Cardiac Direct Reprogramming Gene Therapy for Ischemic Injury

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Abstract

Heart failure is a major cause of death, with an estimated prevalence of 38 million patients worldwide. Although many treatment strategies have been proposed, the inability of cardiomyocytes to regenerate has been the main therapeutic roadblock. Direct cardiac reprogramming has the ability to convert non-myocytes into working myocardial cells, thereby generating induced cardiomyocytes at the site of injury. Cardiac reprogramming has been shown to improve cardiac function after myocardial infarction (MI) in rodents, however reprogramming of human cells has proven much more complex.

We have successfully developed a first-in-class single vector for the advancement of human direct cardiac reprogramming into the clinic using an optimized human reprogramming cocktail, cassette and viral capsid. We achieved an optimized reprogramming cocktail comprised of three factors by screening and multiple rounds of cassette engineering to maximize the conversion of human cardiac fibroblasts (hCFs) into cardiomyocytes (CMs), *in vitro*. Additionally, viral capsid engineering increased hCF transduction efficiency, such that our lead candidate efficiently reprograms hCFs, *in vitro*, yielding cells that exhibit calcium transients and express CM-specific genes. Critically, our lead candidate also infects and converts resident non-myocytes into CMs *in vivo*, improving cardiac function in rodent MI models. The clinical cassette has been further improved to selectively express the cocktail components for the greatest product safety. Current and future studies will assess the optimal timing of viral vector delivery, route of administration, and dose-dependent activity in various animal models.

Miniaturized MYOCD (My$^{\Delta 3}$) + ASCL1 Drive Efficient Cardiac Reprogramming

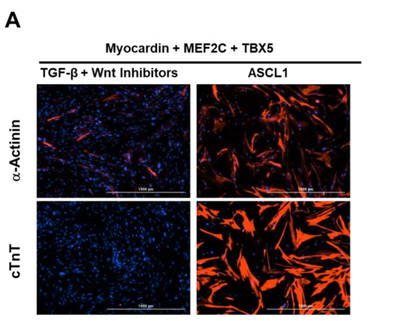

Figure 1: Miniaturized MYOCD (My$^{\Delta 3}$) + ASCL1 Drive Efficient Cardiac Reprogramming and Fit into a Single AAV Vector.
A) Addition of ASCL1 (Acheate-Scute Complex-Like 1) to the Myocardin (My), MEF2C (M), and TBX5 (T) cardiac reprogramming cocktail, greatly increased the number of cells positive for the cardiac markers α-actinin and cardiac Troponin T (cTNT) compared to My/M/T treated cells with TGF-β and Wnt inhibitors. Immunofluorescence analysis was performed three weeks after retroviral infection of human cardiac fibroblasts (hCFs). **B)** MYOCD + ASCL1 (My/A) is as efficacious in a mouse acute myocardial infarction (MI) model as My/M/T. Mice were injected epicardially with retroviral combinations as indicated immediately following permanent ischemic injury by LAD ligation (n = 15/group). **C)** Replacement of full-length MYOCD with My$^{\Delta 3}$ in the AAV cassette increased cardiac fibroblast reprogramming capacity ~6-fold on average based on RNA comparison of MyA and My$^{\Delta 3}$A-treated cells three weeks post-infection (n = 3). **D)** This effect was also observed at the protein level based on quantitative immunofluorescence of induced cardiac markers three weeks post-infection (n = 3). All AAV cassettes were packaged in an AAV2-based capsid.

AAV: My$^{\Delta 3}$A Preserves Cardiac Function

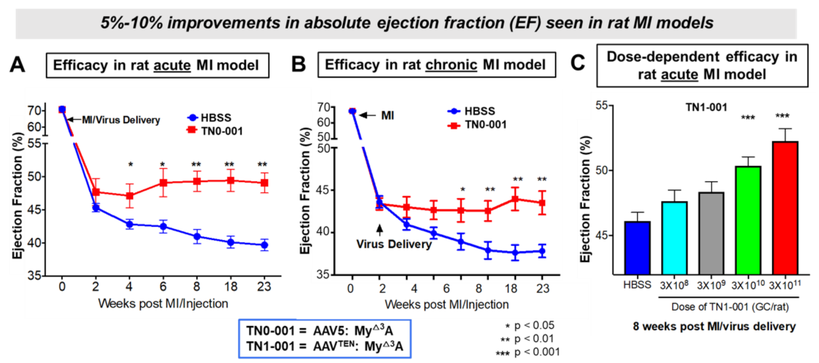

Figure 2: AAV: My$^{\Delta 3}$A Preserves Cardiac Function Following Rat Myocardial Infarction (MI). **A)** AAV5: My$^{\Delta 3}$A (TN0-001) preserved cardiac function when dosed with 1.2 x E11 GC immediately following LAD-ligation (or acute model), compared to vehicle-treated controls (HBSS) (n = 8 rats/groups). Treated animals exhibited a benefit of ~10% ejection fraction over time. **B)** TN-001 is also efficacious when rats were dosed with 5 x E11 GC two weeks after LAD-ligation (chronic model, n = 10 rats/groups). 23 weeks after surgery, or 21 weeks post-injection, rats treated with TN0-001 exhibited an average 6% benefit in ejection fraction. **C)** AAV^{TEN}: My$^{\Delta 3}$A (TN1-001) exhibited dose-dependent efficacy based on % ejection fraction over four orders of magnitude in the acute model (8 rats/group).

Selectively Expressing My$^{\Delta 3}$A in hCFs

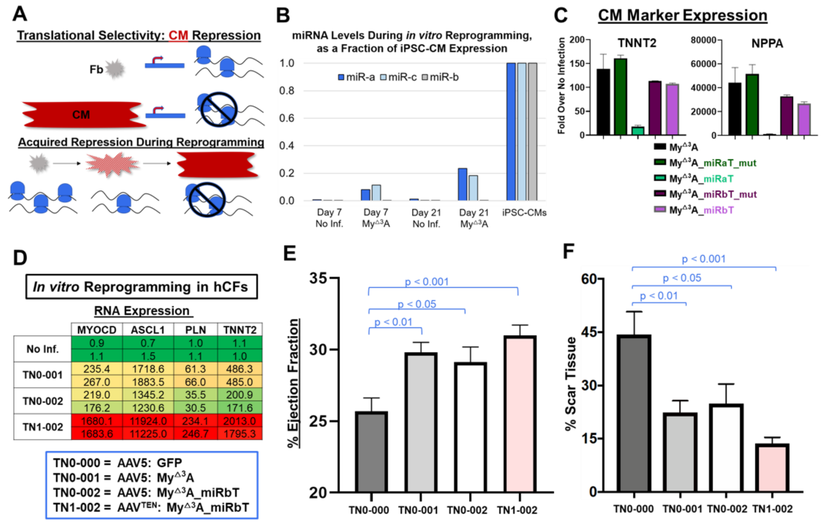

Figure 3: Selectively Expressing My$^{\Delta 3}$A in Human Cardiac Fibroblasts.
A) CM-repression strategy for selectively expressing locally delivered agents in cardiac fibroblasts. The addition of cardiac-specific miRNA binding sites mediates translational repression of the transgene cassette in CMs and ultimately in induced cardiomyocytes. **B)** To determine which cardiac miRNAs might be viable candidate repressors, we assessed the timing of cardiac miRNA emergence during hCF reprogramming with My$^{\Delta 3}$A and compared the levels to those present in human iPSC-CMs. miRNAs levels were assessed by qPCR (n = 2). **C)** Unlike miR-a targeted UTRs, miR-b targeted UTRs support robust reprogramming *in vitro*. My$^{\Delta 3}$A_miRbT and My$^{\Delta 3}$A_miRbT_mut were able to drive robust reprogramming *in vitro* based on RNA analysis after three weeks of reprogramming. Cells were infected with an AAV2 variant at MOI 640k (n =2). **D)** *In vitro* reprogramming in hCFs indicated significantly greater infectivity and potency of TN1-002 compared to TN0-002 based on RNA analysis performed seven days post-infection at MOI 1.2M (n = 2). **E)** Echocardiography data at eight weeks post-injection (1.2 x E11 GC) indicated a significant increase in all groups relative to the GFP-encoding negative control in a mouse MI model (n = 10-13 mice/group**).** **F)** Analysis of fibrosis based on trichrome staining. Quantification of the % of the cardiac cross-section that is fibrotic across all groups indicated a significant reduction in all treatment groups (n = 6-8 hearts/group, five sections/heart).

TN1-002 Improves Cardiac Function in Pigs

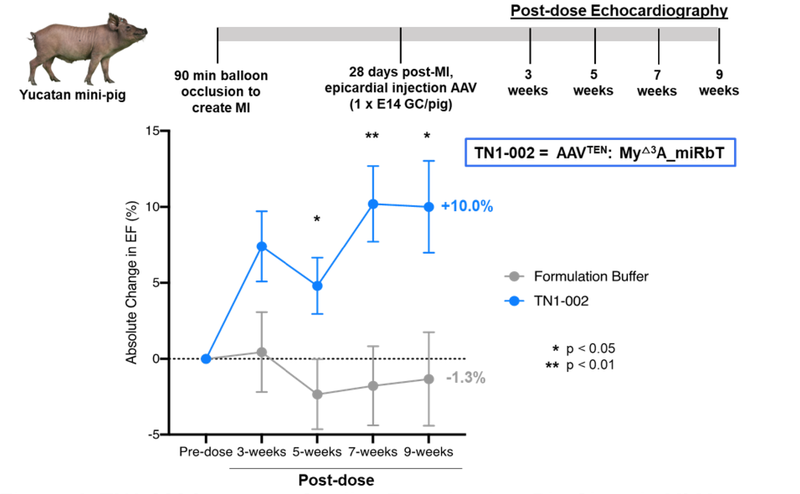

Figure 4: TN1-002 Improves Cardiac Function in a Pig Chronic MI Model.
TN1-002 (My$^{\Delta 3}$A_miRbT) was injected epicardially into the border zone of pig hearts 28 days after a 90-minute balloon occlusion to introduce ischemic injury. In parallel, infarcted pigs were also injected with formulation buffer, so that each group consisted of ten animals. Pigs that received TN1-002 demonstrated significant improvement in ejection fraction relative to control 5-weeks, 7-weeks and 9-weeks post-injection. Thus, treatment with the cardiomyocyte-detargeted reprogramming cocktail resulted in an average 10% improvement in cardiac performance above pre-dose baseline.

miR-133 Boosts hCF Reprogramming

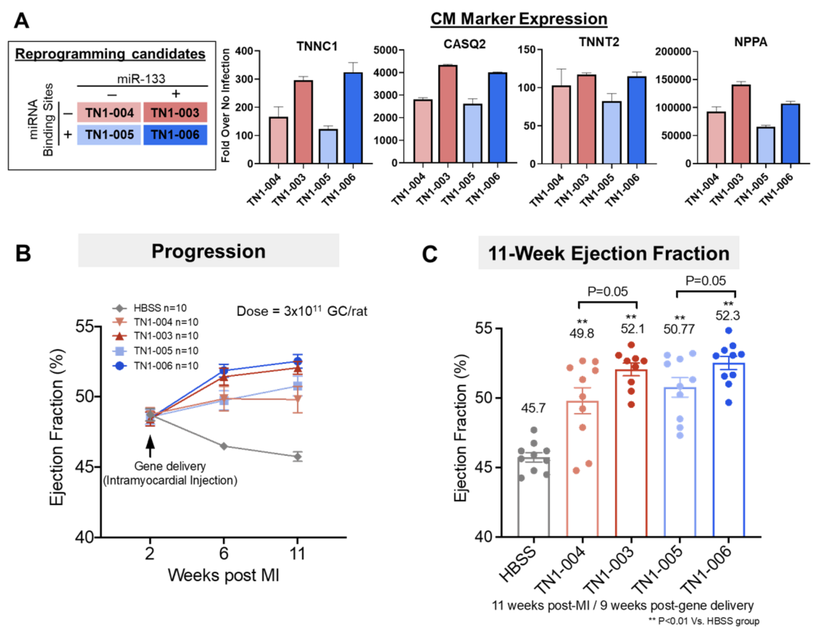

Figure 5: miR-133 Addition Boosts hCF Reprogramming and Efficacy in a Chronic Rat MI Model. **A)** The addition of miR-133 resulted in a two-fold increase in cardiac markers (TN1-003 and TN1-006). Further, with the addition of miR-133, there was no difference in potency between the My$^{\Delta 3}$A and My$^{\Delta 3}$A_miRbt cassettes, indicating the retained utility of the miR-b targeting strategy with boosted reprogramming. RNA expression analysis was performed three weeks post-infection at MOI 640k (n = 2). **B)** %Ejection fraction over time in a rat chronic MI model. Injections were performed two weeks after LAD-ligation (n = 10 rats/group). **C)** % ejection fraction nine weeks post-injection indicated significant efficacy in all test groups, with TN1-003 and TN1-006 performing significantly better than cassettes lacking miR-133.