Cardiac AAV:PKP2 Gene Therapy Reduces Ventricular Arrhythmias, Reverses Adverse Right Ventricular Remodeling, Improves Heart Function, and Extends Survival in a *Pkp2*-deficient Mouse Model of Arrhythmogenic Right Ventricular Cardiomyopathy

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Abstract

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a familial cardiac disease associated with ventricular arrhythmias and an increased risk of sudden cardiac death in adults and children. ARVC has an estimated prevalence in the general population of 1:2000 with the mean age of presentation before 40 years of age. Progressive disease eventually leads to moderate to severe RV dysfunction in approximately 45% of patients. Clinical management of ARVC patients includes lifestyle modification, pharmacological treatment, catheter ablation, ICDs, and heart transplantation. Based on publicly available information to date, we believe there are no approved treatments that address the underlying genetic causes of this disease. Therefore, a new treatment paradigm is needed to prevent disease onset and disease progression.

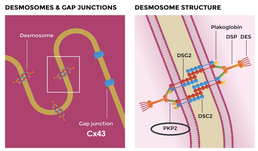
Desmosome gene *Plakophilin-2 (PKP2)* is the most frequently mutated gene responsible for up to 45% of ARVC cases. Our goal is to examine whether adeno-associated virus (AAV)-mediated delivery of the wild-type (WT) transgene prevents ARVC development in a cardiac specific knock-out mouse model of *Pkp2*. Both *PKP2*-deficient human iPSC-derived cardiomyocytes and a *Pkp2-cKO* mouse model were developed to identify the molecular, structural, and functional signatures that recapitulate human disease phenotypes.

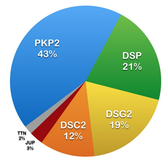
A single dose of cardiac AAV:PKP2 gene delivery significantly improves life span of *Pkp2-cKO* ARVC mice by 1) restoring expression of desmosome components; 2) preventing and reversing adverse right ventricular remodeling; 3) maintaining and improving ventricular functions; 4) preventing cardiac fibrosis and 5) reducing ventricular arrhythmia event frequency and severity. Additional studies on AAV:PKP2 gene therapy efficacy show a dose-dependency in disease modification and survival benefit. Safety evaluation of AAV:PKP2 in WT mice shows no adverse effects on cardiac function and no changes in tissues examined. Our preclinical results demonstrate that cardiac AAV:PKP2 gene therapy may be a promising therapeutic approach to treat ARVC patients with *PKP2* mutations. TN-401, Tenaya's AAV:PKP2 clinical drug candidate, is currently advancing into IND-enabling studies.

Introduction

PKP2 Mutations are the Predominant Genetic Cause of ARVC

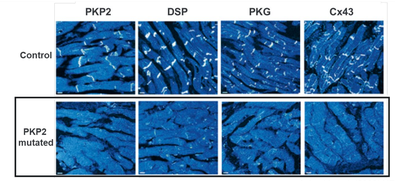
~70K patients with PKP2 mutations in the U.S. alone





https://www.medmolgen.uzh.ch/de/research/arvcs.html

PKP2 Mutation is Associated with Reduction of Protein Levels and Loss of Desmosome Structure in Human Hearts



Asimaki et al. (2009) NEJM

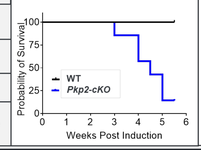
Results

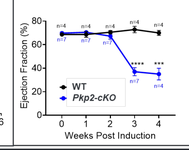
Cardiac-specific *Pkp2* Knockout Mouse Model Recapitulates Human ARVC Phenotypes

Cardiomyocyte-specific-*Cre-ER(T2), Pkp2* $^{fl/fl}$

Cerrone et al. (2017) Nat. Comms.

	Premature Mortality	Decreased LV Ejection Fraction	Increased Spontaneous PVCs
Life Span			
Cardiac Function			
Electro-physiology			
Fibrosis			
Molecular Desmosome Signature			

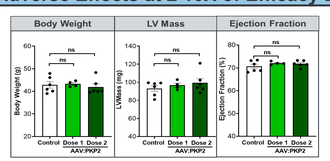


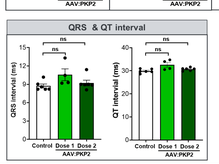


Animal	Week Post Induction		
	Week1	Week2	Week3
1	0	5	>100
2	0	12	12
3	0	2	Died
4	0	66	>100
5	0	20	>100
6	0	1	11
7	4	5	>100

Pkp2-cKO mouse licensed from Dr. Mario Delmar and NYU

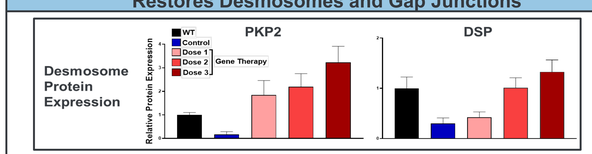
AAV:PKP2 6wks Safety Study in WT Mice Shows No Adverse Effects at ≤ 10X of Efficacy Dose

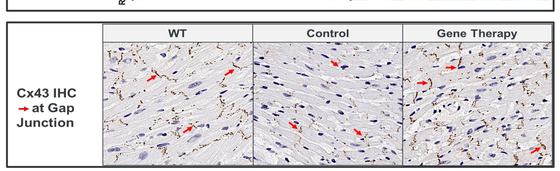




*Histology analysis shows no changes in heart, lung, liver, pancreas, brain, kidneys and skeletal muscle examined.

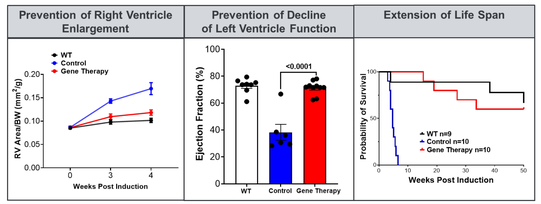
Single Dose Cardiac AAV:PKP2 Gene Therapy Restores Desmosomes and Gap Junctions



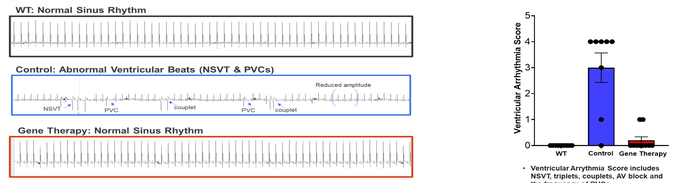


Cardiac AAV:PKP2 Gene Therapy Improves Symptoms of ARVC and Extends Life Span of *Pkp2*-cKO ARVC Mice

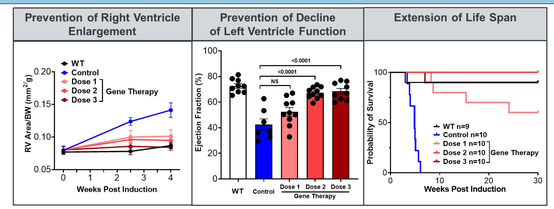
Single Dose Cardiac AAV:PKP2 Gene Therapy Demonstrates Disease Modification and Survival Benefit



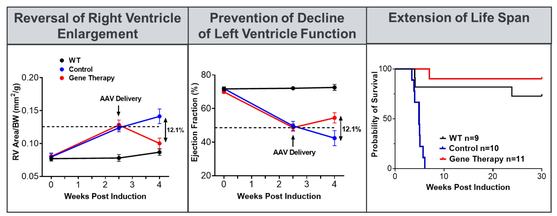
Single Dose Cardiac AAV:PKP2 Gene Therapy Reduces Ventricular Arrhythmias



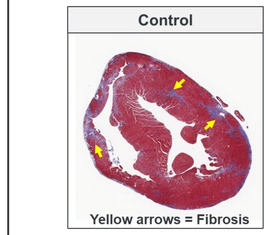
Dose Dependency of Cardiac AAV:PKP2 Gene Therapy is Shown in Disease Modification and Survival Benefit

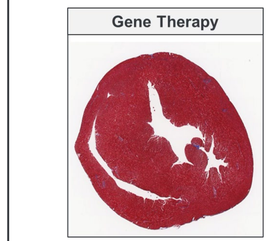


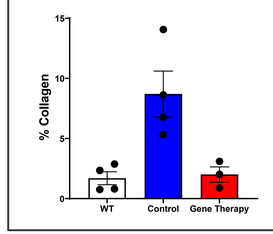
Single Dose AAV:PKP2 Treatment Improves Heart Function after Onset of Disease



Single Dose Cardiac AAV:PKP2 Gene Therapy Prevents Fibrosis







Summary

TN-401, a first-in-class gene therapy, has the potential to offer a profound, disease-modifying approach to address the key aspects of ARVC, including arrythmias, progression of adverse heart remodeling and overall survival.

- Preclinical results using a cardiac-specific *Pkp2* knockout mouse model provide encouraging evidence that cardiac AAV:PKP2 gene therapy may be a promising therapeutic option for ARVC patients with *PKP2* mutations
  - In a mode of disease onset, cardiac AAV:PKP2 gene therapy 1) Reduces ventricular arrhythmias; 2) Demonstrates disease modification and increased survival; 3) Restores desmosomes and gap junctions; 4) Prevents fibrosis
  - In a mode of disease progression, cardiac AAV:PKP2 gene therapy 1) Reverses RV enlargement and prevents decline of LV function; 2) Demonstrates increased survival
  - Preliminary safety evaluation of AAV:PKP2 in wild-type mice shows no adverse effects on cardiac function and no changes in tissues examined
- TN-401, Tenaya's AAV:PKP2 clinical candidate, is currently advancing into IND-enabling studies
- Further studies are needed to support these findings