HDAC6 inhibition reduces cardiac fibrosis, enhances mitochondrial function and demonstrates comparable efficacy as empagliflozin in a mouse model of heart failure with preserved ejection fraction

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Background

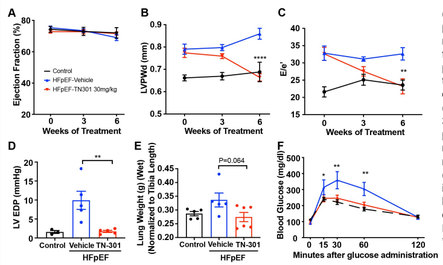
Heart failure with preserved ejection fraction (HFpEF) is a major health problem associated with high morbidity and mortality, yet there are few effective therapies. Previously, we demonstrated that TN-301 (TYA-11631), a histone deacetylase 6 (HDAC6)-selective orally bioavailable inhibitor, improved cardiac structure and function in mouse models of HFpEF and improved glucose metabolism and inflammatory markers in a mouse model of obesity. In this study, we aimed to characterize the underlying mechanism of action for how TN-301 improves cardiac function. Using a mouse model of established diastolic heart failure, we sought to understand whether the observed improvements in diastolic dysfunction are due to direct impacts on the heart or associated with improvement in overall systemic metabolism and inflammation.

Methods

To recapitulate systemic and cardiovascular features of HFpEF in humans, we induced diastolic dysfunction with a combination of moderate transaortic constriction and 12 weeks of high-fat diet. After the HFpEF phenotypes were established, mice received TN-301 (30 mg/kg) or vehicle orally once daily for 6 weeks. Echocardiography, blood glucose measurements, and end point invasive hemodynamic analyses were performed. Whole transcriptome was analyzed by RNA-seq. Mechanistic studies were conducted with human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) and primary human cardiac fibroblasts in vitro. In a separate cohort, head-to-head efficacy comparison with Empagliflozin (10mg/kg), the first approved drug in clinic for HFpEF, was conducted in the mouse HFpEF model.

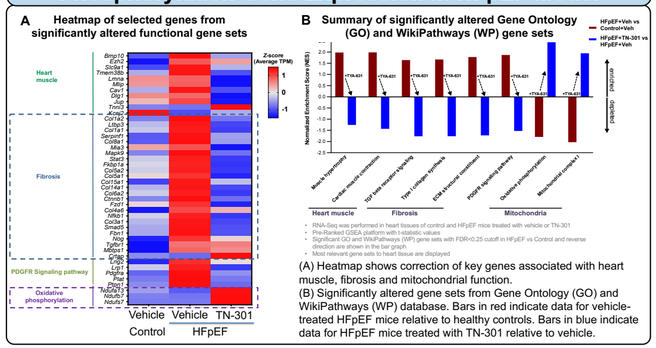
Results

TN-301 Reverses Pre-existing LV Hypertrophy and Diastolic Dysfunction in HFpEF Model



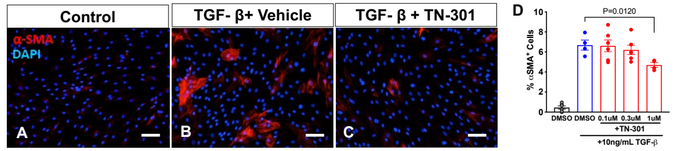
(A - D) Daily oral dosing of TN-301 preserved ejection fraction (A), normalized cardiac structure and heart function during the 6-week dosing period as measured by LV wall thickness (B), E/e' (C) and end diastolic pressure (D) in HFpEF mice.
(E) Lung weight was reduced by TN-301, suggesting improvements in pulmonary congestion and LV remodeling, consistent with the reduction of filling pressure.
(F) Glucose tolerance was markedly improved by TN-301 in HFpEF mice, bringing the levels closer to that of controls.
Error bars = SEM. *P < 0.05, **P<0.01, ****P<0.0001.

TN-301 Corrects Dysregulated Fibrosis and Oxidative Phosphorylation Gene Expression in HFpEF Model



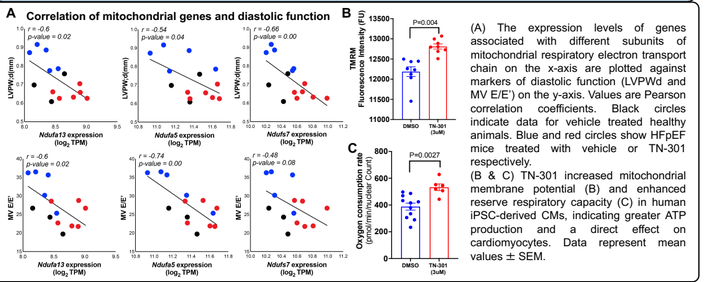
(A) Heatmap shows correction of key genes associated with heart muscle, fibrosis and mitochondrial function.
(B) Significantly altered gene sets from Gene Ontology (GO) and WikiPathways (WP) database. Bars in red indicate data for vehicle-treated HFpEF mice relative to healthy controls. Bars in blue indicate data for HFpEF mice treated with TN-301 relative to vehicle.

TN-301 Dose-dependently Prevents Fibroblast Activation from TGF- β in Primary Human Cardiac Fibroblasts



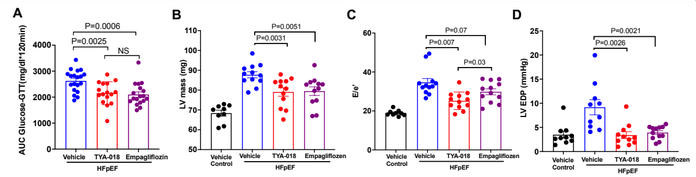
(A-C) Immunostaining of α-SMA in human cardiac fibroblasts (Control, A),or were treated with Vehicle (B) or 1μm TN-301 (C) in the presence of TGF-β (10ng/ml). Red: α-SMA; Blue: DAPI. Scale bars = 50 μm.
(D) TN-301 (1μM) effectively reduced TGF-β induced human cardiac fibroblast activation as measured by α-SMA +cells. α-SMA staining count was determined by blinded analyses. Each point represents 9 images per well. Data represent mean values ± SEM.

TN-301 Increases Mitochondrial Membrane Potential and Spare Respiratory Capacity in Human iPSC-CMs



(A) The expression levels of genes associated with different subunits of mitochondrial respiratory electron transport chain on the x-axis are plotted against markers of diastolic function (LVPWd and MV E/E') on the y-axis. Values are Pearson correlation coefficients. Black circles indicate data for vehicle treated healthy animals. Blue and red circles show HFpEF mice treated with vehicle or TN-301 respectively.
(B & C) TN-301 increased mitochondrial membrane potential (B) and enhanced reserve respiratory capacity (C) in human iPSC-derived CMs, indicating greater ATP production and a direct effect on cardiomyocytes. Data represent mean values ± SEM.

Comparable Efficacy of HDAC6i *Vs.*SGLT2i in HFpEF



(A) Single oral dose 15mg/kg TYA-018 (a HDAC6 selective inhibitor with similar potency to TN-301) significantly improved glucose tolerance to similar levels as Empagliflozin (SGLT2 selective inhibitor, 10mg/kg) in the established mouse HFpEF model.
(B, C, D) Chronic treatment with TYA-018 for 9 weeks reduced LV hypertrophy (B), and diastolic function as measured by E/e' (C) and end diastolic pressure (D). The effects were comparable to Empagliflozin. Data represent mean values ± SEM.

Conclusion

Our results show that the HDAC6 inhibitor TN-301 reverses pre-existing diastolic dysfunction through multiple pathways in the heart associated with fibrosis and mitochondrial dysfunction, which both contribute to HFpEF pathogenesis such as hypertrophy and diastolic heart failure. These results also confirm that TN-301 has a direct benefit on the heart mitochondrial energy metabolism in HFpEF models, and that the improvements seen are associated with increased oxidative phosphorylation, mitochondrial membrane potential and spare respiratory capacity in cardiomyocytes. The comparable efficacy and cellular mechanism observed in HFpEF model with HDAC6 inhibition and an SGLT2 inhibitor provides encouraging evidence of the potential translatability of these findings to clinical development. We are developing TN-301 to treat HFpEF patients.