AFTX-201: A Novel Investigational AAV Gene Therapy for Treatment of BAG3 Dilated Cardiomyopathy

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Introduction

Adeno-associated virus (AAV)-mediated gene therapy is a promising modality for cardiovascular disease, but efficient and safe myocardial gene delivery remains a major hurdle to clinical translation. Through a machine learning-guided rational design approach, we identified novel AAV capsids with enhanced cardiac tropism, enabling robust cardiomyocyte transduction at lower doses than wild-type AAV9.

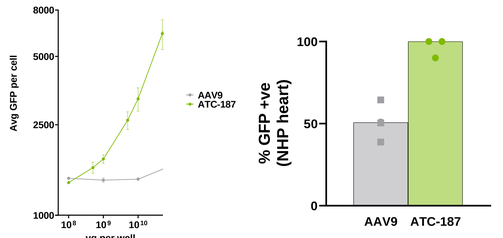
Dilated cardiomyopathy (DCM), characterized by left ventricular dilation and systolic dysfunction, is a leading cause of heart failure and the most common indication for heart transplantation in young patients. Over half of DCM cases have a genetic etiology, including pathogenic variants in BAG3, a co-chaperone protein implicated in sarcomere integrity and cellular stress response. BAG3-associated DCM (BAG3-DCM) is a severe, progressive disorder with no approved disease-modifying therapies.

We are developing AFTX-201, an AAV-based gene replacement therapy using a novel cardiotropic capsid to deliver full-length human BAG3. In a cardiac-specific BAG3 haploinsufficient (*BAG3* cKO$^{+/-}$) mouse model that recapitulates key aspects of BAG3-DCM, a single intravenous administration resulted in significant improvements in cardiac function, with no safety concerns observed. In nonhuman primates, low systemic doses of AFTX-201 achieved therapeutic cardiac BAG3 expression (>80% cardiomyocyte transduction and expression) with minimal off-target expression and no histopathological findings.

AFTX-201 is proposed as an investigational gene therapy for BAG3-DCM. A first-in-human, multicenter, open-label Phase 1/2 study will evaluate the safety, tolerability, and pharmacodynamics of a single intravenous dose in adults with symptomatic BAG3 mutation-associated DCM.

ATC-0187 next generation cardiotropic capsid

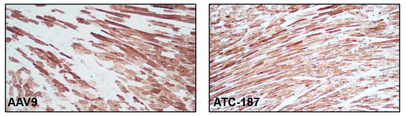
ATC-0187 performance is superior relative to AAV9 in iPSC-derived human cardiomyocytes and NHP heart



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 ventricle from IV delivered AAV9 and ATC-0187 at 3e13 vg/kg in NHPs (28 days in life)

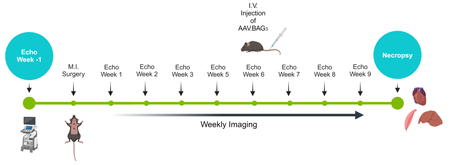
IHC staining of NHP heart sections showing GFP-positive staining in the myocardium



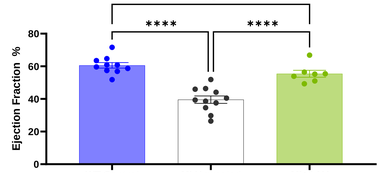
AAV-BAG3 restores normal cardiac function following myocardial infarction

Experimental protocol and BAG3 construct design

AAV expressing human BAG3 under the control of a muscle promoter



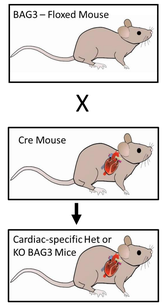
AAV-BAG3 improves cardiac function in MI mice 3 weeks post injection



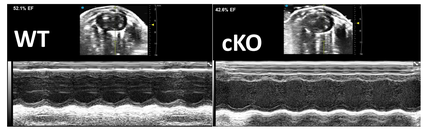
Ejection fraction (EF%) in WT and MI mice (9 weeks post MI surgery and 3 weeks post AAV injection). ****p <0.0001; one-way ANOVA with Tukey's multiple comparisons test. Data are shown as means ± SEM

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

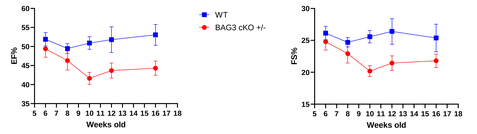
BAG3 Haplo-insufficient conditional KO generation paradigm



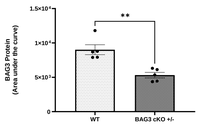
Representative M-Mode ultrasound images from WT and BAG cKO $^{+/-}$ mice at 10 Weeks of age



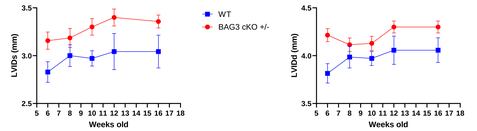
Time course of cardiac dysfunction by ultrasound in BAG cKO $^{+/-}$ mice vs. WT



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

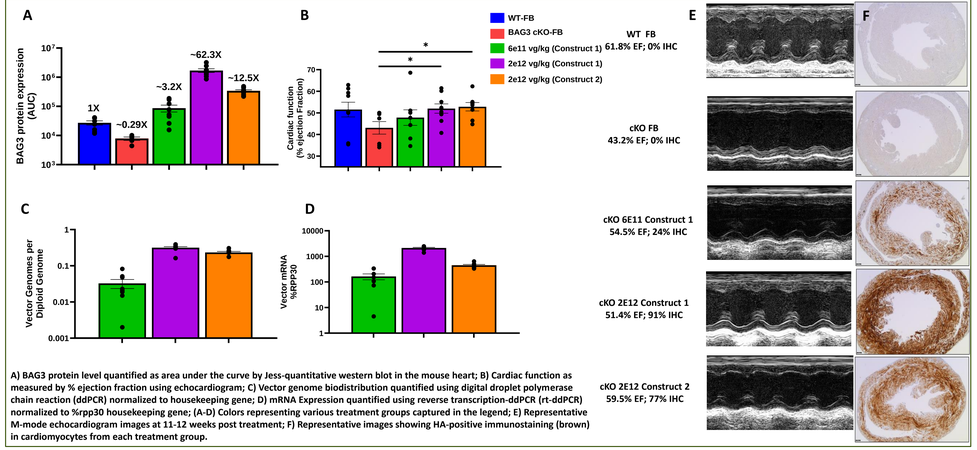


Total BAG3 protein measured in 10-week-old mouse heart lysate by JESS automated Western blot



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

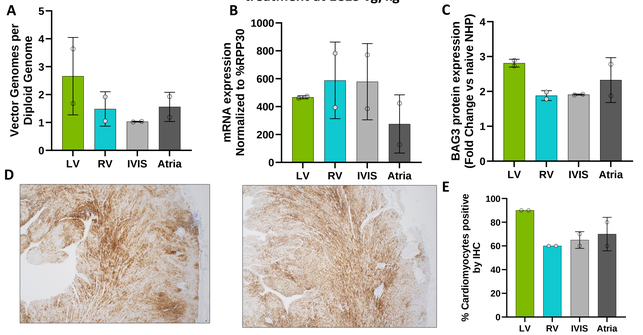
ATC-0187-BAG3 restores cardiac function in *BAG3* cKO$^{+/-}$ mouse model



A) BAG3 protein level quantified as area under the curve by Jess-quantitative western blot in the mouse heart; B) Cardiac function as measured by % ejection fraction using echocardiogram; C) Vector genome biodistribution quantified using digital droplet polymerase chain reaction (ddPCR) normalized to housekeeping gene; D) mRNA Expression quantified using reverse transcription-ddPCR (rt-ddPCR) normalized to %rpp30 housekeeping gene; (A-D) Colors representing various treatment groups captured in the legend; E) Representative M-mode echocardiogram images at 11-12 weeks post treatment; F) Representative images showing HA-positive immunostaining (brown) in cardiomyocytes from each treatment group.

Low systemic dose of ATC-0187-BAG3 shows robust BAG3 expression throughout NHP cardiac tissue

Proof of concept pharmacology, distribution and safety in NHP cardiac tissue after ATC-0187-BAG3 treatment at 1e13 vg/kg



A) Vector genome biodistribution quantified using digital droplet polymerase chain reaction (ddPCR) normalized to housekeeping gene; B) mRNA expression quantified using reverse transcription-ddPCR (rt-ddPCR) normalized to %rpp30 housekeeping gene; C) BAG3 protein level quantified as area under the curve by Jess-quantitative western blot in the ATC-0187-BAG3 treated vs naive NHP heart; D) Representative images showing HA-positive immunostaining (brown) in cardiomyocytes from two ATC-0187-BAG3 treated NHPs. No significant histopathological findings were observed in the NHP hearts (data not shown). E) Histological quantification of % cardiomyocytes positive for protein expression.

Summary and conclusions

- AFTX-201 is an investigational AAV-based gene therapy designed for BAG3 mutation–associated dilated cardiomyopathy (DCM)
- Our novel ATC-0187 capsid enables efficient cardiac transduction at significantly lower doses than wild-type AAV9 (~100% GFP transduction in NHP myocardium at 3e13 vg/kg)
- Proof of concept restoration of cardiac function in myocardial infarction (induced) and BAG3-haploinsufficient (genetic) mouse models.
- In nonhuman primates, systemic, low-dose ATC-0187-BAG3 administration achieved: ~60-90% cardiomyocyte transduction, robust BAG3 expression across heart and no histological abnormalities
- Data support a favorable efficacy and safety profile of AFTX-201 in murine and NHP models
- A Phase 1/2 first-in-human clinical trial is planned to evaluate safety, tolerability and pharmacodynamics in adults with symptomatic BAG3-DCM

References:

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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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