Developing A DWORF Micropeptide Gene Therapy For Heart Failure

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Abstract

Heart failure is a prevalent and chronic disease in which the heart cannot pump enough blood to meet the body's needs. Defective intracellular calcium homeostasis with reduced SERCA (sarco/endoplasmic reticulum calcium-ATPase) activity plays a crucial role in contractile dysfunction in the failing myocardium. Dwarf open reading frame (DWORF) is a muscle-specific micropeptide that enhances SERCA activity by displacing SERCA inhibitors such as phospholamban (PLN). Indeed, overexpression of DWORF in transgenic models, as well as with delivery of DWORF using adeno-associated virus (AAV), has been demonstrated to correct calcium cycling abnormalities, cardiac contractility defects, and reverse adverse ventricular remodeling in multiple mouse models of heart failure, highlighting the potential of DWORF as a therapeutic for heart failure.

Tenaya Therapeutics has developed a series of AAV cassettes designed to express varying amounts of DWORF at a given viral dose and identified the tolerable threshold of DWORF expression in healthy mice. We have tested our gene therapy candidates in a well-characterized dilated cardiomyopathy (DCM) mouse model due to genetic deletion of the muscle-specific LIM domain protein (MLP). AAV-delivered DWORF mitigated the contractile dysfunction and attenuated pathological remodeling in this DCM model. AAV:DWORF cassettes expressed higher, but well-tolerated, levels of DWORF, supporting the greatest degree of efficacy that was durable out to 24 weeks. Our preclinical results suggest DWORF gene therapy may represent a novel means of normalizing calcium homeostasis and limiting disease progression

DWORF Gene Therapy Program

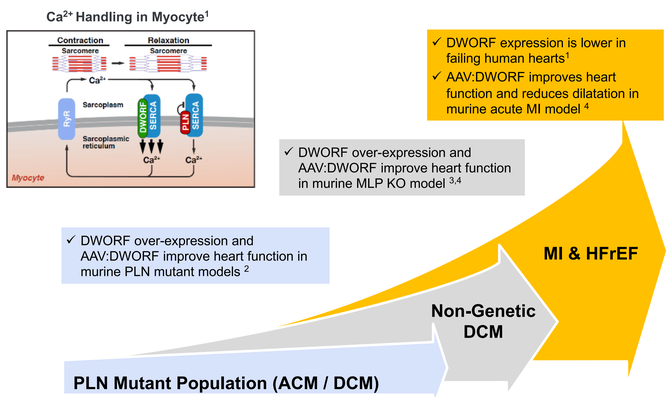

Figure 1. Tenaya's DWORF Gene Therapy Program. Tenaya is developing an AAV-based gene therapy designed to deliver the *DWORF* gene for patients with heart disease. SERCA pathway is widely considered to be a promising target for heart disease. DWORF is a muscle-specific micro-peptide that enhances SERCA activity by counteracting SERCA inhibitor PLN, resulting in increased Ca2+ cycling and contractility. Proof-of-concept efficacy of DWORF over-expression in multiple mouse models creates broad development opportunities, including genetic-DCM such as *PLN* mutation patient population, non-genetic DCM, and HFrEF. ([1] *Nelson. Science,* 2016; [2] Eijgenraam *Sci Rep* 2020; [3] Makarewich, *Elife,* 2018; [4] Makarewich, *Circulation Research,* 2020)

Cassettes Optimization

Gene therapy cassette optimization helps lower the viral load while boosting or maintaining the therapeutic benefits. In the figure below, we show data for our promoters and cassette engineering efforts that illustrate how we have been able to create a suite of cardiac-specific constructs that are able to mediate significantly higher expression of the *Dworf* gene than can be achieved with a standard cTnT promoter.

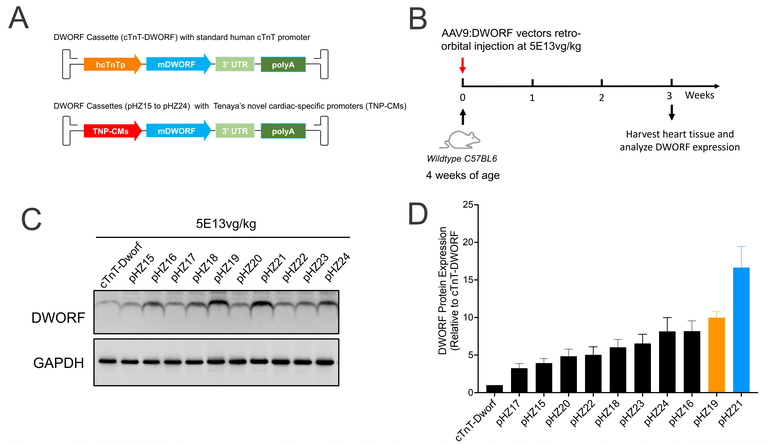

Figure 2. DWORF Expression Cassette Optimization. (A-B) We evaluated AAV9:DWORF constructs with various regulatory elements and arrangements *in vivo* to increase cardiomyocyte DWORF expression. AAV9:DWORF vectors were delivered to four-week-old C57Bl6 mice at 5E13vg/kg dose through retro-orbital injection. Heart samples were collected three-weeks post-injection. DWORF expression was analyzed by Western blot. (C) Representative Western blot images and (D) quantification of DWORF expression. DWORF expression with novel cardiomyocyte-specific promoters and constructs compared with standard cTnT promoter (cTnT-Dworf).

Tolerability of DWORF Gene Therapy

AAV9:pHZ21, the highest expressed and most efficacious DWORF vector is well tolerated in naïve mice up to 2E14vg/kg dose.

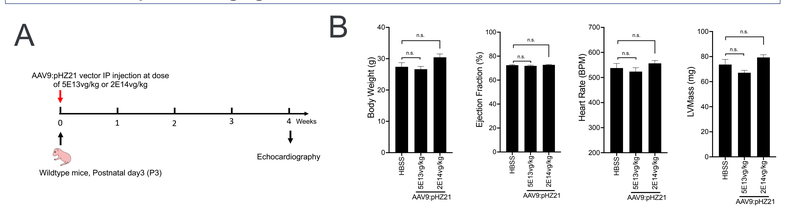

Figure 3. Tolerability of DWORF Gene Therapy in naive mice. (A) Schematic diagram of DWORF gene therapy tolerability study in naïve mice. (B) AAV9:pHZ21 is well tolerated in naïve mice up to 2E14vg/kg dose with no difference in bbody weight, ejection fraction, heart rate, and left ventricular mass (LV Mass).

DWORF Gene Therapy in DCM Model

In a well-characterized DCM mouse model, AAV:DWORF gene therapy utilizing the pHZ19 and pHZ21 constructs resulted in durable improvements in ejection fraction and improved exercise capacity.

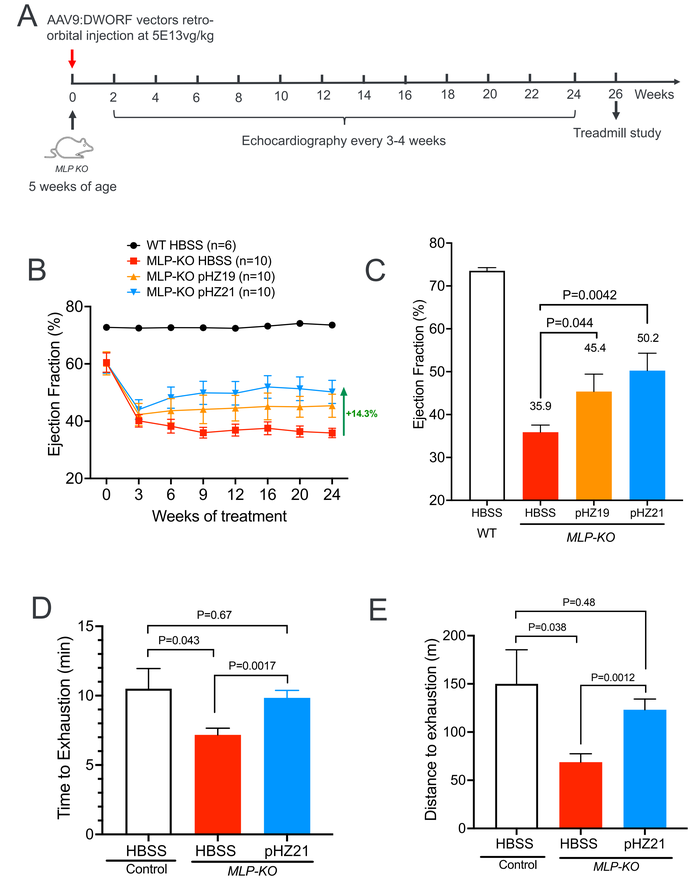

Figure 4. Comparison of Effect of Two DWORF Constructs in a Severe *MLP-KO* DCM Mouse Model. (A) Schematic diagram of DWORF gene therapy efficacy study in *MLP-KO* DCM mouse model. (B-C) AAV9:DWORF constructs containing novel promoters improved EF relative to a saline control in the *MLP*-KO mouse model of DCM. AAV9:DWORF constructs expressing higher levels of DWORF supported the greatest degree of efficacy that was durable out to 24 weeks. (D-E). AAV9:DWORF vector pHZ21 improved exercise capacity, including running distance and time to exhaustion, in the MLP KO DCM mouse model 26 weeks post-treatment.

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