Using Lipid Nanoparticles to Efficiently Deliver CRISPR/Cas9 for Genome Editing

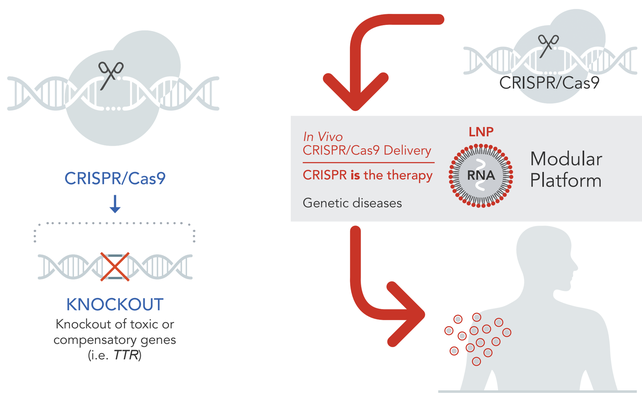
S. C. Alexander, M. Maetani, R. Parmar, K. Wood, M. Pink, J. Seitzer, N. Gardner, A. Kanjolia, T. DiMezzo, E. Rohde, R. Lescarbeau, C. Shaw, J. Eby, J. Moginot, C. Boiselle, I. Liric, K. Walsh, B. Han, B. Murray, S. Roberts, D. Crawford, E. Essig, M. McCaman, Y. Chang

Intellia Therapeutics, Cambridge, MA, USA

INTRODUCTION

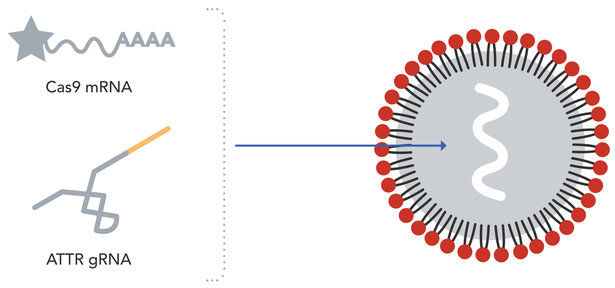
Transthyretin amyloidosis (ATTR) is a progressive disease caused by accumulation of amyloid deposits of misfolded transthyretin (TTR) protein in multiple tissues including the heart, nerves and gastrointestinal tract. Reduction of TTR monomer via stabilization of circulating tetramer and silencing of *TTR* gene expression in hepatocytes of ATTR patients have emerged as successful therapeutic strategies for chronically-administered medicines. As such, specific disruption (or knockout) of the *TTR* gene in hepatocytes using the CRISPR/Cas9 gene editing system is a potentially attractive next-generation treatment for ATTR, which may durably reduce the expression of TTR without the need for chronic therapy.

Objective: To develop NTLA-2001, a lipid nanoparticle (LNP) formulated CRISPR/Cas9 genome editing therapeutic, which targets the human TTR gene for the treatment for ATTR. NTLA-2001 is advancing toward the clinic with an IND submission planned for mid-2020.



METHODS

Lipid Nanoparticles (LNPs)



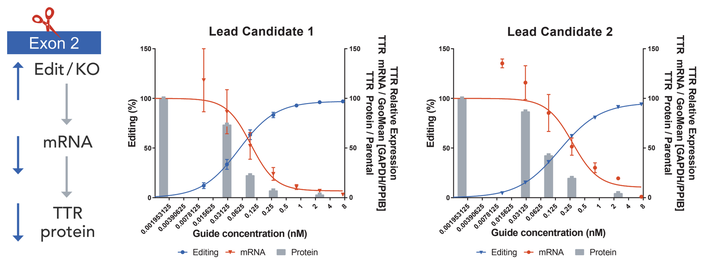
Key Advantages of LNP Delivery

- Large cargo capacity
- Transient expression
- Scalable synthetic manufacturing
- Redosing capability
- Low immunogenicity, well-tolerated & biodegradable

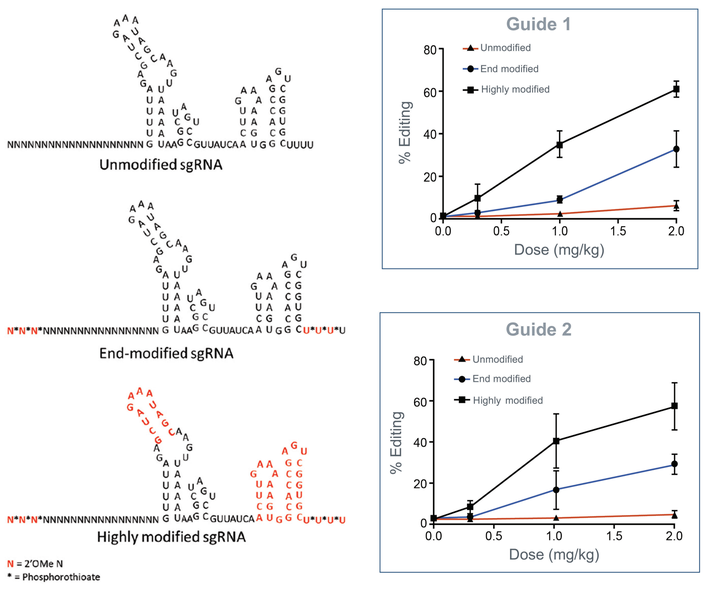
Liver Editing by Next Generation Sequencing (NGS): Genomic DNA (gDNA) was isolated from livers by homogenizing a liver biopsy. gDNA samples are sequenced (NGS) using amplicon sequencing directed to the site of interest. The editing percentage is defined as the total number of sequence reads with indels or substitutions divided by the total number of sequence reads, including wild-type.

RESULTS

Lead Human TTR LNPs Demonstrate On-Target Editing, Reduction of TTR mRNA and TTR Protein in Primary Human Hepatocytes *In Vitro*

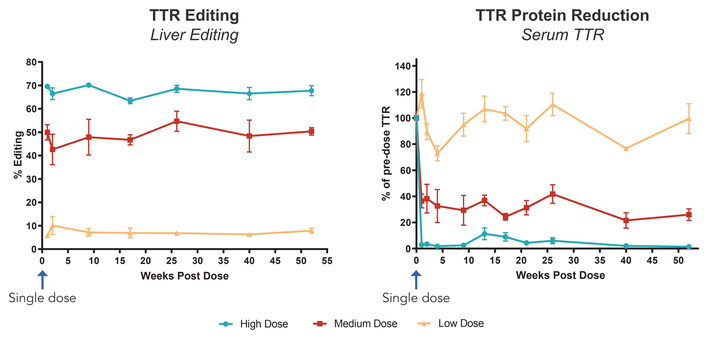


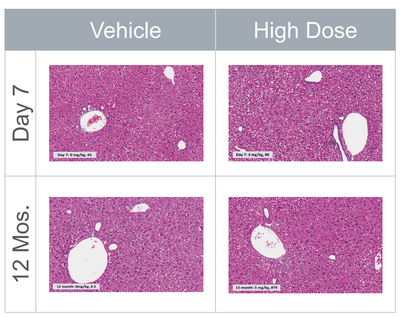
Chemical Modification of sgRNA Plays a Critical Role in the Potency of TTR LNPs Delivered in Mouse



Chemical modifications of sgRNA increase potency relative to end-modified sgRNA

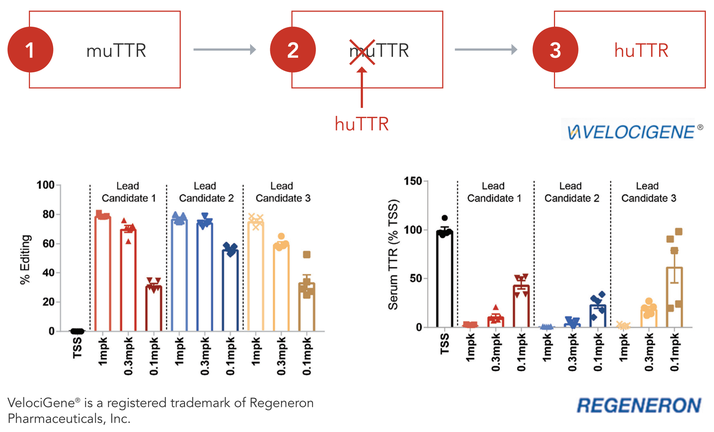
Achieved Persistent Serum TTR Protein Reduction for 12 Months with No Histological Findings in Mouse After a Single Administration of TTR LNPs





- >97% serum TTR reduction is maintained at 12 months following a single administration as measured by ELISA specific for TTR protein
- No transformation or neoplastic changes observed across >100 mice over time up to 12 months

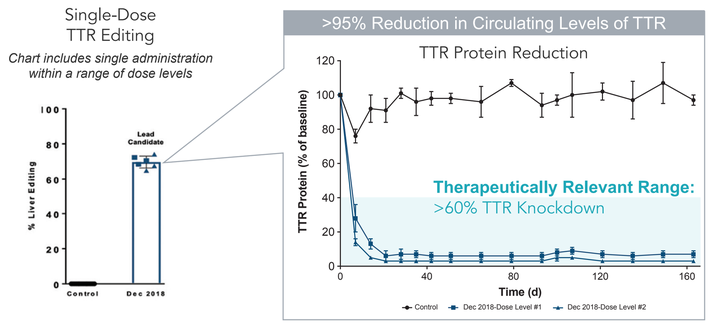
Human Guides Exhibit Robust, Dose-Responsive Liver Editing and Reduction of TTR in huTTR Mice



VelociGene® is a registered trademark of Regeneron Pharmaceuticals, Inc.

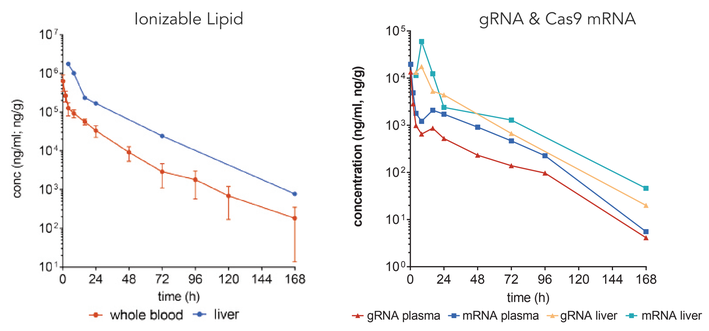
REGENERON

Achieved Therapeutically Relevant and Sustained Serum TTR Protein Reduction of >97% in Non Human Primates (NHP) After a Single Dose of TTR LNPs



Liver editing was determined by NGS from a core needle liver biopsy and circulating serum TTR concentration was determined by an LC-MS/MS assay specific for the TTR protein.

TTR LNPs and Cargo Exhibit 17–24 Hour $T_{1/2}$ and Are Cleared from Circulation and Liver Within 5 days in NHP



Single Dose IV

	Half Life $t_{1/2}$ (h)		
	Lipid	gRNA	mRNA
Plasma	20	21	18
Liver	17	19	24

Ionizable lipid concentration was determined by an LC-MS/MS assay. gRNA and Cas9 mRNA were measured by a qRT-PCR assay with primers and probes specific for the analyte.

CONCLUSIONS

- NTLA-2001 achieves ***significant knockdown of the TTR protein*** by editing the TTR gene across multiple species, including mouse and NHP
  - Twelve month study demonstrated sustained reduction of circulated levels of TTR
  - No significant histopathology findings noted
- Humanized mouse model of hATTR demonstrated a robust dose-responsive liver editing and reduction of TTR after a single dose of LNPs containing the CRISPR/Cas9 components
- NTLA-2001 is ***advancing toward the clinic*** in collaboration with Regeneron Pharmaceuticals, Inc., with an IND submission planned for ***mid-2020***
- Demonstrated the potential of LNP-delivered *in vivo* CRISPR/Cas9 gene editing; suggests that future therapies based on this platform may enable next-generation, curative treatment paradigms for chronic genetic diseases such as ATTR