## Methods

***Animals and drug treatments.*** *Fbn1*$^{mgR/mgR}$ experiments were performed using male mutant mice or sex-matched wild-type (WT) littermates maintained on the mixed 129T2/SvEmsJ;C57Blk/6J genetic background. Mice received 0.6 g/L losartan *ad libitum* in drinking water and the 1D11 antibody by intraperitoneal injection 3 times per week at a dose of 10 mg/kg of body weight; the antibody 13C4 was used as placebo treatment.[1] TAA progression in *Fbn1*$^{mgR/mgR}$ mice was monitored bi-weekly by echocardiography performed using a VisualSonics Vevo 2100 imaging system and a 40-MHz transducer.[3] The tail-cuff method was used to measure blood pressure on conscious WT animals treated with placebo or 1D11; bone mass and quality were evaluated in the same experimental groups according to standard *ex vivo* protocols.[2] The Institutional Animal Care and Use Committees of the Icahn School of Medicine at Mount Sinai and Genzyme Corporation reviewed and approved all animal studies.

***Histomorphometry.*** Proximal ascending aortas harvested from WT and mutant mice were processed, stained and evaluated for the extent of media degeneration based on the combined assessment of wall thickness and wall architecture.[3] Average aortic thickness of 3 representative areas was independently measured and averaged by two observers blinded to genotype and treatment. Likewise, two individuals blinded to genotype and treatment independently counted and averaged the number of free ends along elastin-stained lamellae at 3 equally distant rings along the vessel's length. Aortic wall architecture was graded giving thickness and number of free ends equal weight according to a severity scale for a combined score ranging from 1 (normal elastic fiber morphology) to 5 (diffuse elastic fiber disruption).

***Biochemistry.*** Protein extracts were prepared from frozen aortic tissues and processed for immunoblots as previously described.[4] Antibodies against phosphorylated and non-phosphorylated Smad2/3 and Erk1/2 proteins (Cell signaling) were diluted 1:1000 in Tris-buffered saline, pH 7.4, and 0.1% (v/v) Tween 20 in the presence of 5% BSA and incubated with the membrane for 12 h at 4 °C. In all cases, Ponceau S Solution staining (Sigma) of the transfer membrane was used as a protein loading control. Immunoreactive products were visualized by chemiluminescence using Clarity Western ECL substrate (Bio-Rad) and their relative intensity was evaluated using Photoshop (Adobe Systems Inc.).

***Statistics.*** Equality of group variances was examined by F-test and Brown-Forsythe test (GraphPad Prism Statistical Software Package). Un-paired two-tailed t-tests were used to determine the statistical significance between two groups assuming significance at $p < 0.05$ with Welch's correction applied when necessary and further confirmed with the non-parametric Mann-Whitney Test. Analyses between multiple groups employed one-way ANOVA with $p < 0.05$ considered statistically significant. Tukey's Multiple Comparison test was used for post-hoc 2-sample comparisons. All values are expressed as mean ± S.D. Overall survival was calculated from weaning through death or collection at the intended time points. Overall survival was evaluated with Mantle-Cox (log-rank) statistical tests (GraphPad Prism); Kaplan-Meier curves were constructed for each treatment.

References:


1. Cook JR, Carta L, Benard L, Chemaly ER, Chiu E, Rao SK, Hampton TG, Yurchenco P, Costa KD, Hajjar RJ, Ramirez F. Abnormal muscle mechanosignaling triggers cardiomyopathy in mice with Marfan syndrome. *J. Clin. Invest.* 2014;124:1329-1339.

2. Carta L, Smaldone S, Zilberberg L, Loch D, Dietz HC, Rifkin DB, Ramirez F. p38 MAPK is an early determinant of promiscuous Smad2/3 signaling in the aortas of fibrillin-1 (*Fbn1*)-null mice. *J. Biol. Chem*. 2009;284:5630-5636.
3. Nistala H, Lee-Arteaga S, Carta L, Cook JR, Smaldone S, Siciliano G, Rifkin AN, Dietz HC, Rifkin DB, and Ramirez F. Differential effects of alendronate and losartan therapy on osteopenia and aortic aneurysm in mice with severe Marfan syndrome. *Hum. Mol. Genet.* 2010;19:4790-4798.
4. Matt P, Schoenhoff F, Habashi J, Holm T, Van Erp C, Loch D, Carlson OD, Griswold BF, Fu Q, De Backer J, Loeys B, Huso DL, McDonnell NB, Van Eyk JE, Dietz HC, GenTAC Consortium. Circulating transforming growth factor-β in Marfan syndrome. *Circulation* 2009;120:526-532.