

Development of NTLA-2001, a CRISPR/Cas9 Genome Editing Therapeutic for the Treatment of ATTR

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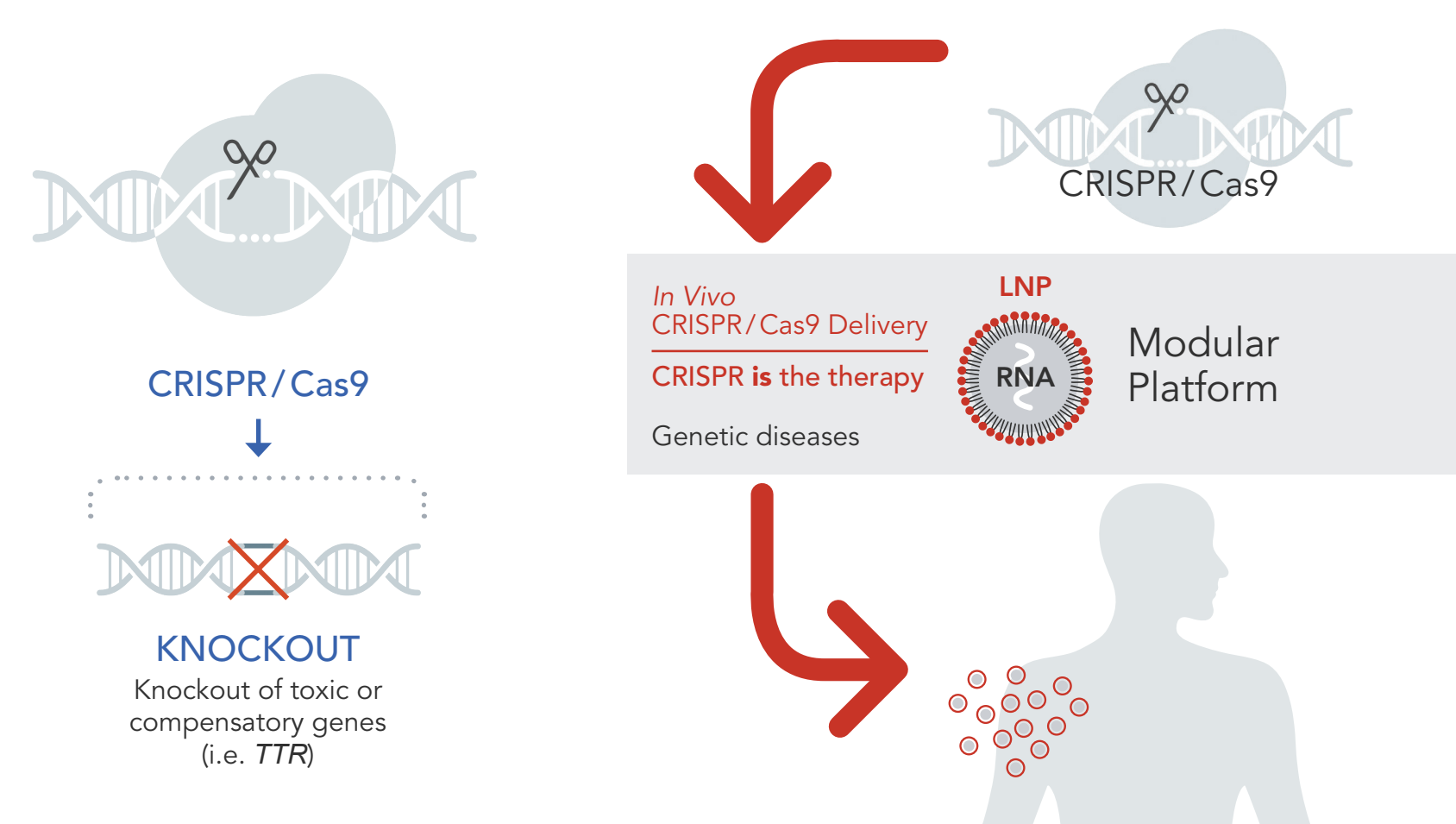
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INTRODUCTION

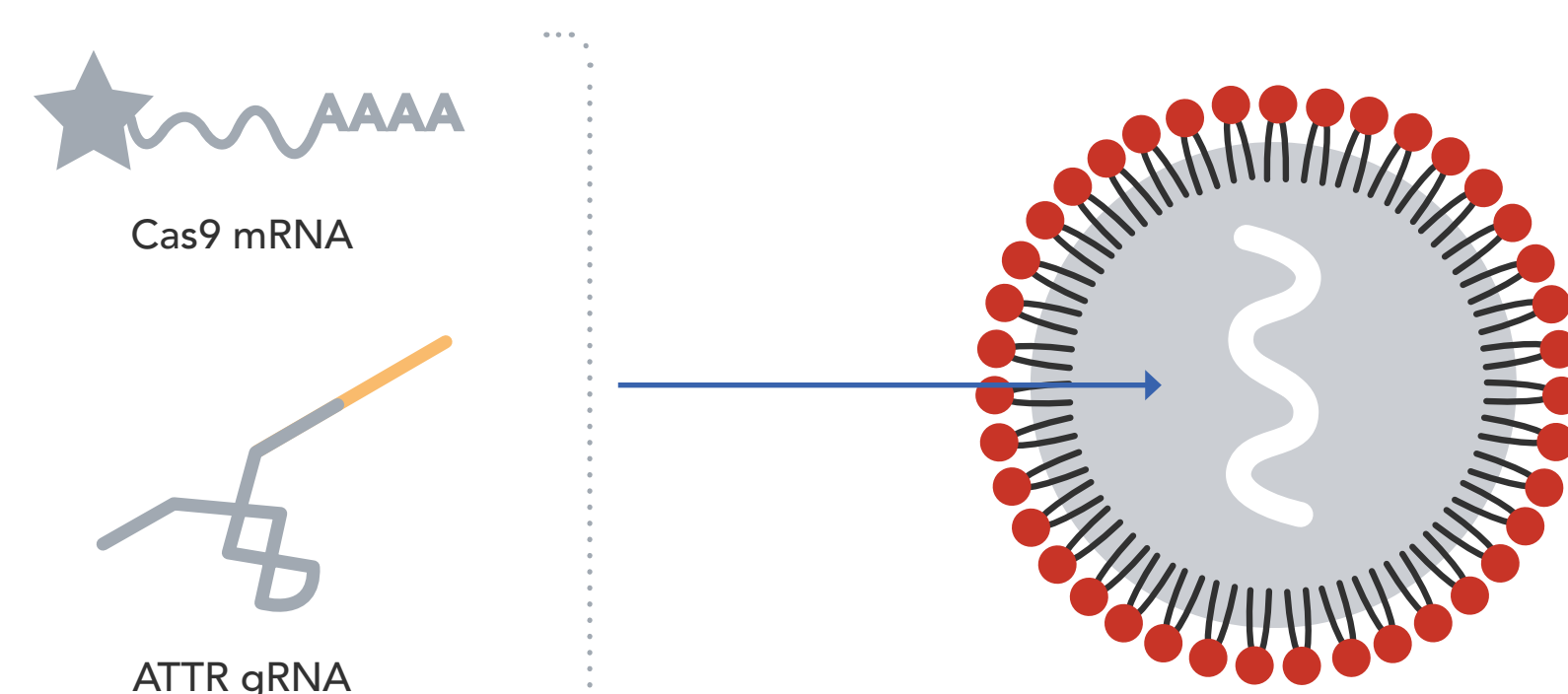
Transthyretin amyloidosis (ATTR) is a progressive disease caused by accumulation of amyloid deposits of misfolded transthyretin (TTR) protein in multiple tissues including the heart, nerves and gastrointestinal tract. Reduction of TTR monomer via stabilization of circulating tetramer and silencing of *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 targeting 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)



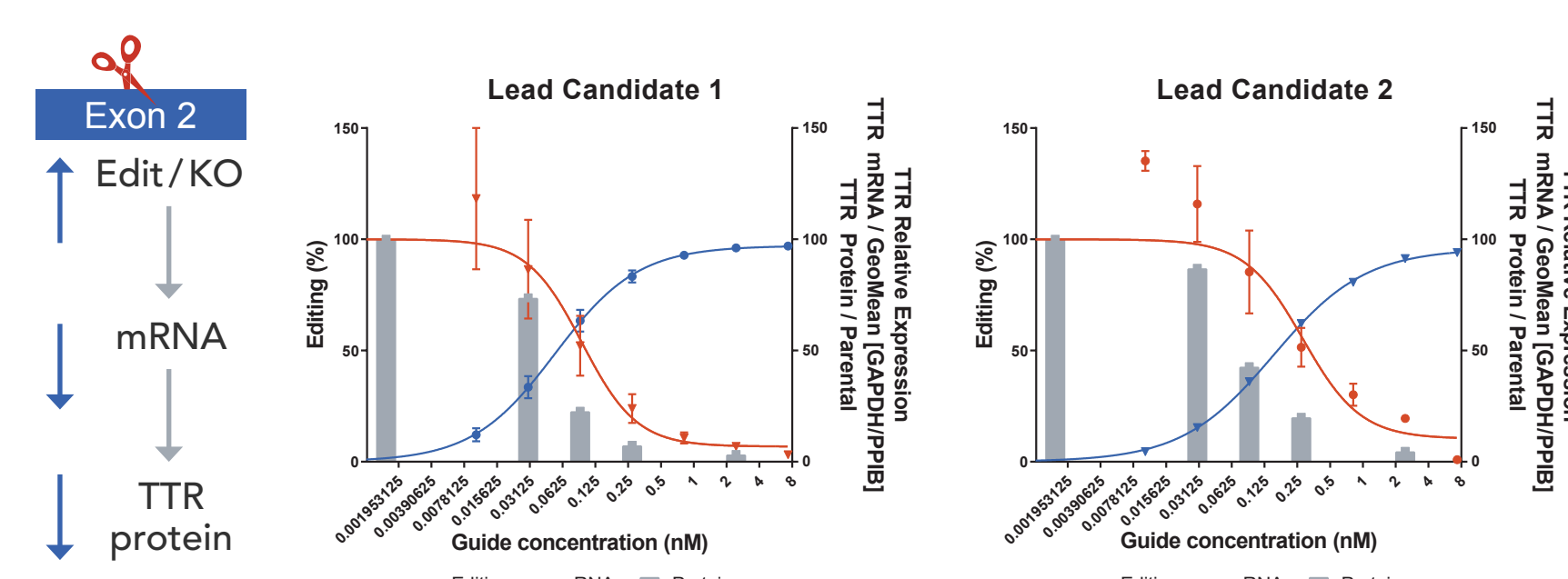
Key Advantages of LNP Delivery

- Large cargo capacity for CRISPR/Cas9
- 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.

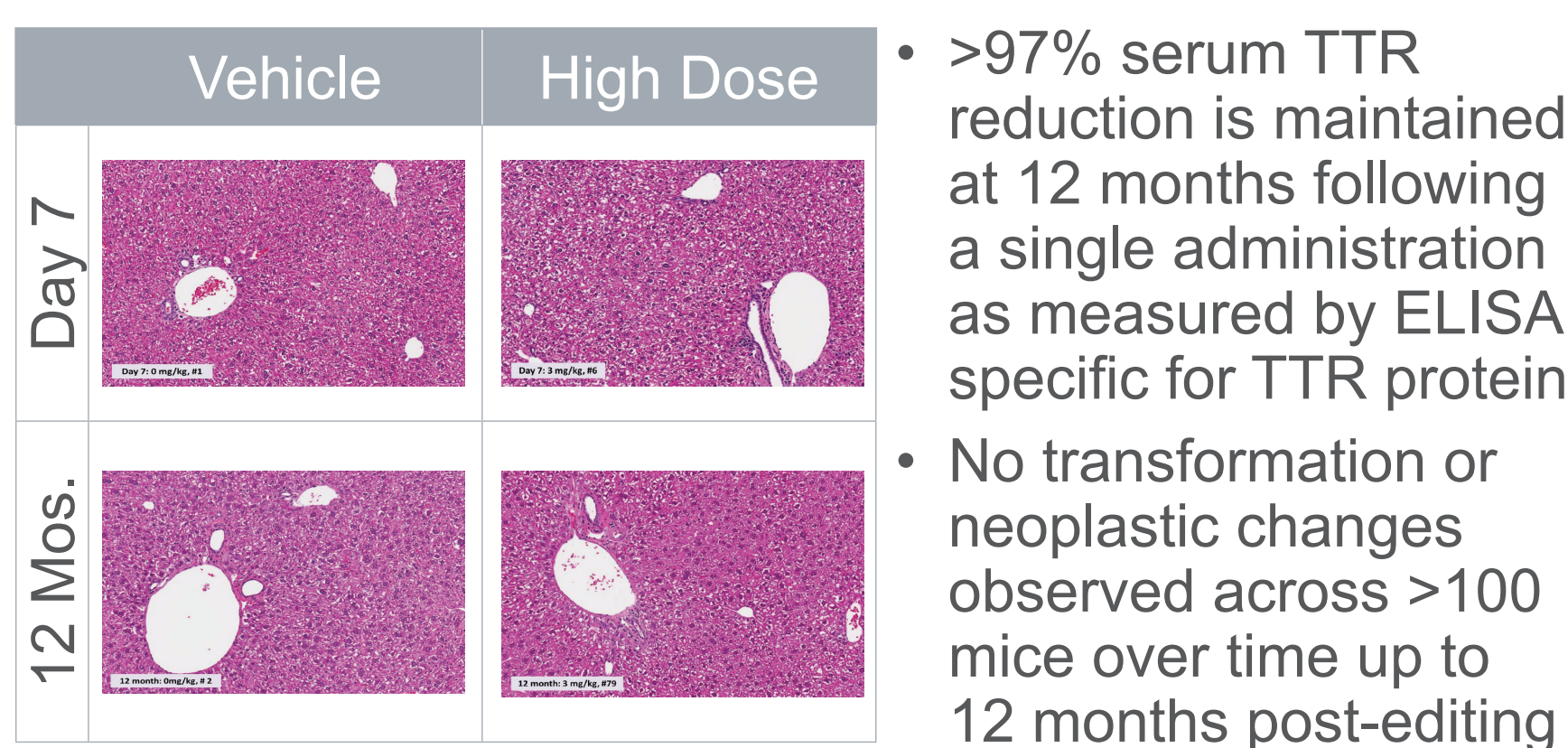
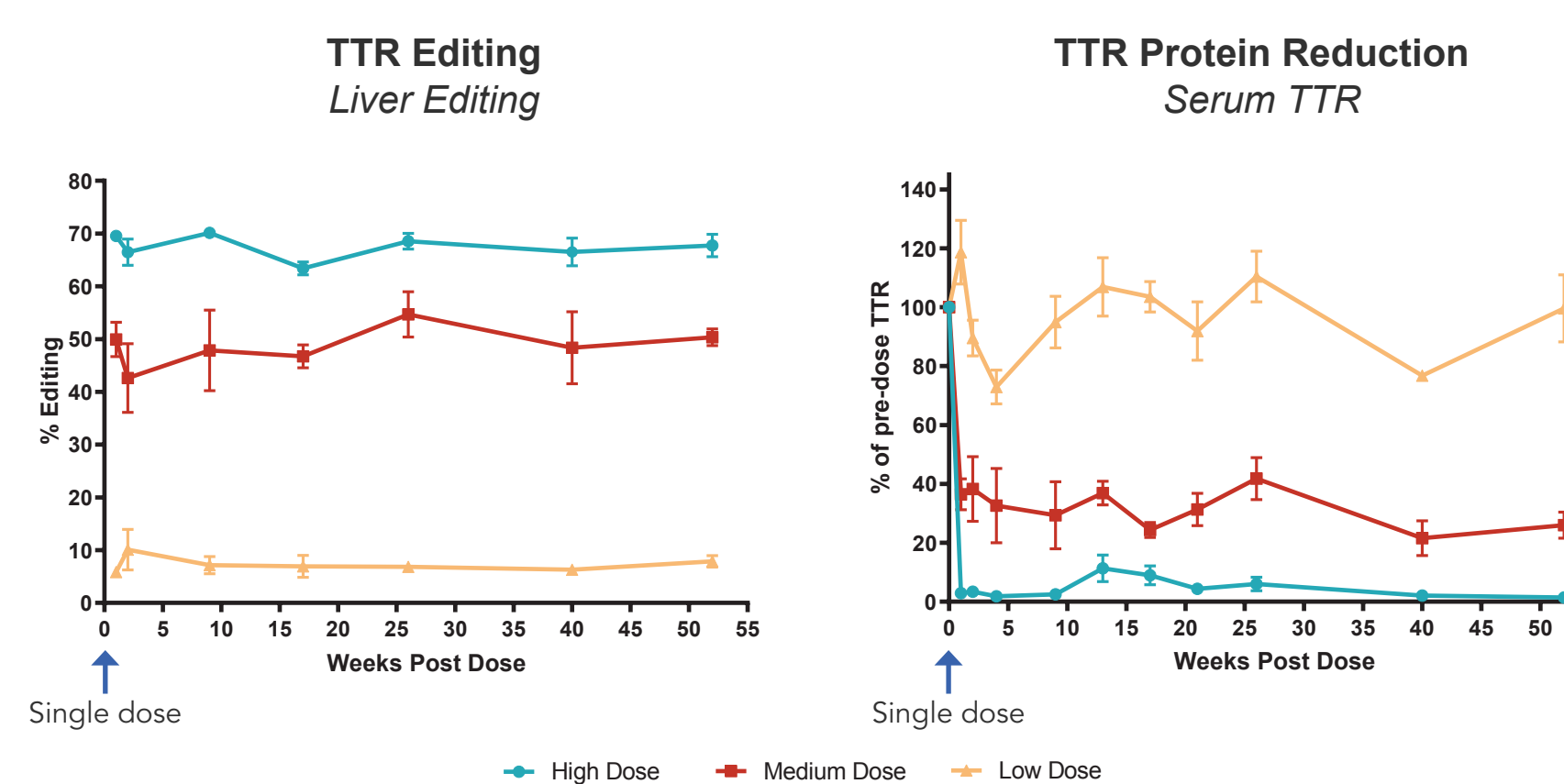
RESULTS

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

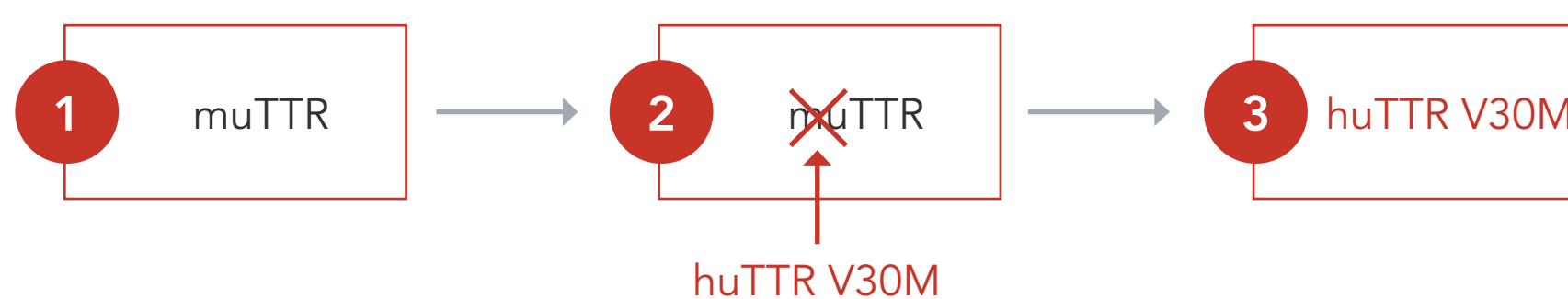


RESULTS

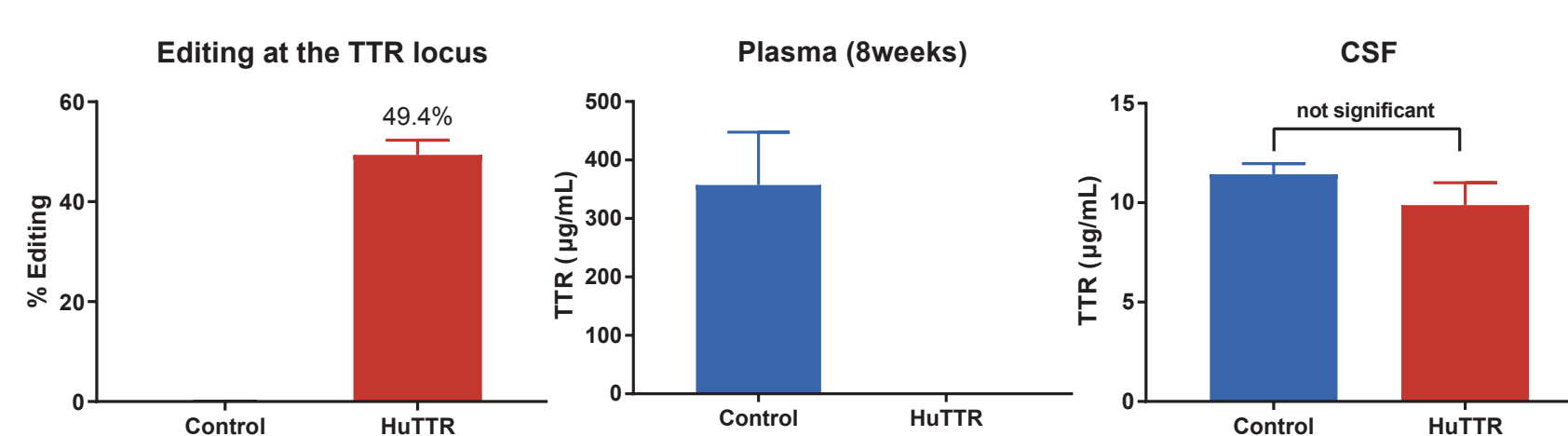
Achieved Persistent Serum TTR Protein Reduction for 12 Months in Mouse After a Single Administration of TTR LNPs with No Histological Findings



Findings from huTTR V30M Mouse Model Study Recapitulate TTR Deposition Phenotype in Tissues and the Nervous System



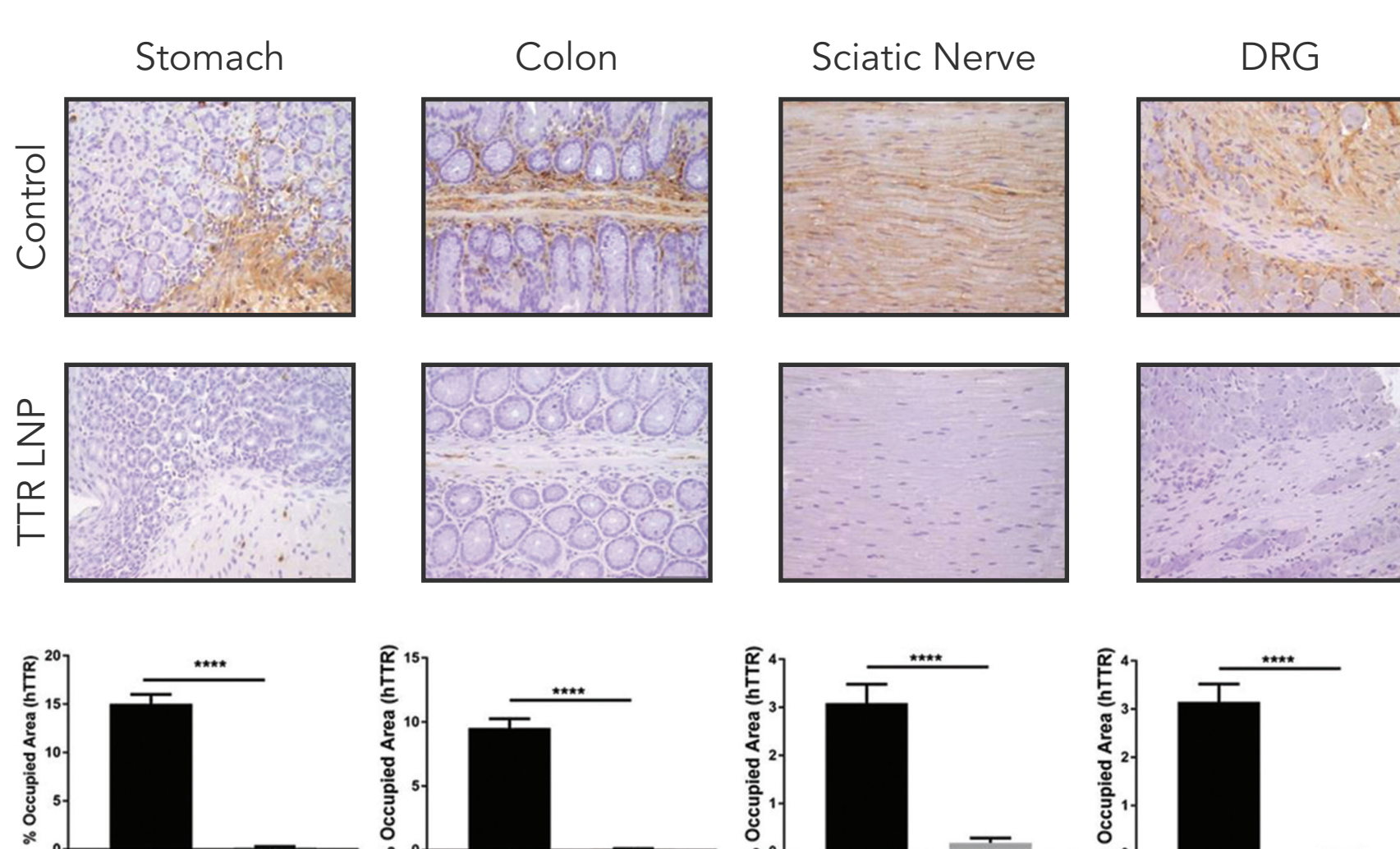
Homozygous for the human mutant V30M TTR transgene in a mouse *TTR*-null background transgenic mice contain approximately ~47 copies of huTTR V30M



- A single administration of TTR LNP resulted in >99% reduction of TTR in plasma at 8 weeks
- Changes in peripheral TTR levels (plasma) had no effect on TTR levels in the CSF of treated mice

Mouse model from Santos et al. 2003; In collaboration with U. of Porto

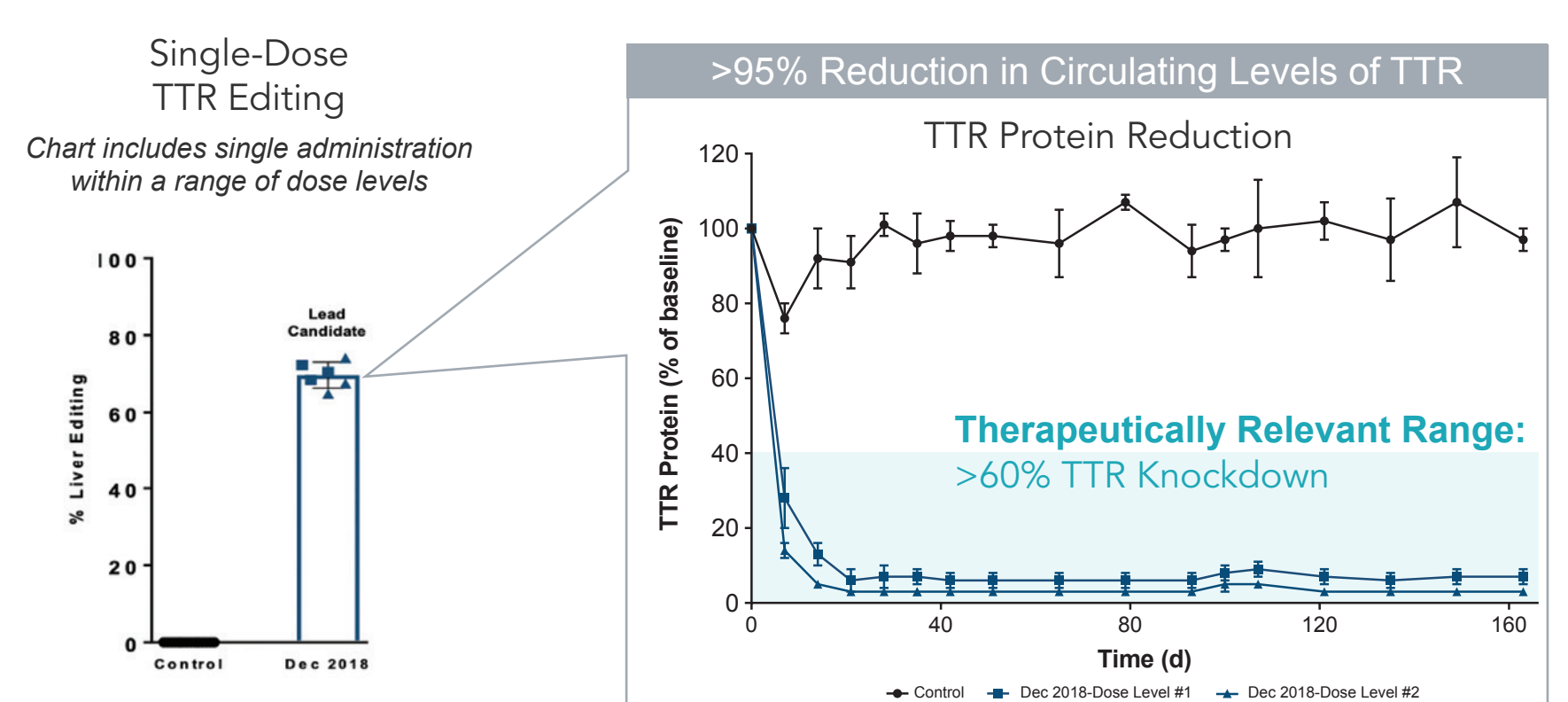
Decreasing Serum TTR by Editing the huTTR V30M Mouse Model Via TTR LNP Dramatically Decreases TTR Deposition in Tissues



- Semi-quantification of human TTR by immunohistochemistry in stomach, colon, sciatic nerve and DRG's.
- Approximately 85% or better reduction in TTR staining was observed across these tissues.

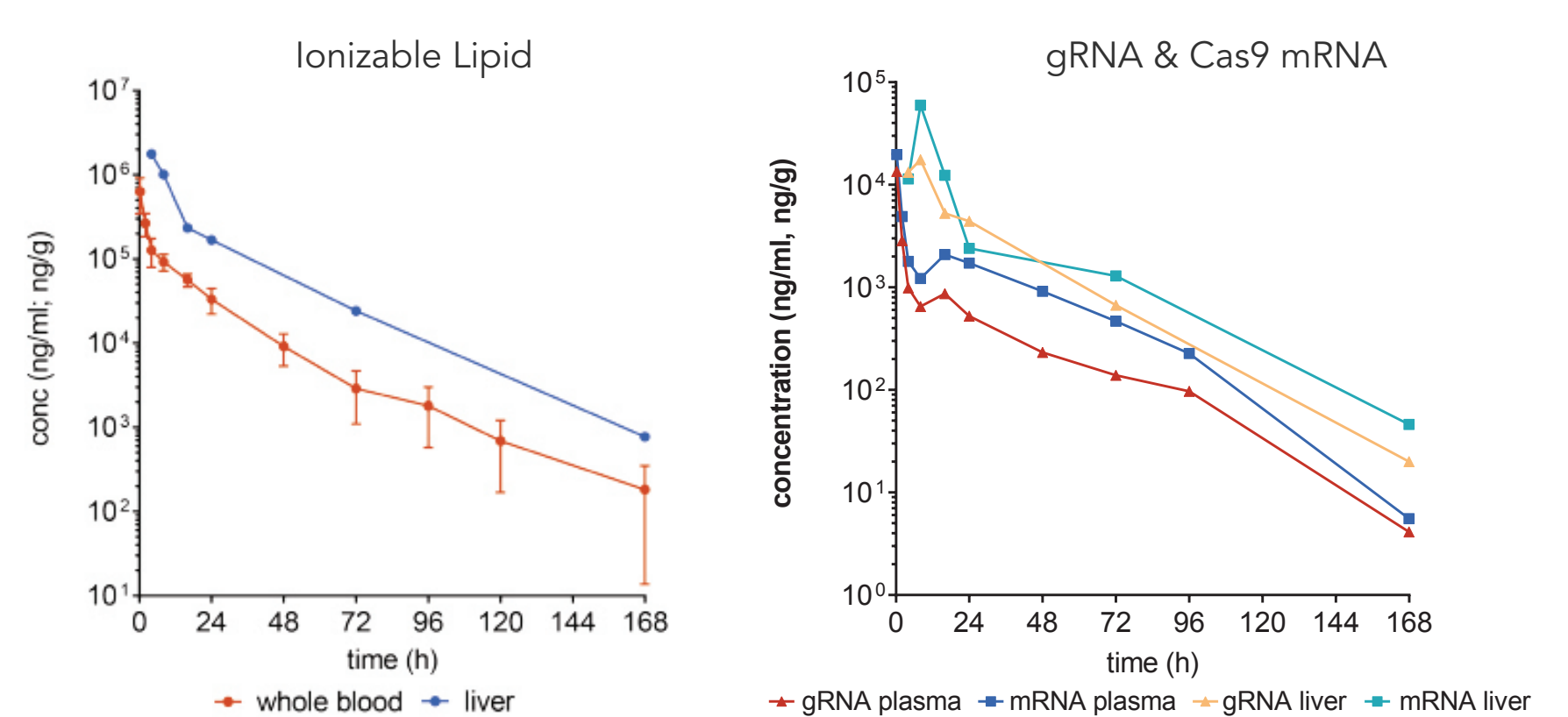
Mouse model from Santos et al. 2003; In collaboration with U. of Porto

Achieved Therapeutically Relevant and Sustained Serum TTR Protein Reduction in Non-Human Primate (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 is **advancing toward the clinic** in collaboration with Regeneron Pharmaceuticals, Inc with an IND submission planned for **mid-2020**
- TTR LNPs enable **significant knockdown of the TTR protein** by editing of the *TTR* gene across multiple species, including mouse and NHP
- In NHP, achieved a therapeutically meaningful level of TTR protein reduction that correlated with robust and significant editing in the liver
- Cas9 mRNA, sgRNA and ionizable lipid are quickly cleared from circulation, with the lipid having plasma and liver half-lives of 20 hours and 17 hours, respectively, in NHP
- Following a **single dose** of LNP-delivered CRISPR/Cas9 in mice:
 - Editing levels achieved that resulted in **>97% reduction** in circulating serum TTR protein
 - Reduction of circulating levels of TTR **sustained for at least 12 months**
 - No significant histopathology findings noted
- Humanized mouse model of hATTR that expresses the V30M mutant form of the human TTR protein demonstrated **rescue of TTR deposition** in multiple tissues after a single dose of LNPs containing the CRISPR/Cas9 components
- 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

