RNA editing of *PNPLA3 I148M* decreases hepatocyte fat content

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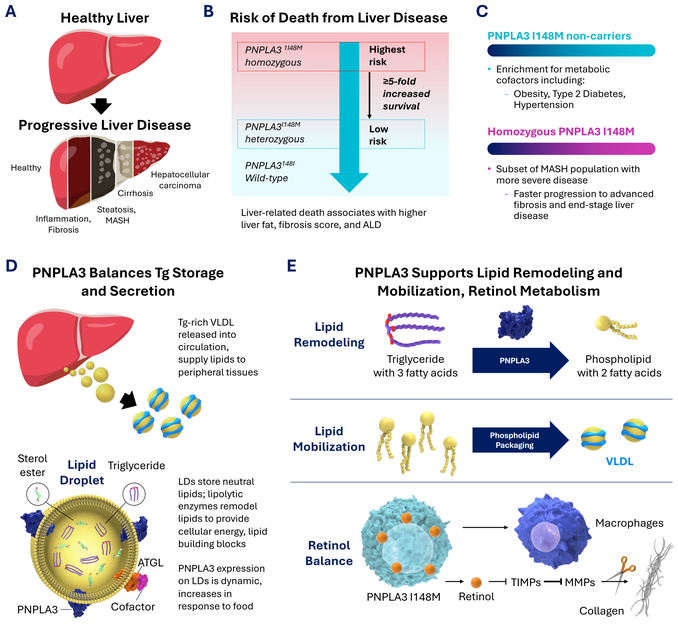
SUMMARY

- The PNPLA3 I148M variant disrupts PNPLA3 function and hepatic lipid homeostasis; it is associated with a substantially increased risk of liver-related deaths, including from MASH (metabolic dysfunction-associated steatohepatitis) and hepatic steatosis.[1-4]
- We have developed RNA editing oligonucleotides, or AIMers, that are designed to edit the base sequence of *PNPLA3 I148M* transcripts and restore functional PNPLA3 protein.
- In primary human hepatocytes homozygous for the *PNPLA3 I148M* variant in monolayer culture, HEPATOPAC® culture, or long-term culture, *PNPLA3* AIMers were potent and supported substantial editing of the *PNPLA3 I148M* transcript, exceeding the ~50% threshold expected to lower risk for liver disease by restoring a heterozygous PNPLA3 I148M phenotype.
- RNA editing with PNPLA3 AIMers significantly decreased lipid droplet density in human hepatocytes in HEPATOPAC® and long-term cultures. RNA editing decreased lipid droplet density more than a small-interfering RNA (siRNA), which decreased the expression of the *PNPLA3 I148M* transcript.
- In wild-type mice that do not express the human *PNPLA3 gene*, a single dose of multiple PNPLA3 AIMers achieved concentrations in the liver that are expected to support substantial editing based on pharmacokinetic-pharmacodynamic (PK-PD) observations in primary human hepatocytes.
- Together, these preclinical data support the advancement of a *PNPLA3 RNA* editing approach for the treatment of genetic liver disease caused by a homozygous *PNPLA3 I148M* variant.
- Wave is advancing WVE-008 as an investigational treatment for genetically defined liver disease. We expect to file a CTA in 2026.

INTRODUCTION TO MASH AND PNPLA3

- *PNPLA3 I148M* is the strongest genetic risk factor for liver disease, including MASH (**Figure 1A**).[1-4]
- The *PNPLA3 I148M* variant (rs738409 G) is common worldwide, with >9 million homozygous individuals in US and Europe. Heterozygous individuals have lower risk of liver disease, and >5-fold increase in survival compared to homozygotes (**Figure 1B**).[1-4]
- Up to 25% of all MASH cases are homozygous for *PNPLA3 I148M* (**Figure 1C**).[5]
- In hepatocytes of the liver, PNPLA3 helps to balance triglyceride and phospholipid storage and secretion by supporting lipid remodeling and mobilization, and retinol metabolism (**Figure 1D,E**).[6]
- The *PNPLA3 I148M* allele disrupts PNPLA3 function and hepatic lipid metabolism.[6]

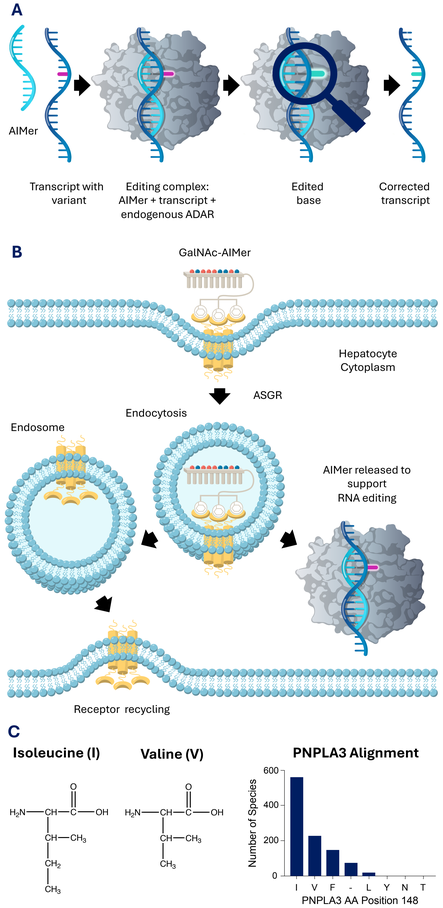
Figure 1. PNPLA3 I148M variant increases risk for liver disease



INTRODUCTION TO RNA EDITING

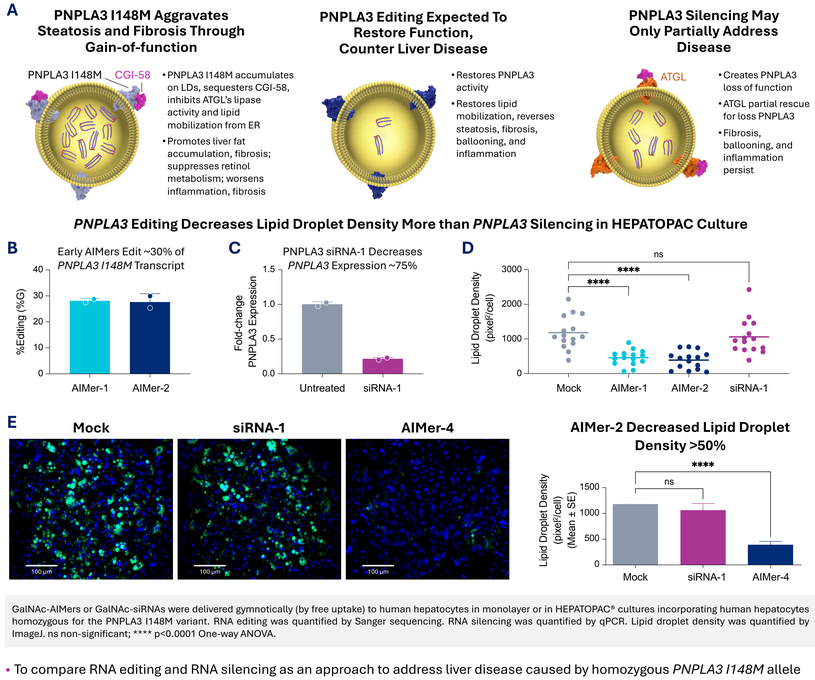
- We have developed RNA editing oligonucleotides, called AIMers, that direct efficient and specific A-to-I RNA editing of mRNA with endogenous ADAR (adenosine deaminase acting on RNA) enzymes (**Figure 2A**).[7,8]
- These AIMers are conjugated to *N*-Acetylgalactosamine (GalNAc) to facilitate delivery to liver hepatocytes (**Figure 2B**).
- PNPLA3 GalNAc-AIMers are designed to convert the methionine in PNPLA3 I148M, which suppresses enzymatic activity by 80%, to valine, which is expected to restore access to the active site and PNPLA3 enzymatic activity (**Figure 2C**). Bioinformatic analysis shows that this amino acid in PNPLA3 is valine in over 200 species, which further supports the notion that an I148V substitution will be functional (**Figure 2C**).
- Emerging evidence showing that PNPLA3 plays an important role in hepatic lipid metabolism[6] suggests that an RNA editing approach that restores PNPLA3 protein function may be preferable to silencing *PNPLA3* expression for the treatment of liver disease driven by this variant.

Figure 2. RNA editing and delivery to liver hepatocytes



RESULTS

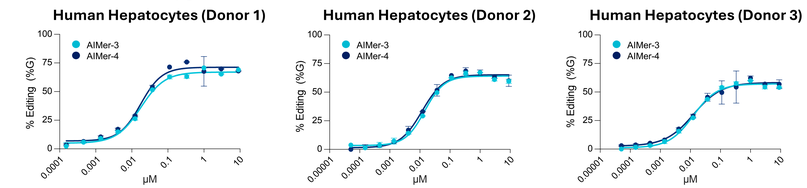
Figure 3. PNPLA3 I148M variant impairs lipase activity, inhibits triacylglycerol hydrolysis through gain-of-function to promote hepatic steatosis



GalNAc-AIMers or GalNAc-siRNAs were delivered gymnotically (by free uptake) to human hepatocytes in monolayer or in HEPATOPAC® cultures incorporating human hepatocytes homozygous for the PNPLA3 I148M variant. RNA editing was quantified by Sanger sequencing. RNA silencing was quantified by qPCR. Lipid droplet density was quantified by ImageJ. ns non-significant; **** p<0.0001 One-way ANOVA.

- To compare RNA editing and RNA silencing as an approach to address liver disease caused by homozygous *PNPLA3 I148M* allele (**Figure 3A**), we evaluated PNPLA3 AIMers and a PNPLA3 (siRNA-1), which was designed to silence expression of *PNPLA3* transcripts regardless of genotype, in HEPATOPAC® cultures incorporating human hepatocytes homozygous for the *PNPLA3 I148M* allele. HEPATOPAC® cultures better replicate the physiological environment of the liver than monolayer hepatocytes in primary culture.
- AIMer-1 and AIMer-2 are early, non-optimized PNPLA3 GalNAc-AIMers. AIMer-1 and AIMer-2 directed ~30% editing of the *PNPLA3* transcript (**Figure 3B**). siRNA-1 decreased expression of *PNPLA3* transcripts by ~75% (**Figure 3C**).
- AIMer-1 and AIMer-2 significantly decreased lipid droplet density (>50%, p<0.0001) compared to mock treatment, whereas siRNA-1 did not (**Figure 3D**).
- AIMer-4, a more advanced PNPLA3 GalNAc-AIMer, significantly decreased lipid droplet density (>50%, p<0.0001) compared to mock treatment, whereas siRNA-1 did not (**Figure 3E**).
- These data indicate that RNA editing may be preferable to *PNPLA3* silencing to restore healthy lipid metabolism in hepatocytes.

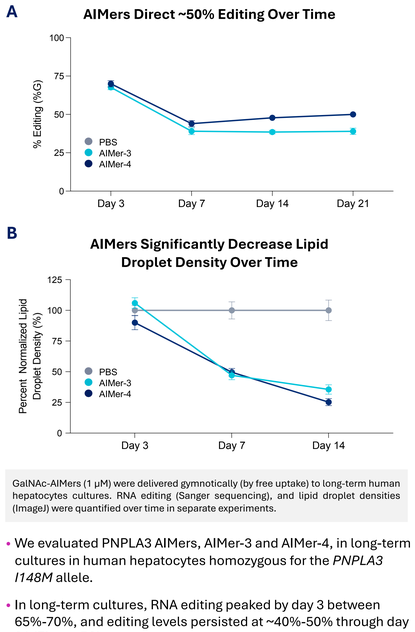
Figure 4. PNPLA3 AIMers support efficient RNA editing in hepatocytes from multiple human donors with homozygous *PNPLA3 I148M* variants



GalNAc-AIMers were delivered gymnotically (by free uptake) to human hepatocytes from three different donors at concentrations ranging from 50 pM to 9 µM for 72 hrs. RNA editing was quantified by Sanger sequencing.

- We evaluated PNPLA3 GalNAc-AIMers, AIMer-3 and AIMer-4, in hepatocytes derived from multiple human donors who were homozygous for the *PNPLA3 I148M* allele.
- *In vitro* half maximal effective concentrations (EC_{50}s) for the AIMers approximated 12 nM, with 60%-70% of the *PNPLA3* transcripts edited at the maximum (**Figure 4**), indicating that the PNPLA3 AIMers are both potent and efficient.

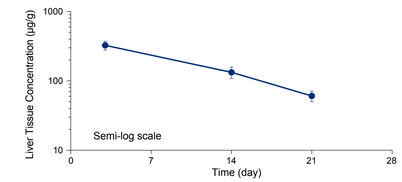
Figure 5. *PNPLA3* RNA editing improves lipid phenotypes in long-term human hepatocyte cultures



GalNAc-AIMers (1 µM) were delivered gymnotically (by free uptake) to long-term human hepatocytes cultures. RNA editing (Sanger sequencing), and lipid droplet densities (ImageJ) were quantified over time in separate experiments.

- We evaluated PNPLA3 AIMers, AIMer-3 and AIMer-4, in long-term cultures in human hepatocytes homozygous for the *PNPLA3 I148M* allele.
- In long-term cultures, RNA editing peaked by day 3 between 65%-70%, and editing levels persisted at ~40%-50% through day 21 (**Figure 5A**).
- In the same cultures, AIMer-3 and AIMer-4 decreased lipid droplet density by 47% and 50% (day 7) and by 65% and 75% (day 14), respectively, with the AIMer (AIMer-2) that supported the most editing having the largest lowering effect on lipid density (**Figure 5B**).
- Together, these data support the hypothesis that *PNPLA3* RNA editing will restore healthy lipid metabolism in hepatocytes.

Figure 6. PNPLA3 AIMers achieve tissue concentrations expected to support *PNPLA3* RNA editing in mice



Wild-type mice were dosed subcutaneously with AIMer-4, and tissue concentrations in liver were quantified by ELISA.

- In wild-type mice, which lack the *PNPLA3 I148M* variant, we assessed concentrations of AIMer in the liver after dosing.
- Based on experiments in hepatocytes, the concentrations of AIMer-4 detected in the liver (**Figure 6**) are expected to yield substantial RNA editing.
- Overall, these data support continued advancement of PNPLA3 AIMers for the treatment of liver disease caused by homozygous *PNPLA3 I148M* variant.

References: 1. Unalp-Arida, A. and Ruhl, C.E. 2020. *Hepatology* doi: 10.1002/hep.31032; 2. Xiang, H. 2021. *J Cancer* doi: 10.7150/jca.57951; 3. Kozlitina, J. and Sookoian, S. 2025. *Liver Int.* doi: 10.1111/liv.16123; 4. Carlsson, B., et al., 2020. *Aliment Pharmacol Ther.* doi: 10.1111/apt.15738; 5. Petta, S., et al., 2025. *Liver Int.* doi: 10.1111/liv.16133; 6. Johnson, S.M. et al., 2024. *Nature Commun.* doi: 10.1038/s41467-024-49224-x; 7. Monian, P. et al., 2022. *Nat. Biotechnol.* doi: 10.1038/ s41587-022-01225-1; 8. Lu, G. et al., 2024, *Nucleic Acids Research* doi: 10.1093/nar/gkae681.
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