A novel AAV gene therapy for treatment of BAG3 dilated cardiomyopathy

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Introduction

Gene therapy with adeno-associated virus (AAV) vectors is a promising therapeutic platform for the treatment of cardiovascular disease. Achieving therapeutically relevant levels of cardiomyocyte transduction with IV delivered AAV vectors at doses that are well tolerated has been the field's most pressing challenge. We have identified novel AAV capsids by a machine learning-guided rational design approach that demonstrates significantly improved cardiac transduction at lower doses compared with wild-type AAV9.

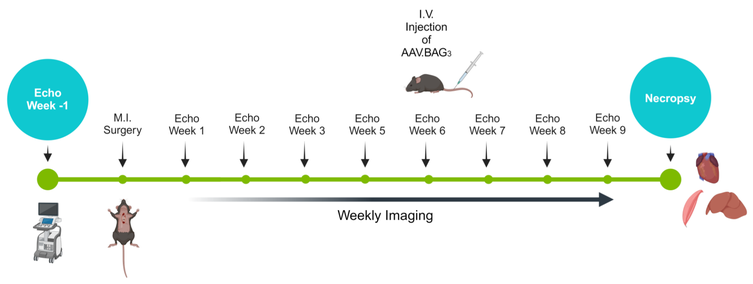
Dilated cardiomyopathy (DCM) has an estimated prevalence of 1 in 250 individuals, with up to 3.6% of cases attributed to mutations in the B-cell lymphoma 2 (Bcl-2) associated anthanogene-3 (BAG3) gene (1). BAG3 is a cochaperone that interacts with members of the heat shock protein (HSP) family and plays an important role in the maintenance of the sarcomere and cardiac contractility. Loss of a single allele can result in protein truncation and reduction in total BAG3 protein levels (haploinsufficiency) and cardiac function. BAG3-DCM represents a significant unmet medical need in a patient population with rapidly progressive cardiac dysfunction for whom no treatments targeting the underlying mechanism of disease exist.

We present proof of concept for an AAV-mediated gene replacement strategy to improve BAG3-related DCM using a novel cardiotropic capsid ATC-0187. This capsid effectively delivers therapeutic levels of BAG3 to the heart at low doses, demonstrating enhanced efficacy compared to wild-type AAV9.

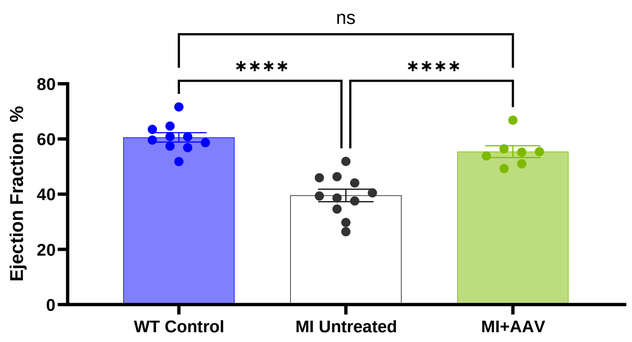
AAV-BAG3 restores normal cardiac function following myocardial infarction

Experimental protocol and BAG3 construct design

AAV expressing human BAG3 under the control of a cardiac promoter



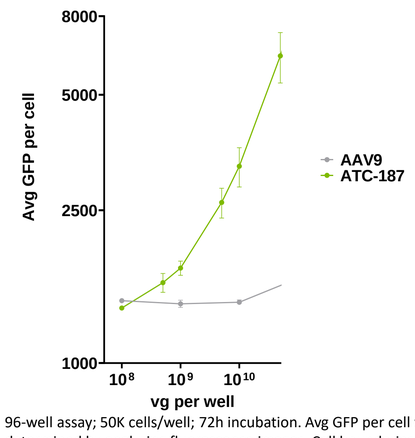
AAV-BAG3 improves cardiac function in MI mice 3 weeks post injection



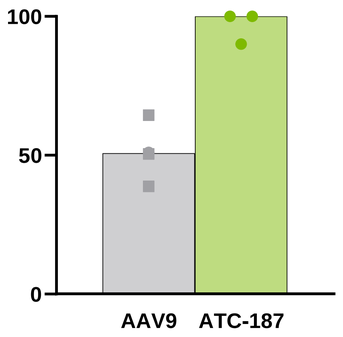
Ejection fraction (EF%) in WT and MI mice (9 weeks post MI surgery and 3 weeks post AAV injection). ****p <0.0001; one-way ANOVA with Tukey's multiple comparisons test. Data are shown as means ± SEM

ATC-0187 next generation cardiotropic capsid

ATC-0187 performance is superior relative to AAV9 in iPSC-derived human cardiomyocytes and NHP heart

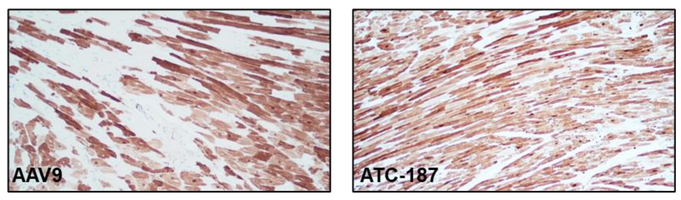


96-well assay; 50K cells/well; 72h incubation. Avg GFP per cell was determined by analyzing fluorescence images. Cell boundaries were defined, and pixel counts within each cell were recorded. The avg pixel intensity was calculated across 25k cells/well



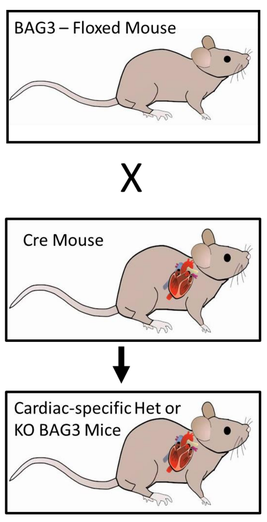
%Cardiomyocytes GFP positive quantified in heart left ventricles from IV delivered AAV9 and ATC-0187 at 3e13 vg/kg in NHPs (28 days in life)

IHC staining of NHP heart sections showing GFP-positive staining in the myocardium

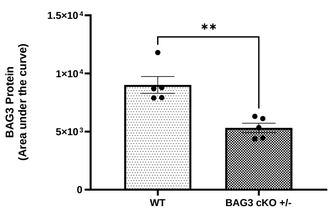


Conditional BAG3 KO mouse with cardiac-specific haploinsufficiency (BAG cKO $^{+/-}$)

BAG3 Haplo-insufficient conditional KO generation paradigm

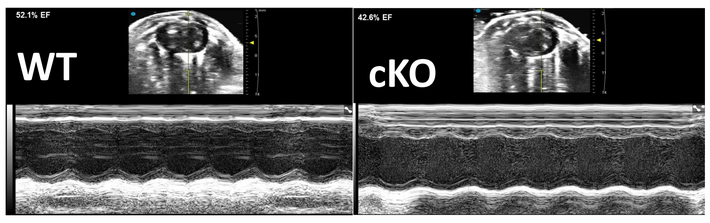


Reduced BAG3 protein expression in BAG cKO $^{+/-}$ mouse heart

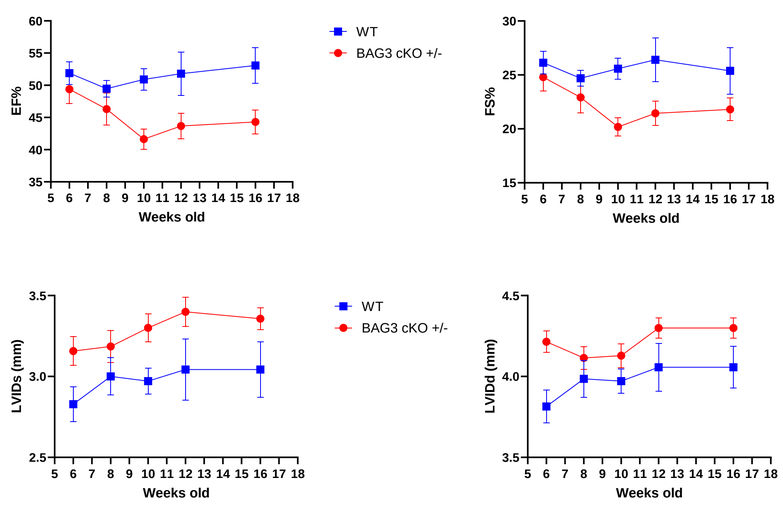


Total BAG3 protein measured in 10-week-old mouse heart lysate by JESS automated Western blot

Representative M-Mode ultrasound images from WT and BAG cKO $^{+/-}$ mice at 10 Weeks of age



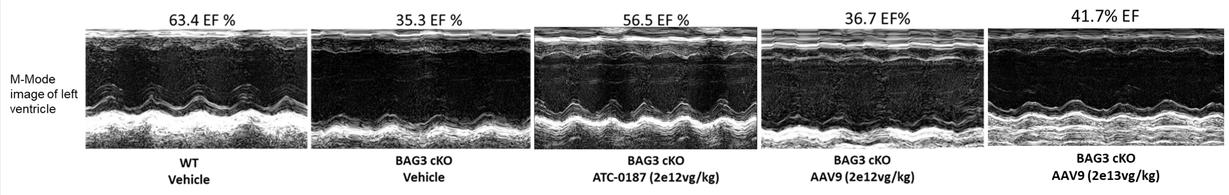
Time course of cardiac dysfunction by ultrasound in BAG cKO $^{+/-}$ mice vs. WT



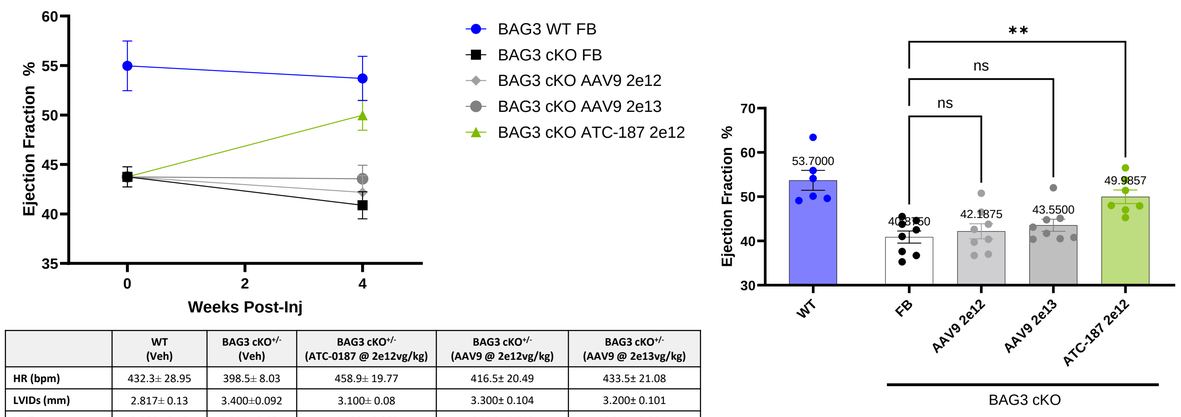
Ejection fraction (EF), fractional shortening (FS), Left ventricular inner diameter during systole (LVIDs), left ventricular inner diameter during diastole (LVIDd)

Restoration of cardiac function in BAG cKO $^{+/-}$ mouse model with ATC-0187-BAG3 treatment

Representative M-Mode ultrasound images from WT and BAG cKO $^{+/-}$ mice 4 weeks post injection



ATC-0187-BAG3 improves cardiac function in BAG cKO $^{+/-}$ mice 4 weeks post injection



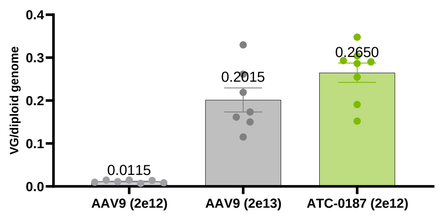
	WT (Veh)	BAG3 cKO$^{+/-}$ (Veh)	BAG3 cKO$^{+/-}$ (ATC-0187 @ 2e12vg/kg)	BAG3 cKO$^{+/-}$ (AAV9 @ 2e12vg/kg)	BAG3 cKO$^{+/-}$ (AAV9 @ 2e13vg/kg)
HR (bpm)	432.3± 28.95	398.5± 8.03	458.9± 19.77	416.5± 20.49	433.5± 21.08
LVIDs (mm)	2.817± 0.13	3.400±0.092	3.100± 0.08	3.300± 0.104	3.200± 0.101
LVIDd (mm)	3.850± 0.12	4.214± 0.074	4.100± 0.073	4.100± 0.041	4.100± 0.114
EF (%)	53.70± 2.24	40.88± 1.37	49.99± 1.53	42.20±1.70	43.6±1.37
FS (%)	27.33±1.43	19.75±0.75	25.10±0.93	20.50±0.938	21.3±0.811
LV Mass (mg)	70.70±5.75	71.13±3.41	75.09±2.05	71.90±1.86	74.4±3.78
LV Mass/BW (mg/g)	2.950±2.95	3.113±0.14	3.188±0.097	3.00±0.103	3.20±0.117

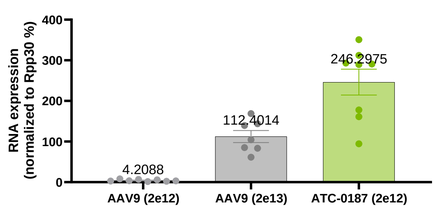
Heart rate (HR), left ventricular inner diameter during systole (LVID;s), left ventricular inner diameter during diastole (LVID;d), ejection fraction (EF), fractional shortening (FS); body weight (BW)

** p <0.01 relative to BAG3 cKO$^{+/-}$ veh; one-way ANOVA with Tukey's multiple comparisons test. Data are shown as means ± SEM

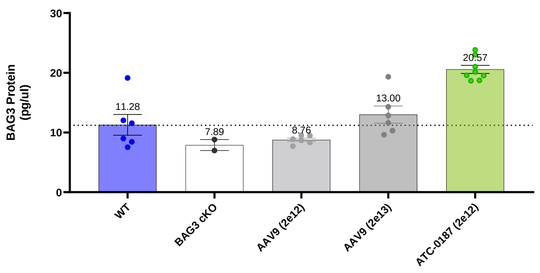
ATC-0187 provides superior transduction and BAG3 expression compared to AAV9

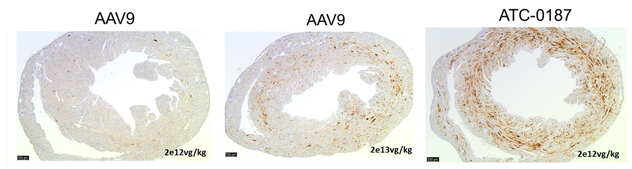
Biodistribution, RNA expression, and protein levels in the BAG3 cKO $^{+/-}$ heart 28 days post injection





ATC-0187 achieves 2X BAG3 protein expression in cKO $^{+/-}$ mouse heart and >50% transduction of the myocardium





Immunohistochemical (IHC) staining of mouse heart sections showing HA-positive staining (brown) in the myocardium. Images taken at 2x

No cardiac histopathology observed at efficacious dose

Summary and conclusions

- We have established proof of concept for an AAV-mediated gene replacement strategy to improve BAG3-related dilated cardiomyopathy (DCM) using both a surgically induced myocardial infarction model and a transgenic mouse model with cardiac-specific haploinsufficiency (BAG cKO $^{+/-}$) with cardiac dysfunction and reduced BAG3 levels similar to BAG3 DCM patients
- Utilizing a next-generation capsid (ATC-0187) with enhanced cardiac tropism in both mouse and NHP, we demonstrated that IV delivery of ATC-0187-BAG3 restored cardiovascular function in the BAG cKO $^{+/-}$ mouse model at doses considerably lower than those previously reported for AAV9 (2,3)
- No adverse in-life observations or test article related histopathologic findings were observed in heart, skeletal muscle or liver
- In contrast, AAV9 demonstrated no efficacy at either equivalent doses or doses that were 10 times higher (comparable to those used in clinical settings) in the BAG cKO +/- mouse model
- This gene therapy approach offers a promising strategy for improving BAG3 dilated cardiomyopathy, and the highly cardiotropic capsid may serve as a novel therapeutic option for the safe and effective delivery of cardiac proteins in cardiovascular disease

References:
1. Domínguez F, et al. Dilated Cardiomyopathy Due to BLC2-Associated Athanogene 3 (BAG3) Mutations. J Am Coll Cardiol. 2018 Nov 13;72(20):2471-2481
2. Knezevic T, Myers VD, Gordon J, Tilley DG, Sharp TE, 3rd, Wang J, et al. BAG3: a new player in the heart failure paradigm. Heart Fail Rev. 2015;20(4):423-34
3. Myers VD, Gerhard GS, McNamara DM, Tomar D, Madesh M, Kaniper S, et al. Association of Variants in BAG3 With Cardiomyopathy Outcomes in African American Individuals. JAMA Cardiol. 2018;3(10):929-38

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