Gene Editing of R14del Mutation in PLN Rescues PLN-R14del-Associated Cardiomyopathy

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Introduction

The Arg14del (R14del) mutation in the Phospholamban (PLN) gene is a common genetic cause of familial dilated or arrhythmogenic cardiomyopathy. PLN plays a crucial role in intracellular calcium homeostasis of the cardiomyocyte by negatively regulating sarco/ endoplasmic reticulum Ca2+-ATPase (SERCA). The R14del mutation creates a dominant active form of PLN and results in both super-inhibition of SERCA activity and PLN protein aggregation. PLN-R14del-associated cardiomyopathy has a poor prognosis, high adolescent mortality, and no effective therapy other than heart transplantation.

In the last decade, CRISPR-Cas9 gene editing emerged to offer a potential new era of curing and preventing human genetic diseases. The ability to correct the specific pathogenetic driver underlying disease, the R14del mutation, through *in vivo* delivery of CRISPR-Cas9 components represents an exciting therapeutic frontier for addressing PLN-R14del-associated cardiomyopathy.

Tenaya has developed a gene therapy designed to deliver both Cas9 and PLN-R14del-specific sgRNA from a single adeno-associated virus (AAV) vector. We first tested our gene editing therapy in patient-specific human iPSC-derived cardiomyocytes (iPSC-CMs) and found that it precisely and efficiently edited the PLN-R14del allele without affecting the wild-type PLN allele. We further tested various doses of our PLN-R14del gene editing therapy in a well-characterized mouse model and found heart function of PLN-R14del mice were rescued to near wild-type levels. Our preclinical results suggest PLN-R14del gene editing may be a promising therapy for PLN-R14del-associated cardiomyopathy.

PLN-R14del Gene Editing Program

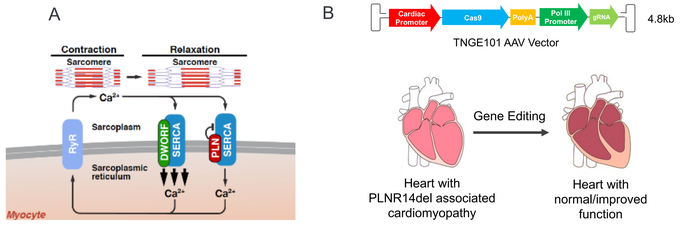
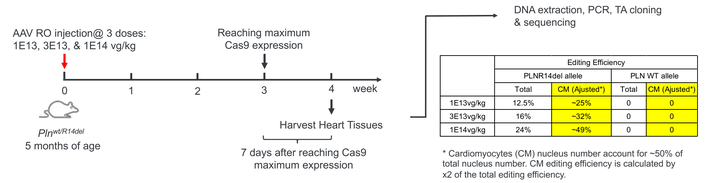


Figure 1. Tenaya's PLN-R14del Gene Editing Program.

(A) Key genes in the calcium handling pathway in cardiomyocytes. PLN negatively regulates SERCA, essential for intracellular calcium homeostasis. The PLN R14del mutation causes misfolded protein aggregation, disrupting heart function. **(B)** Tenaya's development of TNGE101, an all-in-one AAV therapy for PLNR14del-associated cardiomyopathy. TNGE101 combines Cas9 and gRNA in one AAV vector. The gRNA is optimized for specific PLN-R14del targeting while avoiding PLN WT. Cas9 is driven by a cardiac-specific promoter for tissue specificity.

In Vivo Editing Efficiency and Specificity



	Editing Efficiency			
	PLNR14del allele		PLN WT allele	
	Total	CM (Ajusted*)	Total	CM (Ajusted*)
1E13vg/kg	12.5%	~25%	0	0
3E13vg/kg	16%	~32%	0	0
1E14vg/kg	24%	~49%	0	0

* Cardiomyocytes (CM) nucleus number account for ~50% of total nucleus number. CM editing efficiency is calculated by x2 of the total editing efficiency.

Figure 2. Efficient *In Vivo* PlnR14del Gene Editing with Locus Specificity.

Experimental design for *in vivo* gene editing using TNGE101 with gRNA targeting the mouse Pln-R14del locus (mTNGE101) via retro-orbital (RO) injection in PLN WT/R14del heterozygote mice at three different doses (1E13 vg/kg, 3E13 vg/kg, and 1E14 vg/kg). TNGE101 selectively targets the PLN R14del allele in a dose-dependent manner, while leaving the PLN WT allele unaffected.

In Vivo Efficacy in PLN-R1del Mouse Model

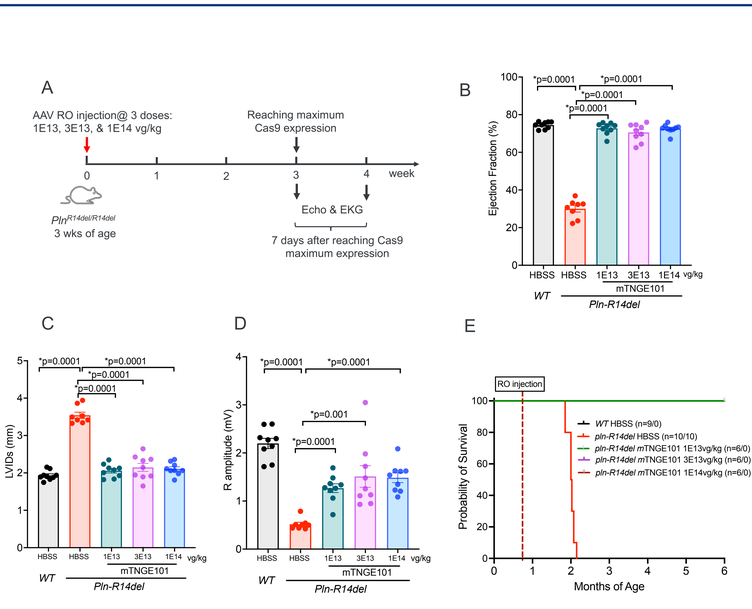


Figure 3. *In Vivo* Efficacy in the PLN-R14del Mouse Model.

(A) Experimental design illustrating the *in vivo* gene editing efficacy using mTNGE101 in PLNR14del homozygote mice. RO injection was employed to deliver mTNGE101 at three different doses (1E13 vg/kg, 3E13 vg/kg, and 1E14 vg/kg). **(B-D)** TNGE101 preserved heart function even at the lowest dose of 1E13 vg/kg, as measured by ejection fraction (EF), left ventricular internal dimension in systole (LVIDs) and R amplitude. **(E)** Ongoing studies demonstrate 100% survival in mice treated with TNGE101 for up to 6 months.

Gene Editing Reduces Cardiac Fibrosis

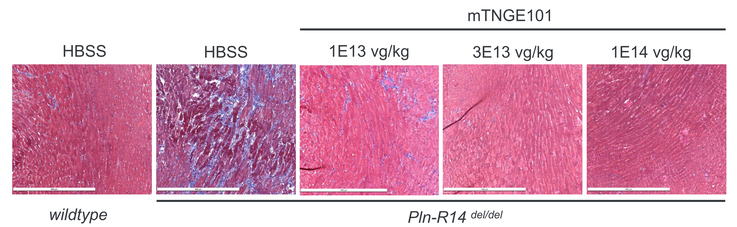


Figure 4. mTNGE101 Reduces Cardiac Fibrosis in the PLN-R14del Mouse Model. Trichrome staining of hearts from 2-month-old mice, 5 weeks after viral administration, reveals a notable decrease in cardiac fibrosis following treatment with mTNGE101 at various doses compared to the vehicle control (HBSS).

Gene Editing Reduces PLN Protein Aggregates

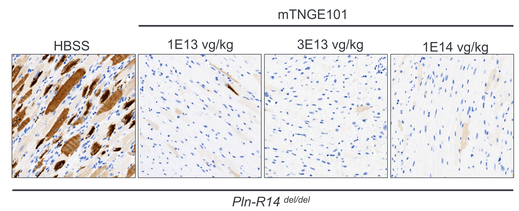
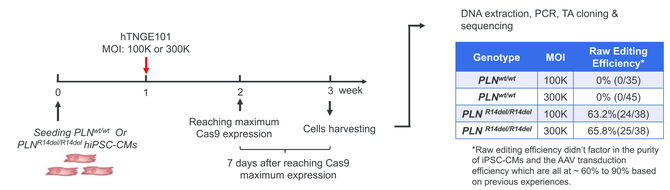


Figure 5. mTNGE101 Reduces PLN Protein Aggregates in the PLN-R14del Mouse Model. DAB staining of the hearts of 2-month-old mice, 5 weeks after viral administration, demonstrates a significant decrease in PLN protein aggregation following treatment with mTNGE101 at various doses compared to the vehicle control (HBSS).

Editing Efficiency and Specificity in hiPSC-CM



Genotype	MOI	Raw Editing Efficiency*
PLNwt/wt	100K	0% (0/35)
PLNwt/wt	300K	0% (0/45)
PLN R14del/R14del	100K	63.2%(24/38)
PLN R14del/R14del	300K	65.8%(25/38)

*Raw editing efficiency didn't factor in the purity of iPSC-CMs and the AAV transduction efficiency which are all at ~ 60% to 90% based on previous experiences.

Figure 6. Efficient PLN-R14del Gene Editing with Locus Specificity in Human Wildtype (WT) or PLN-R14del iPSC-CMs Model.

Experimental design depicting the gene editing process using TNGE101 with gRNA targeting the human PLNR14del locus (hTNGE101) in both PLN WT/WT and R14del/R14del human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Gene editing results demonstrate the efficient and selective targeting of the PLN-R14del allele by hTNGE101, while leaving the PLN WT allele unaffected.