Development of TN-501, an AAV-Delivered Gene Editing Therapy for *PLN-R14del* Cardiomyopathy

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TN-501 For *PLN-R14del* Gene Editing

▶TN-501 is a precise *in vivo* gene editing therapy that inactivates the mutant *PLN-R14del* allele to correct the underlying genetic cause of *PLN-R14del*-associated cardiomyopathy, showing strong preclinical efficacy in improving cardiac function and survival.

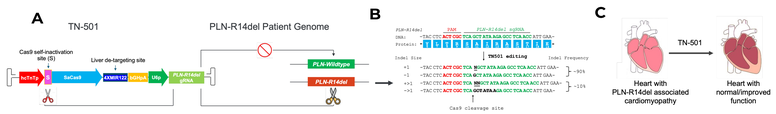

Figure 1. Tenaya's TN-501 *PLN-R14del* Gene Editing Program.

(A) PLN-R14del-associated cardiomyopathy, caused by a mutation in the *Phospholamban* (*PLN*) gene, disrupts calcium handling in cardiomyocytes, leading to life-threatening arrhythmias, progressive heart failure, and high mortality rates. Current treatment options, limited to standard heart failure and arrhythmia management, fail to address the underlying genetic defect. TN-501 is an *in vivo* gene editing therapy designed to specifically inactivate the mutant *PLN-R14del* allele, eliminating the "poison peptide" effects of the mutant protein while preserving wild-type allele function which is sufficient for normal heart function. TN-501 utilizes a cardiac-selective human cardiac troponin T promoter (hcTnTp) to specifically drive the expression of *Staphylococcus aureus Cas9* (*SaCas9*) in cardiac tissues. A human U6 promoter (U6p) controls the expression of a *guide RNA* (*gRNA*) specifically optimized to target the human *PLN-R14del* locus with high specificity and efficiency. Self-inactivation site (S) and liver-detargeting element (4XMIR122) are engineered into the cassette to minimize off-target effects while maintaining robust on-target activity. **(B)** TN-501 creates indels (insertions or deletions) at the *PLN-R14del* locus, disrupting the *PLN-R14del* disease allele to mitigate its toxic effects. The vast majority of indels result in frameshift mutations, which effectively silence the disease-causing allele. **(C)** As a one-time Adeno-Associated Virus (AAV)-based gene editing therapy that addresses the root genetic cause of *PLN-R14del* cardiomyopathy, TN-501 offers a promising treatment for this severe condition with limited treatment options.

Cassette Designs and Optimizations

▶TN-501 is an all-in-one AAV9-based gene editing therapy that has been iteratively optimized to enhance cardiac specificity and safety through Cas9 self-inactivation and liver-detargeting elements.

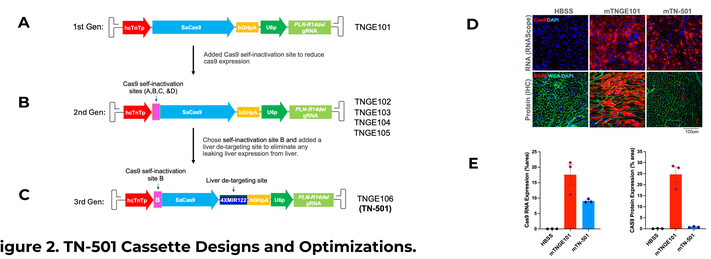

Figure 2. TN-501 Cassette Designs and Optimizations.

(A) 1st Gen all-in-one AAV PLN-R14del gene editing cassette, TNGE101. It employs a cardiac-specific hcTnT promoter (hcTnTp) to drive expression of Staphylococcus aureus Cas9 (saCas9), and a human U6 promoter (U6p) to express a guide RNA (gRNA) optimized for specific targeting of the PLN-R14del locus. **(B)** 2nd Gen all-in-one AAV PLN-R14del gene editing cassettes. To achieve long-term deactivation of Cas9 expression, four cassettes (TNGE102, 103, 104 and 105) were created by fusing distinct Cas9 self-inactivation sites to the 5' end of the Cas9 gene. These self-inactivation sites were designed by modifying the PLN-R14del gRNA target site, allowing the same PLN-R14del gRNA to target both the PLN-R14del gene and Cas9. **(C)** 3rd Gen all-in-one AAV PLN-R14del gene editing cassette, TN-501 (TNGE106). To eliminate any expression from the liver, a 4x miR-122 binding site was added between the Cas9 gene and the bovine growth hormone polyadenylation (bGHpA) signal in the TNGE103 cassette. **(D-E)** Self-inactivating mTN-501 (m: constructs designed with the mouse *Pln-R14del* gRNA) markedly reduces Cas9 protein expression, with only modest reduction in Cas9 mRNA. Red: Cas9 RNA or protein; green: WGA; blue: DAPI.

TN-501 Specifically Edits *PLN-R14del* in Cardiomyocytes

▶TN-501, or its murine surrogate (mTN-501), efficiently and specifically edits the *PLN-R14del* locus in human iPSC-derived cardiomyocytes (iPSC-CMs) and the heart. No germline editing was detected.

Figure 3. Editing Kinetics and Biodistribution of TN-501 and Its Murine Surrogate

(A) Comparison of *in vitro* editing efficiency kinetics between AAV9:hTNGE101 and TN-501 (AAV9:hTNGE106) in *PLN*$^{R14del/R14del}$ iPSC-CMs. **(B)** Kinetics of *Pln-R14del* Gene Editing by mTN-501 (AAV9:mTNGE106, 3E13vg/kg) in *Pln*$^{R14del/R14del}$ mice across several organs.

Cardiac Fibrosis and PLN Aggregation

▶mTN-501 resolves existing cardiac fibrosis and PLN protein aggregation and prevents their further accumulation.

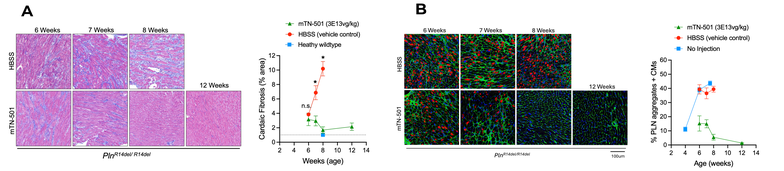

Figure 4. mTN-501 Resolves Existing Cardiac Fibrosis and PLN Protein Aggregation and Prevents Their Accumulation in the *Pln*$^{R14del/R14del}$ Mouse Model.

Retro-orbital (RO) injection was used to deliver mTN-501 at a dose of 3E13 vg/kg to 4-week-old *Pln*$^{R14del/R14del}$ mice. Trichrome staining **(A)** and PLN DAB staining **(B)** were performed on heart tissues collected at 6, 7, 8, and 12 weeks of age. Age-matched 8-weeks-old wildtype littermates were included as healthy controls. Both Trichrome staining, used to assess cardiac fibrosis, and PLN staining, used to evaluate PLN protein aggregation, showed a marked reduction following mTN-501 treatment at various time points, compared to the HBSS (vehicle control) group, which showed a progressive increase in both fibrosis and PLN aggregation. These results demonstrate that mTN-501 not only prevents the development of cardiac fibrosis and new PLN protein aggregation but also reverses existing pathological changes in the *Pln*$^{R14del/R14del}$ mouse model.

Cardiac Function and Survival

▶mTN-501 preserves cardiac function and improves survival.

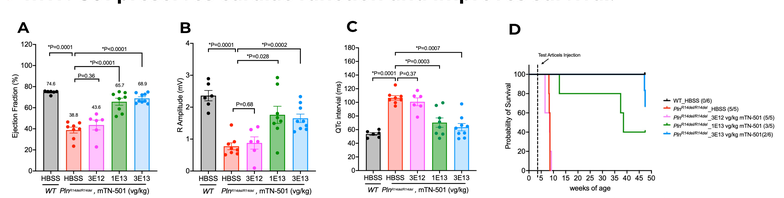

Figure 5. mTN-501 Preserves Cardiac Function and Improves Survival.

Retro-orbital injection was used to deliver mTN-501 (AAV9:mTNGE106) at doses of 3E12, 1E13, and 3E13 vg/kg to three-week-old wild-type (WT) or *Pln*$^{R14del/R14del}$ mice. Echocardiography and electrocardiogram assessments were performed at 7 weeks of age (4 weeks post-injection), and mortality was monitored up to 50 weeks. Measurements of ejection fraction **(A)**, R-wave amplitude **(B)**, and QTc interval **(C)**, at 7 weeks showed that mTN-501 preserved cardiac function near WT levels, in contrast to the vehicle-treated *Pln*$^{R14del/R14del}$ mice. **(D)** mTN-501 significantly improved survival in *Pln*$^{R14del/R14del}$ mice in a dose-dependent manner, with benefits sustained up to 50 weeks.

Tolerability Evaluation in Mice

▶TN-501 and mTN-501 were well tolerated and showed no adverse effects on heart function, body weight, mortality, or organ pathology in WT C57BL/6 mice at 10x the efficacious dose.

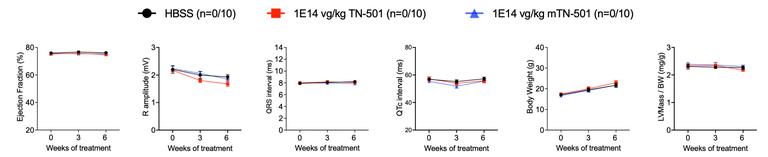

Figure 6. Safety Evaluation of TN-501 and mTN-501 in Adult C57BL/6 WT Mice.

The progression of ejection fraction, R-wave amplitude, QTc interval, QRS interval, and body weight demonstrated that 1E14 vg/kg of AAV9:mTNGE106 or TN-501 did not affect heart function; there were no abnormalities in heart function when compared with the vehicle control group in C57BL/6 WT mice. There was no evidence of hypertrophy upon treatment, measured by left ventricular (LV) mass normalized to body weight.

Immune Responses Evaluation in Adult Mice

▶The addition of a self-inactivation site and liver de-targeting reduced anti-Cas9 antibody responses, while ELISpot assays revealed no significant differences in T cell responses between the HBSS (vehicle control) and mTN-501-treated groups.

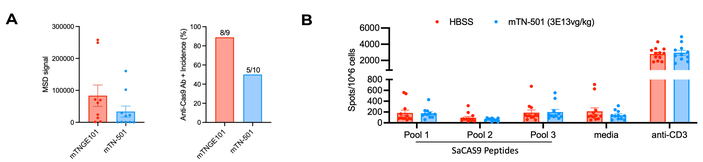

Figure 7. Long-term Immune Responses Evaluation of mTN-501 and its Prototype in Adult mice.

(A) mTN-501 (3E13 vg/kg), or AAV9:mTNGE101 (3E13vg/kg) were administered to WT or *Pln*$^{R14del/R14del}$ mice. Serum samples were collected approximately one year post-dosing, and total anti-Cas9 antibodies were measured using an MSD assay. mTN-501 induced lower anti-Cas9 antibody titers and incidence compared to AAV9:mTNGE101 at the same dose, indicating that transient Cas9 expression is less immunogenic than constitutive expression. **(B)** A total of 217 overlapping 15-mer peptides spanning the Cas9 coding region of TN-501 were synthesized and grouped into three peptide pools for testing. Splenocytes were harvested from mice treated with mTN-501 approximately one year prior, as well as from HBSS-treated WT controls. ELISpot assays revealed no significant differences in T cell responses between the WT HBSS treated group and the *Pln*$^{R14del/R14del}$ mTN-501 treated group.

CONCLUSION

- **TN-501 is a one-time AAV9 gene editing therapy designed to target the root cause of *PLN-R14del* cardiomyopathy.**
- **It achieved cardiac-specific, transient Cas9 expression with no germline editing in mouse model, using self-inactivation and liver de-targeting strategies.**
- **mTN-501 was effective even at 1E13vg/kg dose, improving cardiac function, reducing fibrosis and PLN aggregation, and preventing disease progression, ultimately restoring survival close to WT levels.**
- **TN-501 demonstrated a favorable safety profile, including at least a 10-fold therapeutic index, reduced anti-Cas9 antibody responses compared with an earlier-generation vector, and little observed activation of Cas9-specific T cells.**