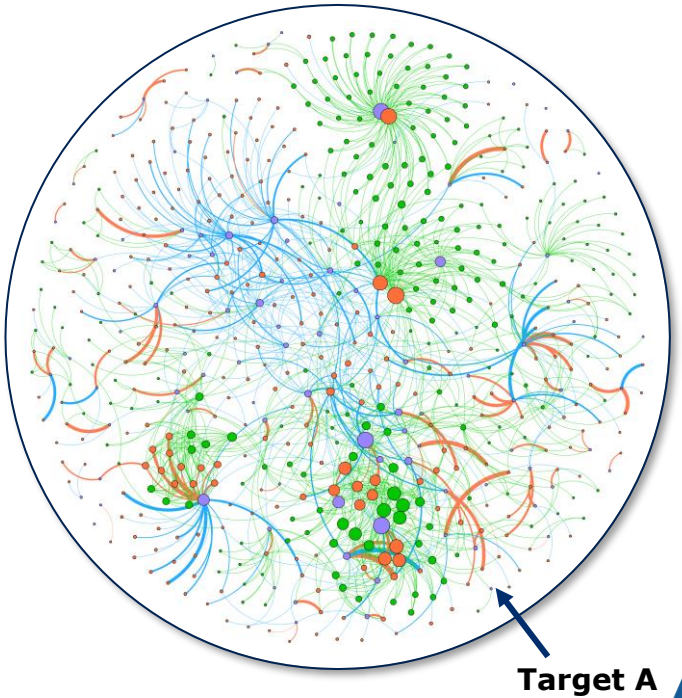
Substantial upregulation of protein induces weight loss

- ~3-fold upregulation of Target A protein with GalNAc-AIMer led to weight reduction in DIO mice

Significant Weight Loss



Hyperlipidemia and Energy Intake Network

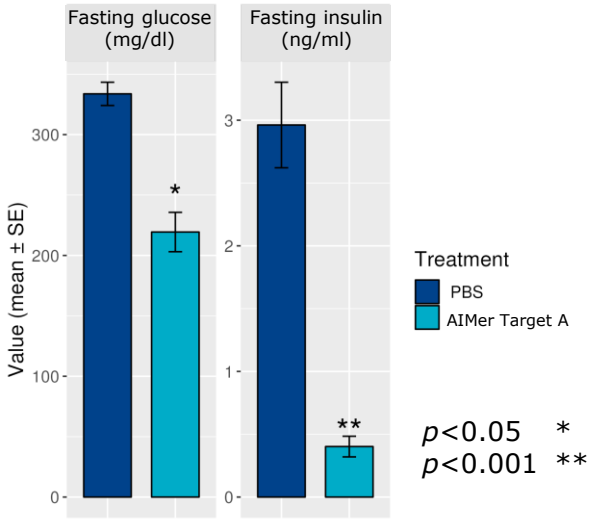


Body weight data were analyzed using a linear mixed effects model to assess the fix effects of diet, time and treatment, controlling for the initial day 0 body weight (continuous covariate) and subject (random effect). Fasted glucose and insulin data (from study termination, day 31) was analyzed using Welch's two-sided t-test. Significance was evaluated at $p<0.05$.

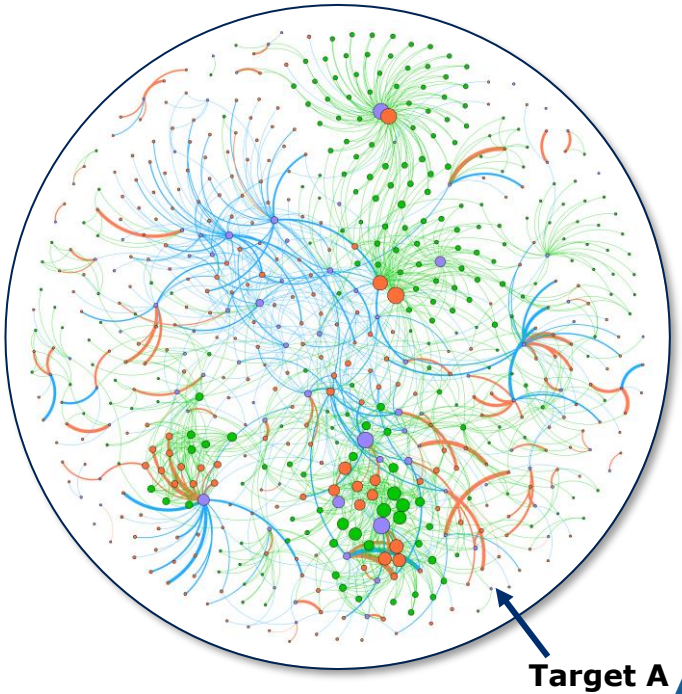
Upregulation of Target A protein improves insulin sensitivity

- ~3-fold upregulation of Target A protein with GalNAc-AIMer led to improved insulin sensitivity in DIO mice

Improved Insulin Sensitivity



Hyperlipidemia and Energy Intake Network



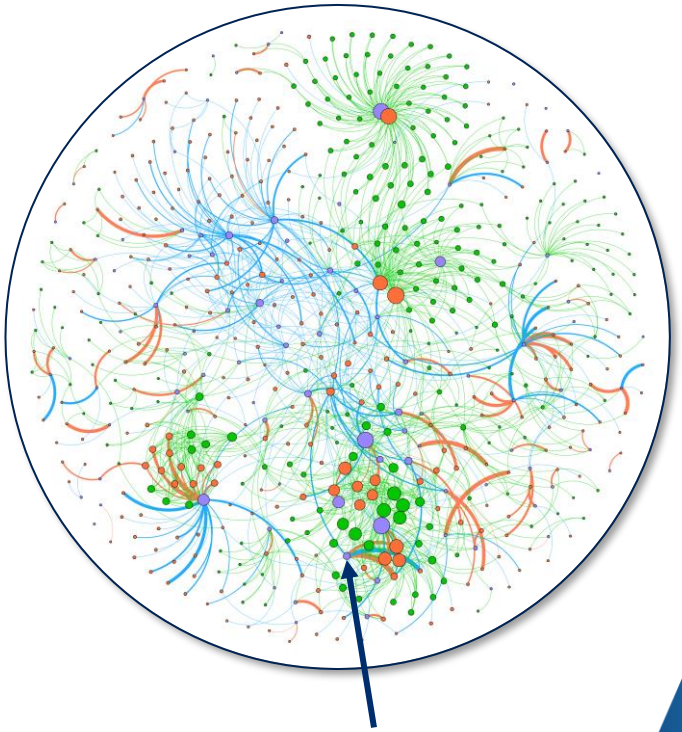
Body weight data were analyzed using a linear mixed effects model to assess the fix effects of diet, time and treatment, controlling for the initial day 0 body weight (continuous covariate) and subject (random effect). Fasted glucose and insulin data (from study termination, day 31) was analyzed using Welch's two-sided t-test. Significance was evaluated at $p<0.05$.

Target B upregulation offers a first-in-class therapeutic approach for hyperlipidemia

Target B

- Liver target for upregulation
- Hyperlipidemia; first-in-class therapeutic approach
- Estimate ~3 million target patients in US and Europe
- Serum biomarkers available to assess target engagement and efficacy
- Potential clinically meaningful benefit of >2 fold upregulation of target mRNA

Hyperlipidemia and Energy Intake Network



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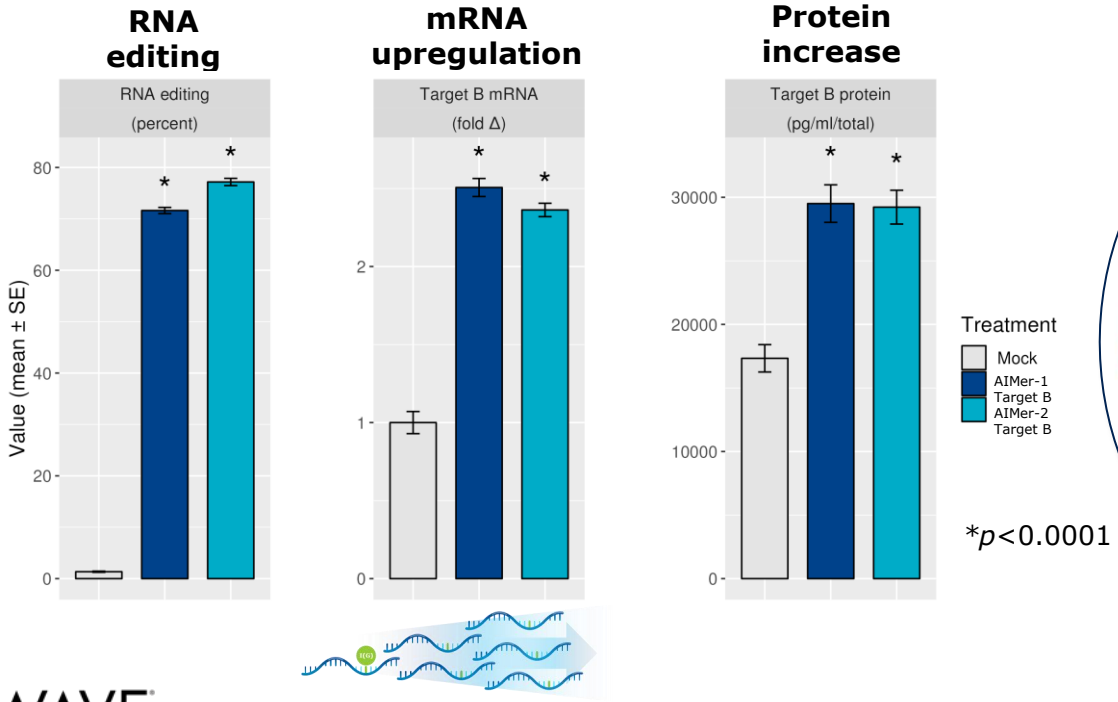
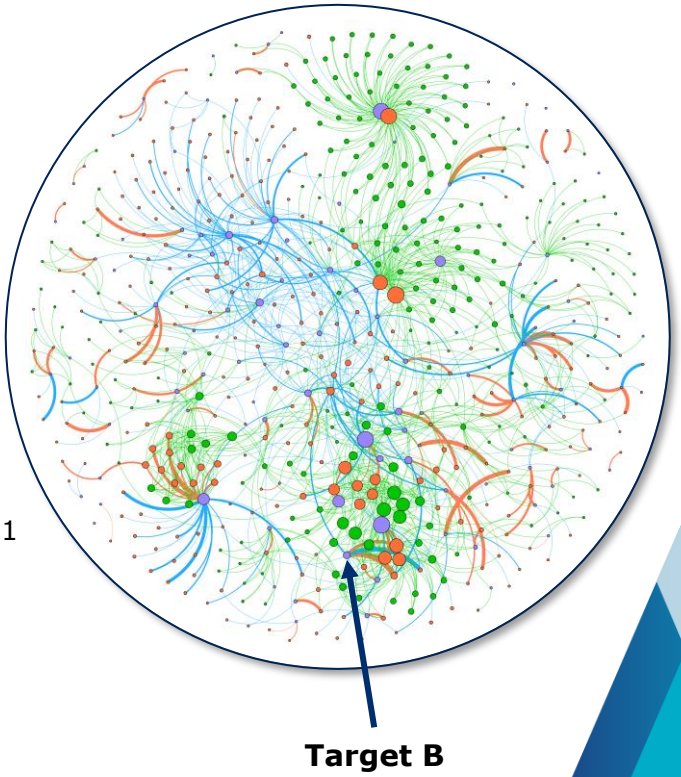
>70% editing achieves ~2-fold upregulation with corresponding increase in protein

Primary human hepatocytes *in vitro*



Hyperlipidemia and Energy Intake Network

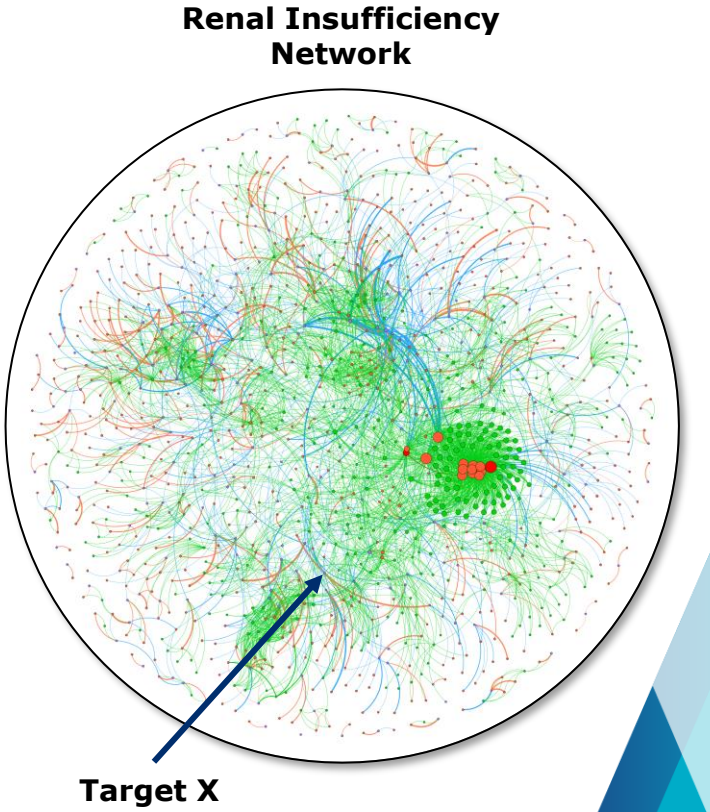


Variable were analyzed with ANOVA and Tukey's HSD. Significance was evaluated at $p<0.0001$.

Upregulation of liver Target X stops decline in kidney function

Target X

- Liver target for upregulation
- Target X produces a secreted protein to treat kidney disease
- Estimate ~170K target patients in US and Europe
- Therapeutic rationale supported by genetic insights, PheWAS, and observational data
- Plasma biomarkers available to assess target engagement
- ~2-fold upregulation in secreted protein expected to be clinically meaningful



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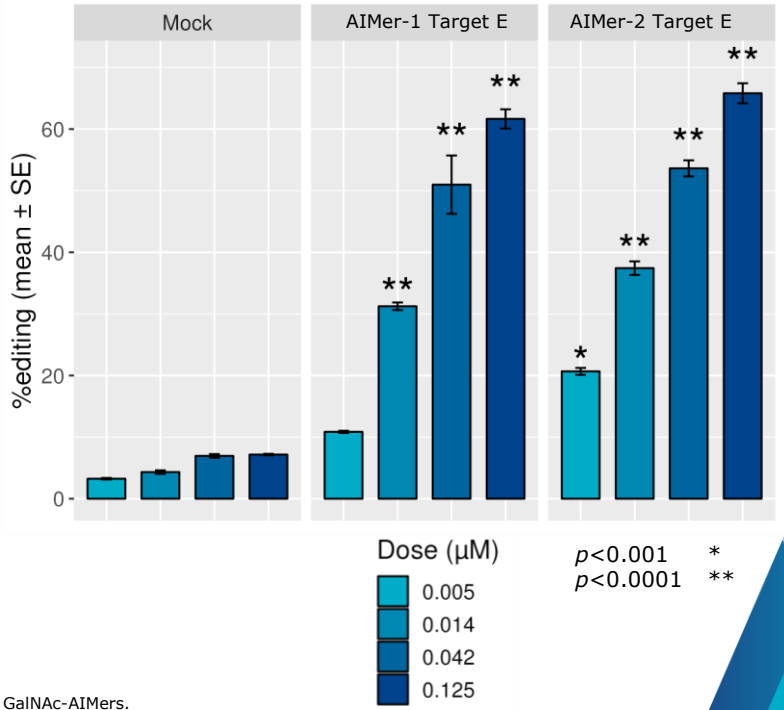
Building on success of AATD: Target E correction restores normal metabolism in rare genetic disease

Target E

- Liver target for correction
- Rare genetic disease
- High unmet need population not addressed with current therapeutic options
- ~17,000 patients addressable with correction approaches in US and Europe
- Fully translatable serum biomarker
- ~15-30% editing expected to deliver clinically meaningful benefit

Proof-of-concept RNA editing in human primary hepatocytes



Two-way ANOVA (Treatment, Dose) and Tukey's HSD. Significance was evaluated at $p<0.05$, GalNAc-AIMers.

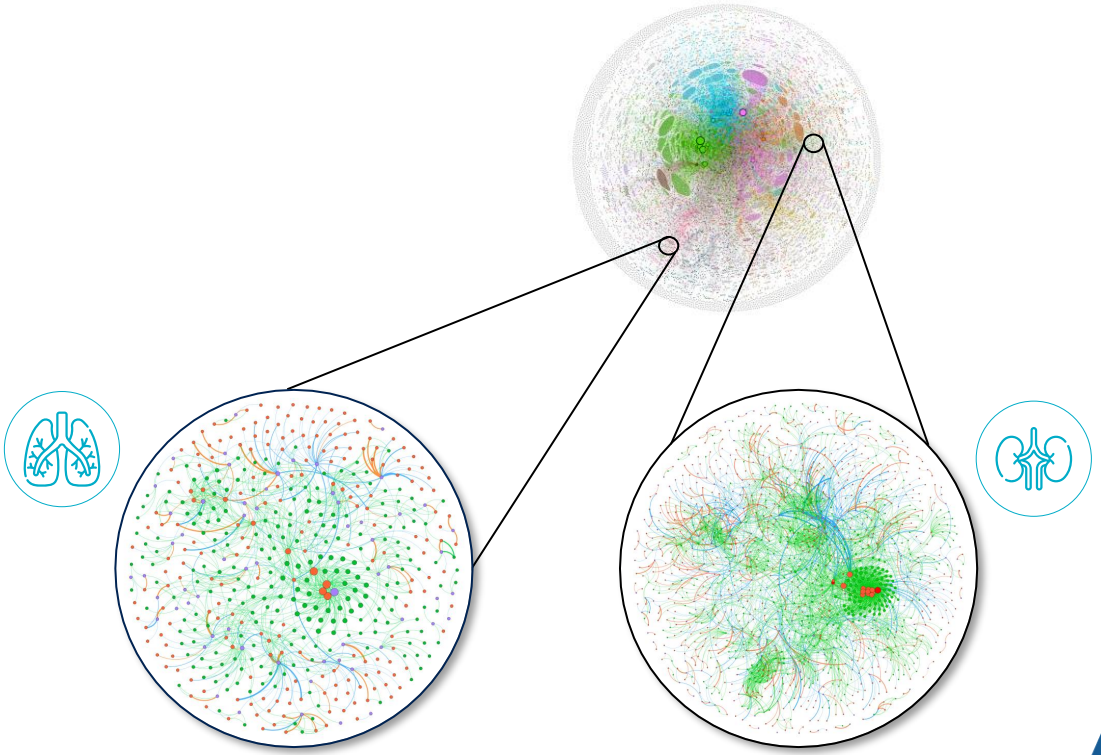
AIMer targetable diseasomes in extra-hepatic organs

Hepatic

- Target A
- Target B
- Target X
- Target E

Extra-hepatic

- Target F
- Target G



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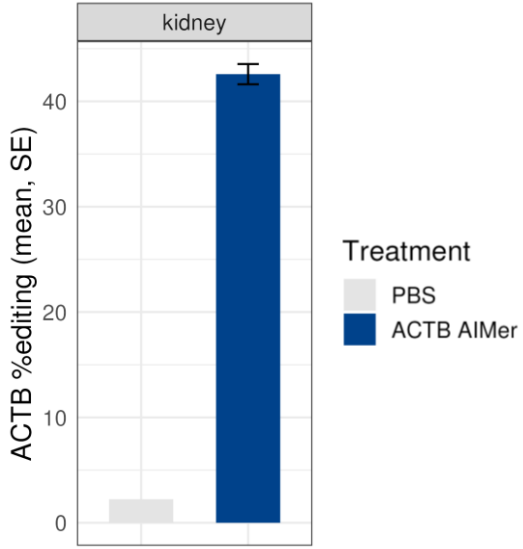
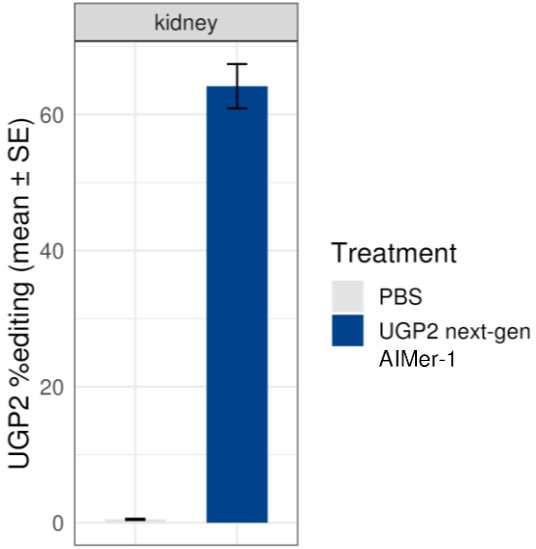
AIMers deliver to proximal and distal convoluted tubules of kidney and achieve substantial editing

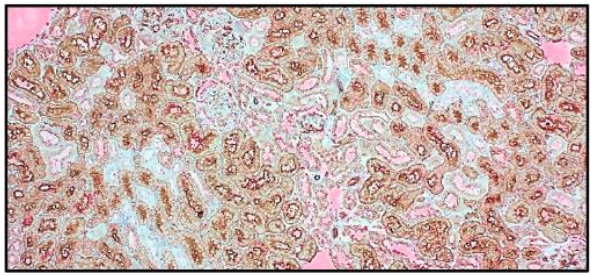
~40% editing of ACTB in NHP 1-week post-single dose (SC)



~60% editing of UGP2 in mice 1-week post single dose (IV)



AIMers (red) accumulated in proximal convoluted tubules (brown) in the NHP kidney following subcutaneous administration

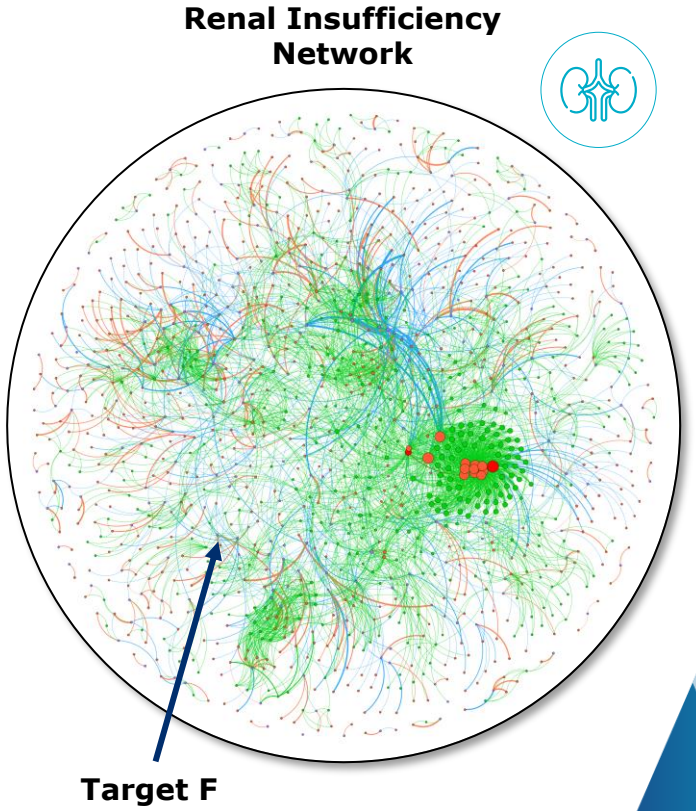


Left: Monian *et al.*, 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1; SC: subcutaneous; IV: intravenous

Upregulation of Target F restores kidney function in a rare genetic kidney disease

Target F

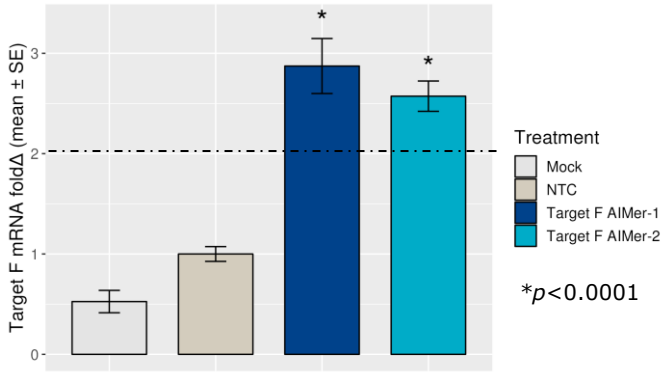
- Kidney target for upregulation
- Rare genetic kidney disease that leads to ESRD and need for dialysis / transplantation; High unmet need with few treatment options currently available
- ~85K patients in US and Europe addressable with upregulation approach
- Urinary biomarkers available to assess upregulation
- Clinically meaningful benefit may be achieved with 2-fold upregulation

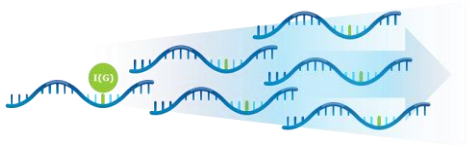


ESRD: End Stage Renal Disease

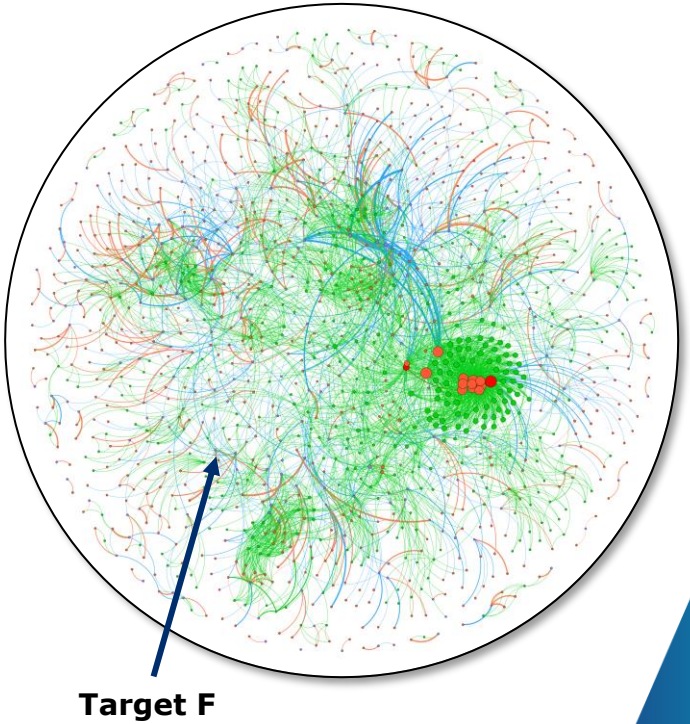
Achieved >2-fold upregulation of Target F mRNA *in vitro* with RNA editing

Upregulation of Target F mRNA in Human kidney tubular epithelial cells





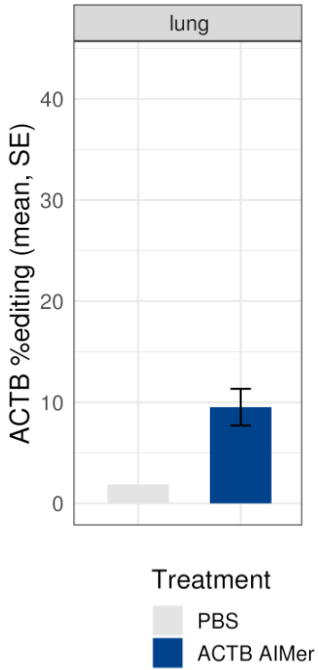
Renal Insufficiency Network



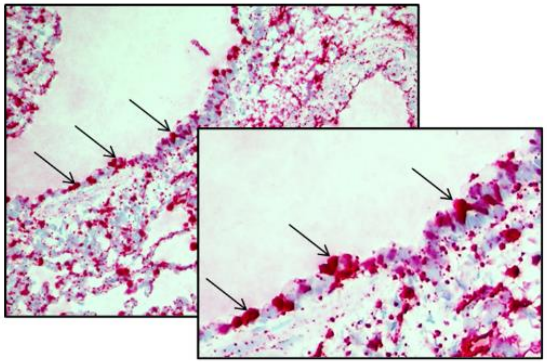
One-Way Anova; samples compared to NTC with Tukey's HSD test. Significance evaluated at $p<0.0001$.

Proprietary AIMer modifications enhance delivery to lung tissue and achieve significant editing *in vivo*

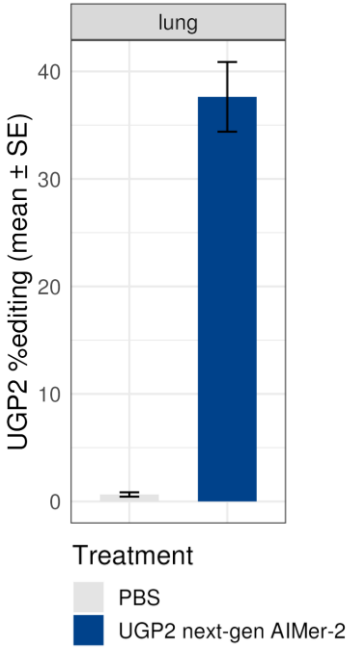
~10% editing of ACTB in NHP
1-week post-single dose (SC)



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



>35% editing of UGP2 in mice
1-week post single dose (IV)



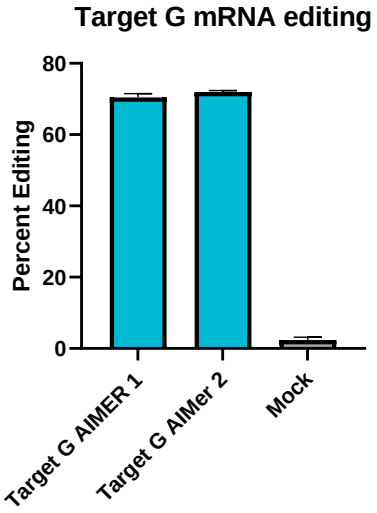
Left: Monian *et al.*, 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1

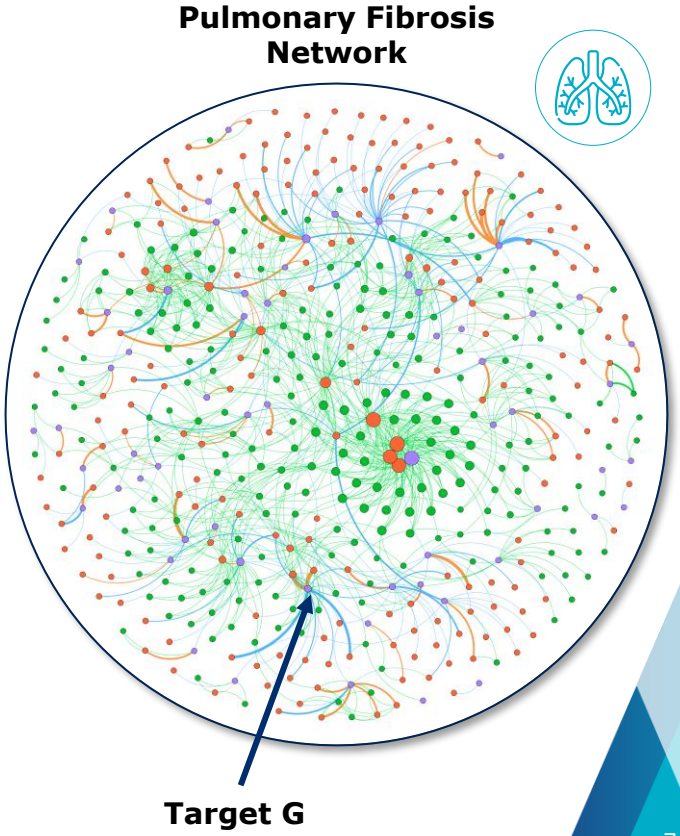
Correction of Target G mutation restores protein function in patients with a genetic lung disease

Target G

- Lung disease target for correction
- Genetic lung disease with target patient population not addressed with available therapies
- ~5K patients amenable to correction approaches in US and Europe
- Clinically meaningful benefit expected with 20% correction
- Established clinical regulatory pathway

In vitro





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Multiple RNA editing opportunities to build high-value pipeline beyond WVE-006

- The Edit-verse is substantial and still expanding
- Advancing work for a diverse set of undisclosed targets addressing areas of high unmet need, including both rare and prevalent diseases

Potential to advance any combination of targets into preclinical development

	Hepatic (GalNAc-AIMers)				Extra-Hepatic (AIMers)	
	Target A	**Target B**	**Target X**	**Target E**	**Target F**	**Target G**
Approach	Upregulation	Upregulation	Upregulation	Correction	Upregulation	Correction
Tissue	Liver	Liver	Liver	Liver	Kidney	Lung
Therapeutic Area	Metabolic	Metabolic	Renal	Rare	Renal	Rare
Estimated Patients (US and Europe)	~90M	~3M	~170K	~17K	~85K	~5K

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Closing Remarks

Paul Bolno, MD, MBA
President and CEO

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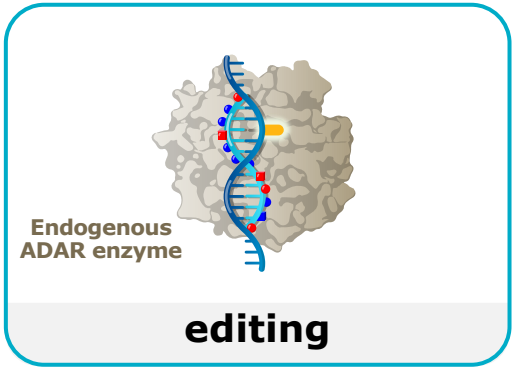
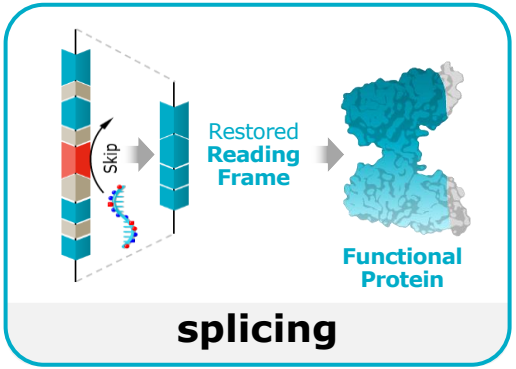
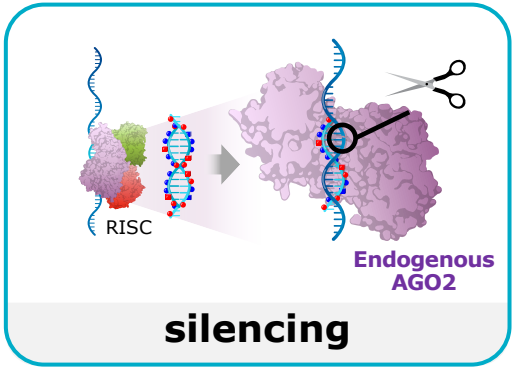
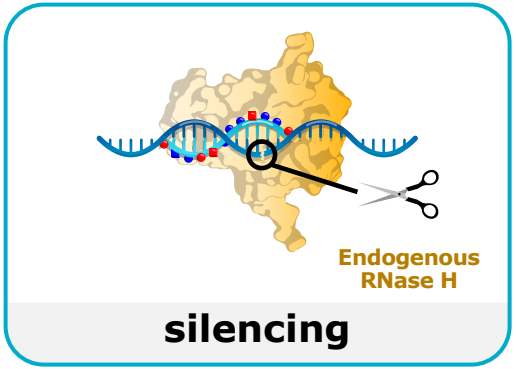
Wave is building the leading RNA medicines company









- Most versatile RNA medicines platform (PRISM™) in the industry
- Best-in-class nucleic acid chemistry applicable across modalities
- Ability to access novel / untapped areas of disease biology
- Platform learnings and clinical validation continue to increase probability of success

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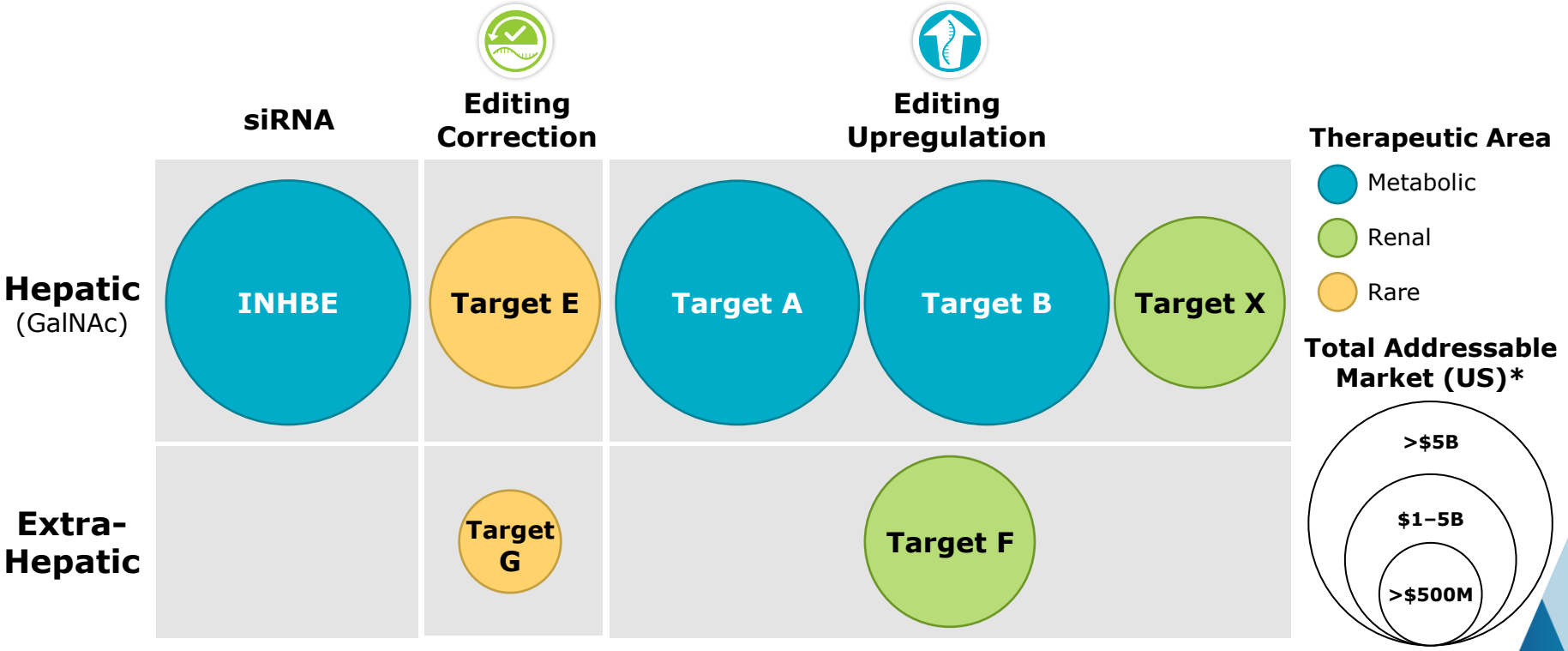
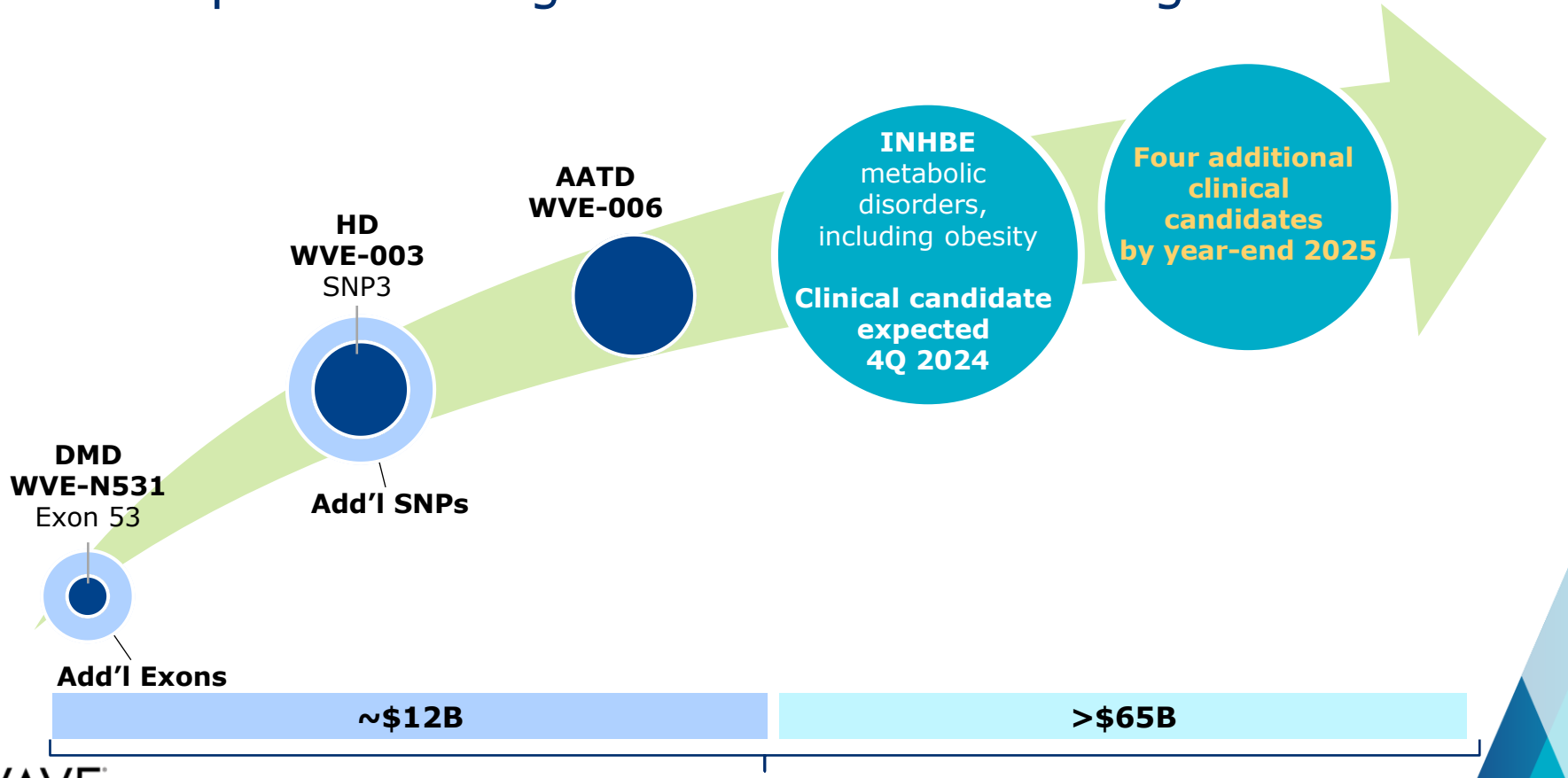
Anticipate five new clinical candidates by year-end 2025



*Assumes 100% share of addressable patients

Wave is poised for significant and sustained growth



Note: Bubble size illustrative of size of total addressable US market (assuming 100% share of addressable patients)

Paul Bolno, MD, MBA
President and CEO

Anne-Marie Li-Kwai-Cheung
Chief Development Officer

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

Q&A

Kenneth Longo
Vice President,
Discovery Data Science

Ginnie Yang
Senior Vice President,
Translational Medicine

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Thank you

For more information:
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