TRIAL OF BETA BLOCKER THERAPY (ATENOLOL) VS. ANGIOTENSIN II RECEPTOR BLOCKER THERAPY (LOSARTAN) IN INDIVIDUALS WITH MARFAN SYNDROME

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OVERVIEW (ABSTRACT)

Marfan syndrome (MFS) is a systemic disorder of connective tissue with autosomal dominant inheritance and a population prevalence of approximately 1 per 5,000. Cardiovascular pathology, including aortic root dilation, dissection, and rupture is the leading cause of mortality in the MFS. Several studies have shown that beta blockers (BB) reduce the rate of aortic growth. Although advances in therapy have improved life expectancy, individuals with MFS continue to suffer significant cardiovascular morbidity and mortality. Recent studies in a *FBN1*-deficient mouse model of MFS with a susceptibility to aortic dilation and dissection, similar to that seen in humans with MFS, showed that postnatal treatment with losartan, an angiotensin II receptor blocker (ARB), normalized aortic root growth and aortic architecture, preventing aortic aneurysms and premature death. The Pediatric Heart Network's proposed multi-institutional, randomized clinical trial will compare aortic root growth and other short-term cardiovascular outcomes between subjects receiving either atenolol or losartan.

Individuals with MFS who meet Ghent diagnostic criteria, between the ages of 6 months and 25 years, with BSA-adjusted aortic root Z-score > 3.0 and without previous aortic surgery, will be eligible for inclusion in this study. Participants will be randomly assigned to receive either atenolol or losartan, with randomization stratified by attainment of maximal height and BSA-adjusted aortic root Z-score at study entry. Dynamic allocation will be used to ensure treatment arms are balanced by center. Data will be collected at baseline and at 6, 12, 24, and 36 months after randomization. The primary aim of the study will be to compare the effect of atenolol (BB) therapy to that of losartan (ARB) therapy on the rate of aortic growth and progression of aortic regurgitation. Secondary endpoints include incidence of the following cardiovascular events: aortic dissection, aortic root surgery, and death; progression of mitral regurgitation; left ventricular size and function; echocardiographically-derived measures of central aortic stiffness; skeletal and somatic growth; and incidence of reported adverse drug reactions. The total sample size target of 604 participants will be recruited over approximately 48 months.

A. SPECIFIC AIMS

MFS is a common, heritable disorder of connective tissue with multi-system involvement. Cardiovascular pathology, including aortic root dilation, dissection, and rupture is the leading cause of mortality in the MFS. The best single predictor of adverse cardiovascular outcome is the aortic root dimension at the sinuses of Valsalva. Several studies have shown that BB therapy reduces the rate of aortic growth. Advances in therapy have improved life expectancy; however, individuals with MFS continue to suffer significant cardiovascular morbidity and mortality.

Recent studies in a *FBN1*-deficient mouse model of MFS with a susceptibility to aortic dilation and dissection showed that postnatal treatment with losartan, an ARB, normalized aortic root growth and aortic architecture, preventing aneurysmal dilation and premature death. ARB therapy has the theoretical advantage of modifying the predisposed tissue directly by antagonism of TGFβ, whereas BB therapy is thought to act by reducing the hemodynamic stress on an inherently predisposed tissue. Very limited data are available to assess the efficacy of ARB therapy in reducing aortic complications in humans with MFS. This multi-center randomized clinical trial will compare outcomes in individuals with MFS who are randomized to treatment with either atenolol or losartan.

A.1 Primary Aim

To compare the effect of BB therapy to that of ARB therapy on the rates of aortic growth and progression of aortic regurgitation.

Hypothesis: The rates of aortic growth and progression of aortic regurgitation will be lower in those receiving ARB therapy when compared to those receiving BB therapy.

Primary outcome:

- Rate of change in aortic root (sinuses of Valsalva) BSA-adjusted Z-score

Secondary outcomes:

- Rate of change in aortic root (sinuses of Valsalva) absolute dimension
- Rate of change in ascending aorta absolute dimension and BSA-adjusted Z-score
- Rate of change in aortic annulus absolute dimension and BSA-adjusted Z-score
- Rate of change of aortic regurgitation, measured as change in vena contracta area indexed for BSA

A.2 Secondary Aim

To compare the effect of BB therapy to that of ARB therapy on the incidence of the following cardiovascular events: aortic dissection, aortic root surgery, and death.

Hypothesis: The frequency of the combined endpoint, aortic dissection, aortic root surgery, or death, will be lower in subjects receiving ARB therapy when compared to that in those receiving BB therapy.

Outcomes:

- Aortic dissection, aortic root surgery, or death at 36 months after randomization
- Time to first occurrence of aortic dissection, aortic root surgery, or death up to 36 months after randomization

A.3 Secondary Aim

To compare the effect of BB therapy to that of ARB therapy on the progression of mitral regurgitation.

Hypothesis: The rate of progression of mitral regurgitation will be lower in subjects receiving ARB therapy when compared to those receiving BB therapy.

Outcome:

- Rate of change of mitral regurgitation, measured as change in vena contracta area indexed for BSA

A.4 Secondary Aim

To compare the effect of BB therapy to that of ARB therapy on left ventricular size and function.

Hypothesis: The rate of progression of left ventricular dilation and dysfunction will be lower in subjects receiving ARB therapy when compared to those receiving BB therapy.

Outcomes:

- Rate of change of left ventricular mass, volume, mass to volume ratio, and ejection fraction by two-dimensional echocardiography
- Rate of change of left ventricular end-diastolic and end-systolic dimensions, diastolic septal and posterior wall thickness, left ventricular mass and shortening fraction by M-mode

A.5 Secondary Aim

To compare the effect of BB therapy to that of ARB therapy on echocardiographically-derived measures of central aortic stiffness.

Hypothesis: The rate of change in central aortic stiffness indices will be higher in subjects receiving ARB therapy when compared to those receiving BB therapy.

Outcome:

- Rate of change of ascending aortic elastic modulus and stiffness index

A.6 Secondary Aim

To compare the effect of BB therapy to that of ARB therapy on skeletal and somatic growth.

Hypothesis: Long bone overgrowth will be reduced and weight gain will be improved in subjects receiving ARB therapy when compared to those receiving BB therapy.

Outcomes:

- Rate of change in Z-scores for weight, height, body mass index (BMI), and upper-to-lower segment ratio corrected for age in subjects as determined by availability of Z-scores (see text)
- Rate of change in weight and BMI with covariate adjustment for age in all subjects

A.7 Secondary Aim

To compare the effect of BB therapy to that of ARB therapy on the incidence of drug side effects.

Hypothesis: ARB therapy will be associated with a lower incidence of drug side effects compared to BB therapy.

Outcome:

- Incidence of adverse events and patient-reported symptoms.

B. BACKGROUND

B.1 Previous Studies

B.1.1 The Marfan Syndrome

MFS is a systemic disorder of connective tissue with autosomal dominant inheritance and a prevalence of approximately 1 per 5,000 population [1]. The syndrome shows no racial or gender preference. The cardinal features of this disorder involve the ocular, musculoskeletal, and cardiovascular systems. Cardiovascular pathology, including aortic root dilation, dissection, and rupture and myxomatous valve changes with insufficiency of the mitral and aortic valves, is the leading cause of mortality in the MFS. The majority of fatal events associated with untreated MFS occur in early adult life. In a prospective study of 72 patients in 1972, the median life expectancy was about 45 years [2]. A recent reevaluation of life expectancy in the MFS suggested that early diagnosis and refined medical and surgical management have increased median life expectancy to about 70 years [3]. Nevertheless, MFS continues to be associated with significant morbidity and selected subgroups are refractory to therapy and continue to show early mortality. In a review of 54 patients diagnosed during infancy, Morse *et al.* reported that 89% had serious cardiac pathology, and that cardiac disease was progressive despite standard care (22% died during childhood, 16% before age 1 year) [4, 5]. In the classic form of MFS diagnosed after infancy, it is estimated that up to 90% of affected individuals will have a cardiovascular 'event' during their lifetime, including surgical repair of the aortic root, fatal or non-fatal aortic dissection or mitral valve surgery [6-9]. Ocular and skeletal morbidity is also common [10-12]. Approximately 60% of individuals with MFS have lens dislocation, often requiring surgical lens removal for optimal management. Retinal detachment and glaucoma can cause blindness. Skeletal involvement is evident in nearly all people with MFS. Progressive anterior chest deformity or scoliosis can cause cardiopulmonary dysfunction and commonly requires surgical correction. Joint instability can cause physical disability and predispose to premature arthritis. Between 70 and 90% of individuals with MFS will show radiographic evidence of dural ectasia, most often characterized by progressive dural sac widening in the lumbar and sacral spine [13, 14]. This can be associated with bony erosion and nerve impingement. Common symptoms include low back pain, headache, proximal leg pain, weakness and numbness above and below the knee, and genital/rectal pain. Lung disease most commonly manifests with spontaneous pneumothorax and has been identified in 4 -11% of MFS patients [15, 16]. Pathologic findings include upper lobe bullae with or without diffuse fixed obstructive airway disease that can be progressive and has traditionally been equated with destructive emphysema [17, 18]. The majority of patients with MFS display a marked deficiency in skeletal muscle mass and fat stores despite adequate caloric intake and no evidence for malabsorption [19-22]. The skeletal myopathy observed in a subset of individuals with MFS may

contribute to decreased functional performance, respiratory insufficiency, ocular misalignment, and altered development of the skeleton including kyphosis and scoliosis [19-21]. An increasing challenge is to define the "new" natural history of MFS now that many individuals are surviving their predisposition for early aortic root dissection; already appreciated aging-associated phenotypes include aneurysms or dissection of the descending thoracic and abdominal aorta. Thus, despite advances in our ability to increase the length of life for many individuals with MFS, there is ample opportunity to improve their quality of life.

B.1.2 Etiology of Marfan syndrome

In 1991 a positional-candidate analysis demonstrated heterozygous disease-producing mutations in the *FBN1* gene on chromosome 15q21.1 that encodes fibrillin-1, the major glycoprotein component of extracellular microfibrils [23]. Linkage evidence suggests the absence of locus heterogeneity for the classic Marfan phenotype [24, 25]. To date, over 600 *FBN1* mutations have been reported to an international database [26]. Analyses of patients' tissues and cultured cells revealed diminished amounts of microfibrils [27, 28]. Conventional wisdom held that microfibrils are essential for elastogenesis and that elastic fiber formation is virtually complete after early postnatal life [29-31]. Expression of fibrillin-1 and its close family member fibrillin-2 is significantly down-regulated after early childhood. Taken together, these data suggested a very limited window of opportunity to modulate the pathogenetic sequence in MFS and boded poorly for the development of novel therapeutic strategies. Recent work using genetically engineered mouse models of MFS has tested and ultimately refuted this hypothesis. Importantly, these models recapitulate most phenotypic alterations in MFS including a specific predisposition for progressive deterioration in aortic wall architecture, aortic root enlargement and aortic dissection leading to premature sudden death [32-34]. Despite an early and severe deficiency of microfibrils, mutant mice show normal elastin content and elastic fiber architecture early in postnatal life. With time, they demonstrate elastic fiber fragmentation and disarray, excess production of matrix elements and matrix-degrading enzymes, and inflammation – all features of the so-called "cystic medial necrosis" that has been documented in the human condition [34, 35]. In essence, these data document that fibrillin-1 and microfibrils are not needed for elastic fiber formation, as originally inferred, but rather contribute to elastic fiber homeostasis in postnatal life [32-35]. Inherent to this paradigm is the newly recognized opportunity for productive therapeutic intervention.

Many features of MFS (e.g. bone overgrowth, myxomatous valve changes, craniofacial abnormalities) were difficult to reconcile using pathogenetic models that singularly invoke structural weakness of the tissues. Rather, they more plausibly reflect altered cellular migration, proliferation and/or programmed death. TGFβ rapidly emerged as a potential mediator of these morphogenetic

perturbations. The TGFβs are multipotential cytokines that regulate cell performance and tissue morphogenesis. They are synthesized and secreted by the cell as an inactive precursor complex (termed the large latent complex) that binds to the extracellular matrix and requires regulated activation (i.e., release of free TGFβ) for biologic activity [36, 37]. Because the latent transforming growth factor-beta-binding protein component of the large latent complex has been localized to extracellular microfibrils and specifically binds to fibrillin-1 [38-41], it was reasoned that the morphogenetic abnormalities in MFS might manifest failure of latent complex sequestration and consequent excessive cytokine activation [42]. A mechanistic link between excessive TGFβ activation and signaling and the pathogenesis of disease in the fibrillin-1 deficient state has been demonstrated [42-44]. It was shown that failed lung alveolar septation [42], myxomatous thickening of the mitral valve [43], dural ectasia [45], failed muscle regeneration and muscle hypoplasia (unpublished data from Dietz laboratory) and progressive aortic root dilation [46] correlated with increased TGFβ signaling in fibrillin-1 deficient mice. Moreover, the lung, valve, muscle and aortic phenotypes could be attenuated or prevented in mouse models of MFS by systemic administration of an antibody that specifically antagonizes the activity of the canonical TGFβ ligands (TGFβs 1-3) *in vivo*. These data develop the paradigm that matrix sequestration of cytokines is critical to their regulated activation and that perturbation of this function can contribute to the pathogenesis of heritable disorders of connective tissue, including MFS.

The prominent role of TGFβ dysregulation in the pathogenesis of MFS and related conditions was validated by the clinical manifestations in individuals heterozygous for mutations in either of the two genes that encode the TGFβ receptor (*TGFBR1* and *TGFBR2*). It was found that heterozygous mutations in either receptor subunit cause a distinct but overlapping phenotype (Loeys-Dietz syndrome; LDS; OMIM# 609192) that includes many features of MFS (arachnodactyly, pectus deformity, scoliosis, dural ectasia, ascending aortic aneurysm with dissection), but also many unique findings (e.g. hypertelorism, cleft palate/bifid uvula, craniosynostosis, generalized arterial tortuosity, aneurysms and dissection throughout the arterial tree). Heterozygosity for loss-of-function mutations leads to a paradoxical increase in TGFβ signaling in the aortic wall in affected individuals, identical to that seen in patients with MFS [47] [44].

B.1.3 Treatment of Marfan syndrome

Surgery for aortic aneurysm: The frequency of acute aortic dissection is directly proportional to the maximal diameter of the aorta. Elective surgery to repair the aortic root in patients with MFS is recommended when the maximal aortic diameter in adults or older children reaches 5.0 cm [9, 48]. Additional considerations include the rate of aortic growth and family history of aortic dissection at a

size less than 5.0 cm. Earlier surgical intervention is recommended for individuals with an increase in aortic diameter exceeding 1 cm per year.

Composite surgical replacement of the aortic root and valve was pioneered by Bentall and De Bono in 1968 [49]. Modifications of this technique have evolved with various methods of coronary reimplantation [50]. Gott and colleagues reported outcomes for 675 patients with MFS who underwent aortic root replacement surgery at 10 experienced surgical centers (7 in North America and 3 in Europe) [9]. Mortality for elective surgery was 1.5%, compared to urgent surgery (taking place within 7 days after surgical consultation) with mortality of 2.6%. Mortality was 11.7% among patients who underwent emergent surgery, within 24 hours after surgical consultation.

Because of the risks of thromboembolism and the lifetime requirement for warfarin anticoagulation in the setting of a mechanical prosthetic aortic valve, recent surgical efforts (pioneered by David and colleagues) have attempted to maintain the native aortic valve among eligible patients with MFS [51, 52]. To date, no randomized clinical trials of valve replacement versus valve-sparing aortic root surgery have been performed, and long-term data on the outcomes with valve-sparing surgery are not yet available. Nevertheless, the short-term data are encouraging, with an extremely low rate of operative mortality (equivalent to that seen with composite graft repair) [53-55]. The valve-sparing procedure has seen a tremendous evolution, largely focused upon preserving function of the native aortic valve. Initial attempts to resuspend the valve into the Dacron graft were complicated by damage to the leaflets upon maximal excursion. A remodeling procedure that involved sewing the graft to the top of the aortic annulus showed an unacceptable rate of valve splaying with subsequent dysfunction and re-operation. Most recently, a modified resuspension procedure is being employed with crafting of artificial sinuses above the valve [53, 55, 56]. This has shown excellent short-term results and is now the preferred treatment in all eligible patients with MFS presenting for surgical intervention. The valve-sparing approach is particularly attractive for young women with MFS who anticipate pregnancy since it would preclude the need for coumadin, a known teratogen. Given the current preference for the valve-sparing procedure, the emergence of significant aortic regurgitation is now widely accepted as an additional criterion to proceed with prophylactic surgery. Valve function can often be improved surgically if this operation is performed before development of severe regurgitation.

Medical therapy to modify the progression of aortic dilation: Beta-adrenergic receptor blockade to delay or prevent aortic aneurysm and dissection is commonly used for patients with MFS. This was first proposed in 1971 by Halpern and colleagues [57]. The rationale for this treatment strategy is primarily to decrease proximal aortic shear stress, or dP/dT. BBs are likely beneficial both through

negative inotropic and negative chronotropic effects. The majority of published studies have demonstrated benefit of treatment with BBs in MFS, including in children [58, 59]. The only randomized trial assessing the effect of beta-blockade in patients with MFS was published in 1994 [60]. Using an open-label protocol, the investigators treated 32 of 70 enrolled patients with propranolol using a dose targeted for a heart rate below 100 beats per minute during exercise or resulting in a 30 percent increase in the systolic time interval (corrected for the heart rate). Patients were analyzed for aortic growth, with change in body size accounted for in an aortic ratio by dividing the measured aortic diameter by the diameter predicted from the patient's height, weight, and age [61, 62]. Fewer patients treated with propranolol reached a primary clinical endpoint of aortic regurgitation, aortic dissection, cardiovascular surgery, congestive heart failure, and death (5 in treatment group, 9 in control group). Furthermore, the normalized rate of aortic dilation was lower in the propranolol group compared to the control group (0.023 vs. 0.084 per year, $p<0.001$). In 1995, Silverman and colleagues examined the life expectancy for individuals with MFS. In comparison to 1972, they found an increase in the mean age at death (41 ± 18 vs. 32 ± 16 years; $p = 0.0023$) and the median cumulative probability of survival (72 vs. 48 years) [3]. Use of BBs was positively correlated with prolonged survival.

Many studies have shown increased aortic stiffness and decreased aortic compliance in individuals with MFS [63-71]. The significance of these findings to predisposition for progressive aortic root enlargement and dissection is currently unknown. One study found that increased aortic stiffness correlated with descending but not ascending aortic enlargement and dissection [70]. As in other studies, aortic root size was the best determinant of root dissection. Studies of aortic stiffness measured by echocardiography, magnetic resonance imaging, and cardiac catheterization have shown a heterogeneous response to beta-blockers in patients with Marfan syndrome [63, 69, 70]. Advanced aortic root size correlated with lack of response [70], perhaps highlighting the need for early intervention.

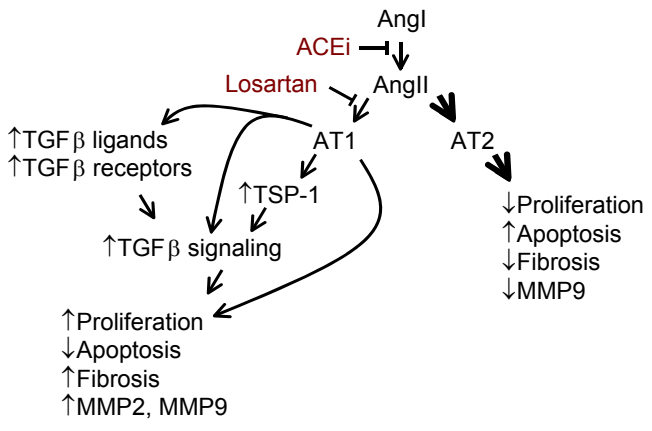
Data from trials that have assessed use of pharmacologic therapy for aortic aneurysm in MFS show that aortic growth is not stopped or reversed, but typically is slowed in response to treatment. The use of BBs does not prevent attainment of other important clinical endpoints including aortic valve dysfunction, surgery, dissection and death in all patients.

B.1.4 Pharmacologic trials in mouse models of Marfan syndrome

Numerous studies describe the ability of ARBs; e.g. losartan, to achieve clinically-relevant inhibition of TGFβ signaling *in vivo* [72-83]. For many disease states, including chronic renal disease and cardiomyopathy, the antifibrotic effects of losartan have been directly linked to TGFβ inhibition, and occur independently of the hemodynamic consequences of drug use. Markers of TGFβ antagonism have included reduced plasma levels of free TGFβ, reduced intracellular initiation of the TGFβ signaling cascade, and reduced tissue expression of TGFβ-responsive genes.

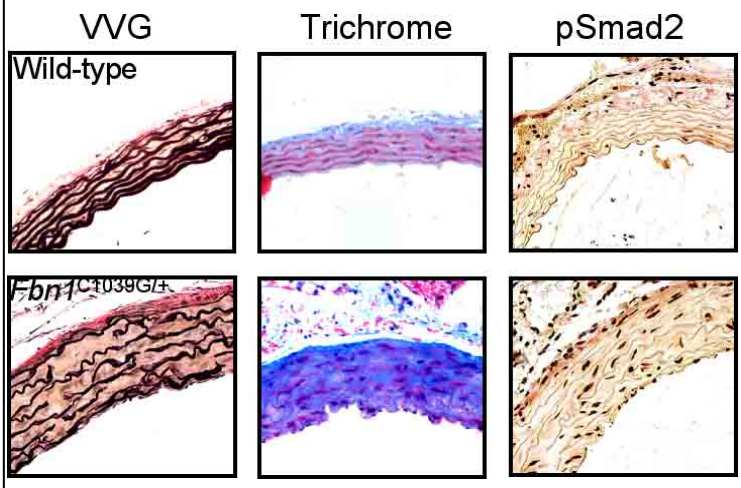
Theoretical and practical evidence supports the use of an ARB (such as losartan), which blocks only AT1 receptors, instead of an angiotensin converting enzyme inhibitor (ACEi). First, the vast majority of studies examining TGFβ antagonism have used ARBs. Second, it is known that signaling through the AT1 receptor initiates multiple relevant pathologic events including expression of thrombospondin-1 (TSP-1, a potent activator of TGFβ signaling [84, 85]), TGFβ ligands and receptors and TGFβ-responsive genes. AT1 signaling also promotes cellular proliferation, fibrosis and expression of matrix metalloproteinases (MMPs) 2 and 9, the very MMPs that have been implicated in the pathogenesis of MFS [35] **(Fig.1)**. Signaling through the AT2 receptor (which would also be blocked with use of an ACEi) antagonizes each of these effects [86], a potentially desirable event. In contrast, selective blocking of the AT1 receptor (as with losartan) actually stimulates AT2 signaling, presumably by relieving competition for ligand binding. Informatively, in a model of angiotensin II-mediated abdominal aneurysm, Daugherty and colleagues showed that selective AT1 blockade prevented vascular disease while selective AT2 blockade augmented both the incidence and severity of aneurysm [87].

Figure 1.
Events Mediated by AT1 Signaling



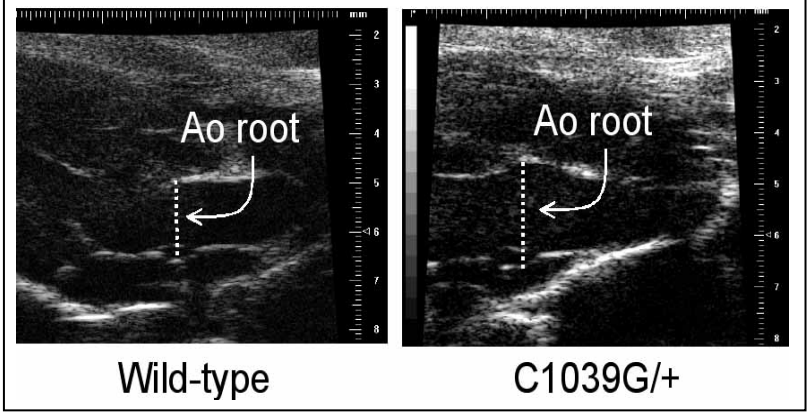
To test the hypothesis that AT1 blockade will provide superior protection to the aorta in MFS, Dr. Hal Dietz's laboratory first comprehensively characterized the aortic wall in fibrillin-1 deficient mice (C1039G/+). At 4 months of age significant elastic fiber fragmentation, wall thickening and excess TGFβ signaling was observed, as shown by increased nuclear accumulation of phosphorylated Smad2 (pSmad2) and increased output of TGFβ-responsive genes (e.g. collagens, as assayed by trichrome staining; **Fig. 2**). Echocardiography was used to monitor aortic root size and growth **(Fig. 3)**. The studies showed that systemic administration of TGFβ-neutralizing antibody could productively modify each of these phenotypes (data not shown).

Figure 2.
Pathology in Fibrillin-1 Deficient Mice



VVG – elastin stain; Trichrome – collagen (blue);
pSmad2 – marker of TGFβ signaling

Figure 3.
Parasternal Long-axis View of Mouse Models at 4 Months of Age



The investigators then randomized cohorts of mutant mice to receive placebo, propranolol or losartan. The doses of propranolol and losartan were titrated to achieve comparable hemodynamic effects (15 - 20% reduction of both heart rate and blood pressure). Oral treatment was initiated at about 2 months of age, after the development of aneurysmal dilation. Over the next 6 months, each animal underwent 3 independent measurements of the aortic root every 2 months. All measurements and analyses were made by observers who were blinded to genotype and treatment arm. At the end of 6 months, all mice were sacrificed for histopathologic analyses.

As shown in **Figs. 4 and 5**, the Dietz lab found that fibrillin-1 deficient mice (C1039G/+) mice showed an accelerated rate of aortic growth compared to wild-type animals ($p<0.0001$). Propranolol-treated mice showed slower aortic growth compared to the placebo group ($p<0.001$), but showed faster growth than the losartan-treated animals ($p<0.02$). Indeed, the rate of growth in losartan-treated animals was indistinguishable from that seen in the wild-type group ($p=0.55$). Propranolol had no impact on aortic wall thickness ($p=0.17$) or architecture ($p=0.47$) when compared to placebo, but losartan had a dramatic effect ($p<0.002$ and $p<0.002$, respectively). Indeed, there was no difference in either variable between wild-type and losartan-treated animals ($p=0.47$ and $p=0.23$, respectively). Similar results were obtained in a separate cohort of animals in which treatment was initiated prenatally (data not shown). Informatively, the improved performance of losartan correlated with reduced TGFβ signaling, as evidenced by decreased nuclear accumulation of pSmad2 in the aortic media **(Fig. 4)**. Dr. Dietz and colleagues concluded that BBs work in this model as suggested in several studies in humans. They slow but do not halt abnormal aortic growth, and do not appear to directly interface with the underlying pathogenetic mechanism. In contrast, ARBs have the potential to completely arrest abnormal aortic growth and

Figure 4. Postnatal losartan rescues aortic wall architecture

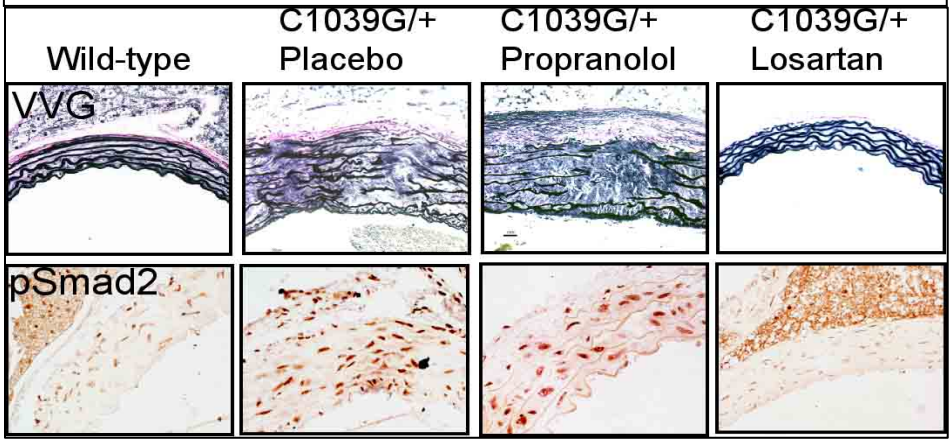
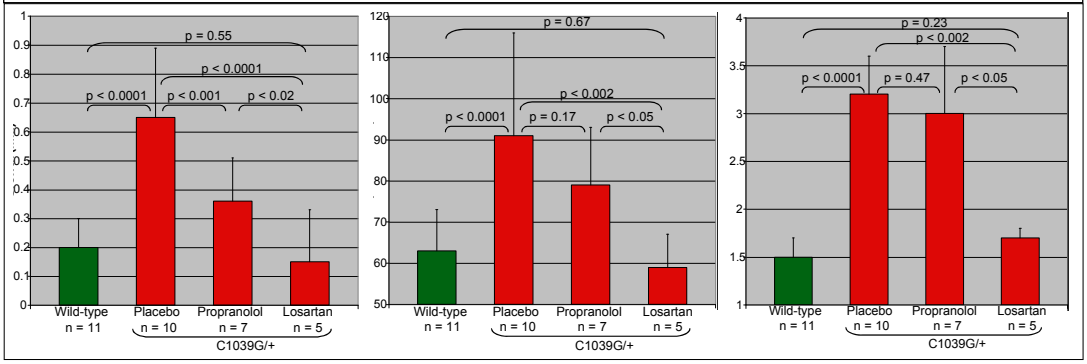


Figure 5. Postnatal Losartan Rescues Aortic Wall Performance



may even promote aortic wall remodeling. The similar hemodynamic effects of BBs and losartan therapy in this trial are suggestive that the particular protection afforded by ARBs is not simply modification of the stress imposed on an inherently predisposed tissue, but rather relates to modification of the underlying predisposition presumably through antagonism of TGFβ.

The C1039G/+ model was selected for analysis because it recapitulates the most common mutational mechanism seen in human MFS (i.e., heterozygosity for a cysteine substitution in a calcium-binding EGF-like domain in fibrillin-1) and shows all of the relevant histopathologic events associated with the human condition. It does not, however, progress to dissection and death within the normal life-span of the mouse. In order to determine whether losartan has the potential to modify this important clinical endpoint, Dr. Dietz's laboratory has initiated a study of the R1/R1 mouse line that is homozygous for a targeted hypomorphic *Fbn1* allele. These mice die due to aortic dissection within the first 6-9 months of life. Although these studies are still in progress, the preliminary data are very encouraging **(Fig. 6)**. They show that by 6 months of age there is 30% and 40% mortality due to aortic dissection in propranolol- and placebo-treated groups, respectively. In contrast, there have been no deaths in the losartan-treated cohort ($p<0.001$). Furthermore, losartan treatment has not been associated with any discernable side effects. Analysis of aortic growth, size and histology is awaiting completion of the study.

Figure 6. R1/R1 Mouse Drug Trial (in progress)

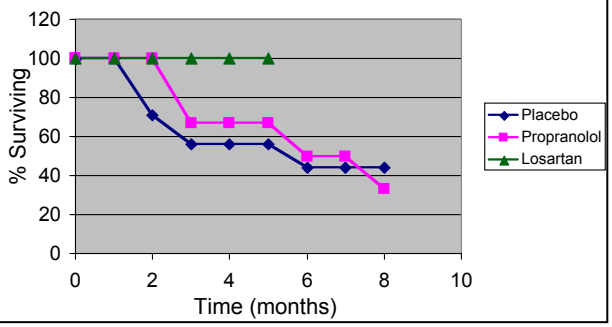
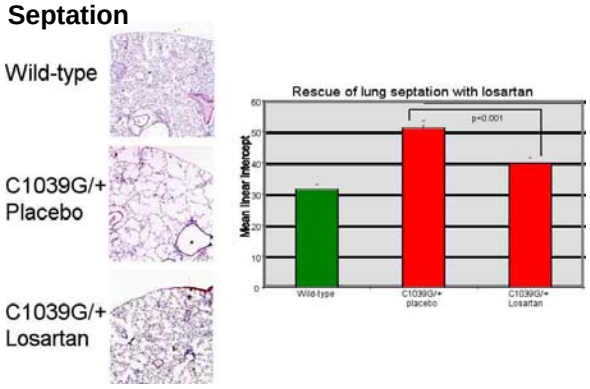


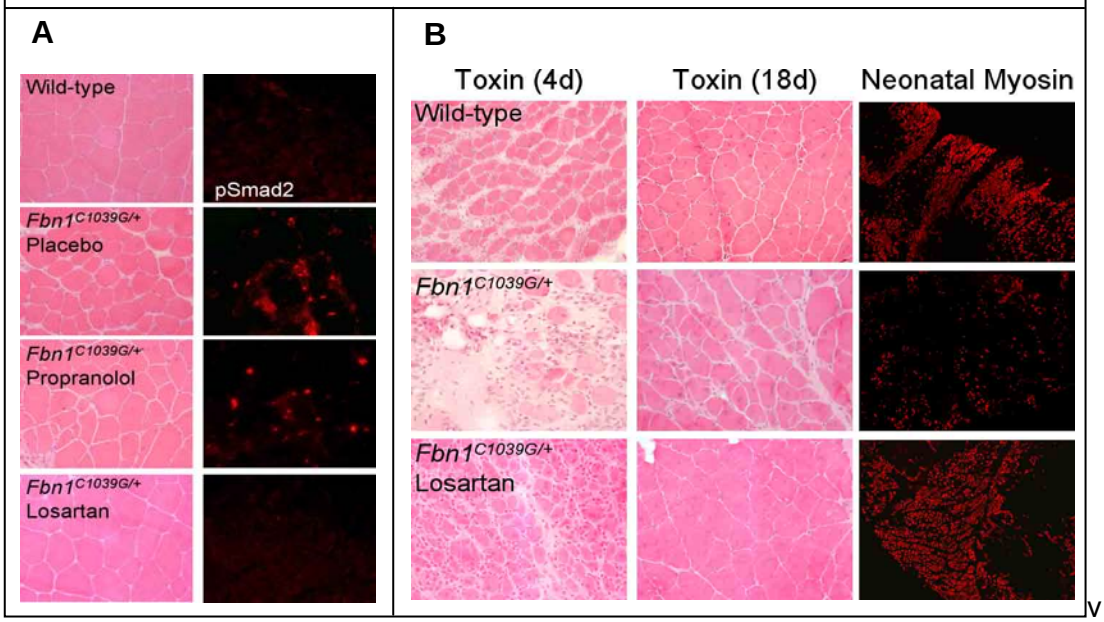
Figure 7. Postnatal Losartan Rescues Lung Septation



Losartan and other tissues in mouse models of Marfan syndrome:

Emphysema in mouse models of MFS reflects failure of distal alveolar septation that correlated with excess TGFβ signaling and is rescued by administration of TGFβ-neutralizing antibodies in vivo [42]. Dr. Dietz's laboratory has explored the basis for muscle hypoplasia and found dramatic evidence of myopathy including reduced and wide variation in muscle fiber size, excess accumulation of matrix between fibers, and reduced capacity for muscle regeneration in response to injury due to impaired satellite cell proliferation and differentiation. Once again, these findings correlated with increased TGFβ signaling, as evidenced by increased pSmad2/3 staining and increased expression of periostin (a TGFβ-responsive gene), and were rescued by administration of TGFβ-neutralizing antibody (data not shown). Given the expression of the AT1 receptor in lung and skeletal muscle, studies were performed to determine whether losartan could also modify these phenotypes. Postnatal losartan treatment achieved a significant reduction in distal airspace caliber, as shown in **Fig. 7**. It also normalized the steady-state architecture of skeletal muscle, with normalization of fiber size and reduced endomysial fibrosis **(Fig. 8A)**. These visual impressions were confirmed upon precise morphometric analyses and vimentin staining, respectively (data not shown). Pre-treatment with losartan also normalized muscle regeneration in response to induced injury **(Fig. 8B)**, as evidenced by normalization of muscle architecture both 4 days and 18 days after injection with cardiotoxin and restored expression of neonatal myosin, a marker of newly regenerated muscle. These effects correlated with reduced TGFβ signaling, as evidenced by reduced nuclear accumulation of pSmad2 **(Fig. 8A)**.

Figure 8. Effect of Losartan on Skeletal Muscle



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B.1.5 Losartan treatment of patients with MFS

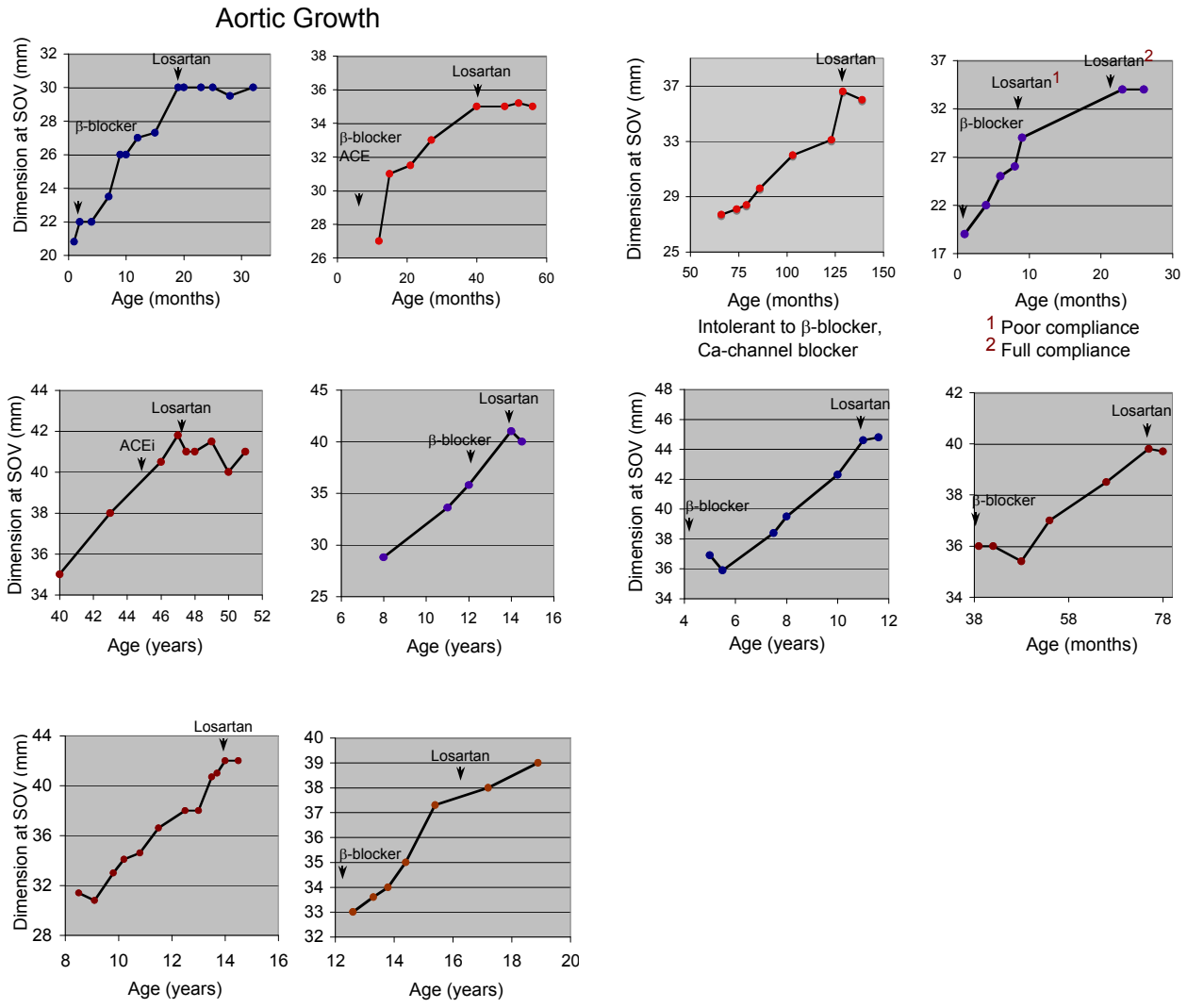
Losartan has proven safe and effective for the treatment of hypertension in both adults and children. An early study showed that the pharmacokinetics of losartan in children > 18 kg (50th percentile for a 6 year old), was similar to that in adults [88] and losartan has been labeled for use by the FDA for the treatment of pediatric hypertension in children down to the age of 6 years. Shahinhar and colleagues studied the dose response relationship in hypertensive children 6 and 16 years of age and showed that a once daily dose of 0.75 mg/kg effectively lowered diastolic blood pressure and that doses up to 1.44 mg/kg were well tolerated [89]. In a subsequent study of hypertensive children between 3 months and 15 years of age, Shaw and colleagues found that the pharmacokinetics of losartan and its active metabolite E-3174 are generally similar in pediatric patients of different ages. No serious adverse experiences were noted, and no patients discontinued treatment due to adverse experiences [90]. Losartan has been administered safely to patients as young as 3 years of age with renal disease [91, 92]. Case reports document the safe use of losartan in infancy [93]. No differences in the character, rate or severity of side-effects were observed in young children, as compared to older children or adults, and the majority of these side-effects resolved with dosage adjustment [94].

Although losartan is most commonly used for treatment of systemic hypertension, it is also routinely used without significant adverse events for treatment of normotensive patients with proteinuria [91, 92]. Losartan has a negligible effect on resting blood pressure and heart rate in healthy volunteers

[95-97]. AT1-receptor blockade with losartan can safely and effectively reduce afterload in symptomatic patients, maximally treated with ACE inhibitors for heart failure [98].

Given these data, Dr. Dietz and colleagues assessed the efficacy of losartan in a small group of patients with MFS. These patients fell into 3 groups: 1) young children with severe and rapidly progressive aortic dilation despite maximal therapy with a BB and/or ACEi; 2) children with classic MFS who proved intolerant to other medications; and 3) adults with MFS who had been incidentally started on losartan for the treatment of hypertension. Although the numbers remain small (total n = 16), the early results are promising. None of the patients has shown progression in aortic size since initiation of therapy with losartan despite prior documentation of steadily progressive aortic dilation on other medications. Available data are shown in **Fig. 9**. No adverse events have been reported in any of the patients receiving losartan alone, or losartan plus BB therapy. Renal and liver function studies have not shown any abnormalities.

Figure 9. Effect of Losartan in Patients with Marfan Syndrome



B.2 Rationale for this Trial

Despite the major advances that have been made in the medical and surgical management of MFS, morbidity and early mortality persist. Moreover, no existing therapy derives from the revolution in our molecular understanding of the pathogenesis of MFS. Existing medical therapies simply aim to reduce hemodynamic stress on an inherently weak and predisposed tissue. Although BBs and other agents can slow the rate of aortic root growth, they do not prevent attainment of important clinical endpoints including surgery, dissection and death. Surgical therapies have associated risk, often require sequential modification and can be associated with significant chronic complications including those associated with anticoagulation or infection. Furthermore, we are only beginning to appreciate the "new" natural history of MFS in an aging population that has survived the predisposition for aortic root dissection. The emerging view is that other vascular segments, prominently the descending thoracic aorta, demonstrate a later predisposition for enlargement and dissection. The appeal of a trial of losartan therapy reflects its rational derivation from elucidation of disease pathogenesis, its novel mechanism of action, its remarkable performance in validated mouse models of MFS and its excellent tolerance profile in other populations including both children and adults with hypertension and/or chronic renal disease. One of the primary goals of the Pediatric Heart Network is to promote evidence-based clinical care. Given the widespread publicity and excitement regarding the performance of losartan in animal models and the lack of practical barriers for its widespread clinical application, there is currently a unique but time-limited opportunity to rigorously and to prospectively assess the utility of this therapy using a randomized study design while clinical equipoise is still maintained. The results of this study will make an important contribution to the management of a relatively common and often devastating disorder, and will establish a model by which the effects of losartan on other organ systems can be assessed in the future. In addition, this study will establish a cohort of patients who can be followed prospectively to assess long-term outcome, and will provide important data to enable future evaluation of interventions in this group of patients.

B.3 Rationale for Selection of Outcome Measures

B.3.1 Normalized and Absolute Aortic Growth

Currently aortic root size and growth rate are considered the best predictors of the risk of aortic dissection and remain the most commonly utilized variables or measures for determining the timing of surgery in both adults and children [9, 48, 99, 100]. The widespread application of this approach has had a very favorable effect, with the life expectancy for an individual with MFS now approaching that of the general population [3]. Current medical therapy with BBs aims to slow the rate of aortic root growth, and all studies have used this measurement in the evaluation of the

efficacy of treatment. Maximal aortic root diameter in the parasternal long axis view is a standard measurement by conventional echocardiography, and has proven both accurate and reproducible in experienced echocardiography laboratories [101]. It is well established that normal aortic root size varies in a manner dependent upon both age and body size [62]. Normative values for age and body surface area are available and widely utilized [62, 102]. Thus there is both a rationale and a mechanism to use rate of change in normalized aortic root dimension (expressed as BSA-adjusted Z-score) as the primary outcome parameter.

We will use the rate of change of absolute aortic dimension as a secondary outcome parameter. The absolute aortic size is the most widely used measurement to estimate risk of dissection and to determine the timing of prophylactic surgery in all age groups. Since, by definition, every patient entering the study will have an aortic root measurement that is excessive for age and body size (Z-score > 3.0), a decrease in the rate of change of the absolute aortic dimension is a meaningful goal and an appropriate outcome parameter to follow.

Although aortic dilation is maximal and often restricted to the sinuses of Valsalva in patients with MFS, dilation of the aortic annulus and the ascending aorta can be observed. Both findings have potential clinical significance. Annular dilation is thought to drive the emergence and progression of aortic regurgitation, and it has been suggested that ascending aortic dilation may reflect a more aggressive cardiovascular course including the anticipated rate of aortic growth and the risk of dissection [103]. For this reason, we will also follow the rate of change of the absolute dimension and Z-score for the aortic annulus and the ascending aorta. Both of these measurements can be reliably made from the parasternal long-axis view (with minor modification for the ascending aorta) and normative values are available [62, 102]. We will also monitor the rate of change of aortic regurgitation, as measured by the cross-sectional area of the vena contracta of the regurgitant jet [104].

B.3.2 Cardiovascular Events (Surgery, Aortic Dissection and Death)

The major clinical cardiovascular endpoints for individuals with MFS are aortic root surgery, aortic dissection and death. Given current medical and surgical practices, aortic dissection and death are exceedingly rare in children and young adults with MFS. Under these circumstances, it is uncertain whether any differences between treatment groups can or will reach statistical significance within the time period of this trial. Nevertheless, these are the outcomes that cardiovascular treatment strategies ultimately aim to avoid, and their incidence should be recorded. It is also possible that losartan use will accelerate rather than retard these outcomes, which would be an unfortunate but

important conclusion of this study. In contrast, aortic root replacement during childhood is relatively common. The timing of surgery generally relates to aortic root size or aortic root growth rate, which we hypothesize will be favorably influenced by losartan. Aortic regurgitation, the third variable that can initiate referral for aortic root surgery, is thought to be driven by progressive stretching of the aortic annulus that is imposed by an expanding aortic root. Thus, it is a reasonable and testable hypothesis that the use of losartan will decrease the incidence of aortic root surgery during the time-frame of this study. The absolute criteria for performing aortic root replacement are somewhat variable, including at participating Centers in the Pediatric Heart Network. It is also anticipated that individuals recruited to this study will vary in the initial size of their aortas and hence will vary in the amount of tolerable aortic root growth prior to reaching a threshold for initiation of surgery. It is not our intention to standardize the criteria for surgery across centers. Rather, it is anticipated that randomization and stratification of patients to each treatment arm will control for variation in both surgical practices and aortic size at the time of presentation.

B.3.3 Mitral Regurgitation

Mitral valve prolapse and regurgitation are commonly observed in patients with MFS. Both manifestations can emerge during postnatal growth and development and progress in severity throughout life [105]. Mitral valve regurgitation with subsequent left heart failure remains the leading cause of cardiovascular surgery and death in individuals with neonatal presentation of severe and rapidly progressive MFS [4, 5, 106]. Mitral valve prolapse and regurgitation can also contribute to left heart dysfunction, the need for surgical intervention and an increased risk of bacterial endocarditis in more classic presentations of MFS. Work in mouse models has shown that excess TGFβ signaling drives mitral valve thickening and prolapse and that these findings can be attenuated by TGFβ antagonism in vivo [43]. It is therefore a reasonable hypothesis that losartan will attenuate the severity and progression of mitral valve dysfunction. Given the ambiguities inherent to defining the severity of mitral valve prolapse using echocardiography, we have elected to focus upon the progression of mitral regurgitation, measured as change in the cross-sectional area of the vena contracta of the regurgitant jet indexed to BSA, as described [104, 107, 108].

B.3.4 Left Ventricular Size and Function

Many factors in MFS can contribute to left ventricular enlargement and dysfunction. Although these include the presence and severity of mitral and aortic regurgitation, a subset of patients show evidence of primary myocardial dysfunction in the absence of valve abnormalities or other sources

of volume overload [109]. Mouse models have shown reduced preload-recruitable stroke work and left ventricular enlargement (unpublished data). As previously stated, there is reason to postulate that losartan will reduce volume overload by reduction of stretching of the aortic annulus, improved mitral valve remodeling and function, and/or by simply reducing afterload. It may also influence the inherent performance of the myocardium through TGFβ antagonism. Left ventricular performance will be monitored by measuring the rate of change of left ventricular mass, volume, mass-to-volume ratio and ejection fraction by two-dimensional echocardiography. We will also monitor the rate of change of left ventricular end-diastolic and end-systolic dimensions, diastolic septal and posterior wall thickness, left ventricular mass and shortening fraction by M-mode echocardiography.

B.3.5 Aortic Stiffness

Multiple studies have demonstrated that aortic stiffness is increased in individuals with MFS [64-66, 69, 70, 110]. Although the pathogenetic significance of this finding is unclear, one study documented correlation between increased aortic stiffness in MFS and dissection of the descending aorta [100]. One worrisome trend that has been observed as individuals with MFS are living longer is an apparent predisposition for descending aortic aneurysm and dissection. Anecdotal evidence suggests that aortic size is not a reliable predictor of this predisposition. Many of the histologic changes observed in the aortic wall of patients and mouse models of MFS predict decreased aortic elasticity and compliance including elastic fiber fragmentation, aortic wall thickening and excess accumulation of extracellular matrix including collagen. Virtually all of these changes were abrogated in mouse models of MFS after administration of losartan. We propose the use of noninvasive echocardiographic methods to measure the rate of change of ascending aortic elastic modulus and stiffness index.

B.3.6 Skeletal and Somatic Growth

Overgrowth of the long bones is a hallmark feature of MFS. This is manifested by an increase in the arm span-to-height ratio and a decrease in the upper to lower segment ratio. Many patients with MFS also have an inherent inability to increase muscle mass and fat stores despite adequate caloric intake and exercise. For skeletal muscle, Dr. Dietz and colleagues have shown that this relates to a TGFβ-induced failure of muscle stem cells (satellite cells) to respond to injury or physiologic signals for hypertrophy in mouse models of MFS. This defect was corrected upon administration of losartan. We propose monitoring of skeletal and somatic growth using simple and routine anthropometric measurements. All of the study centers will need to be trained to perform all

of these measurements for the diagnostic evaluation of patients, so this aim will not impose an undue burden on this study focused on the cardiovascular manifestations of MFS. Furthermore Marfan-specific growth curves have been established and validated [111]. We will monitor the rate of change in Z-scores for weight, height, and BMI, and arm span-to-height ratio and upper-to-lower segment ratio.

B.3.7 Drug Side Effects

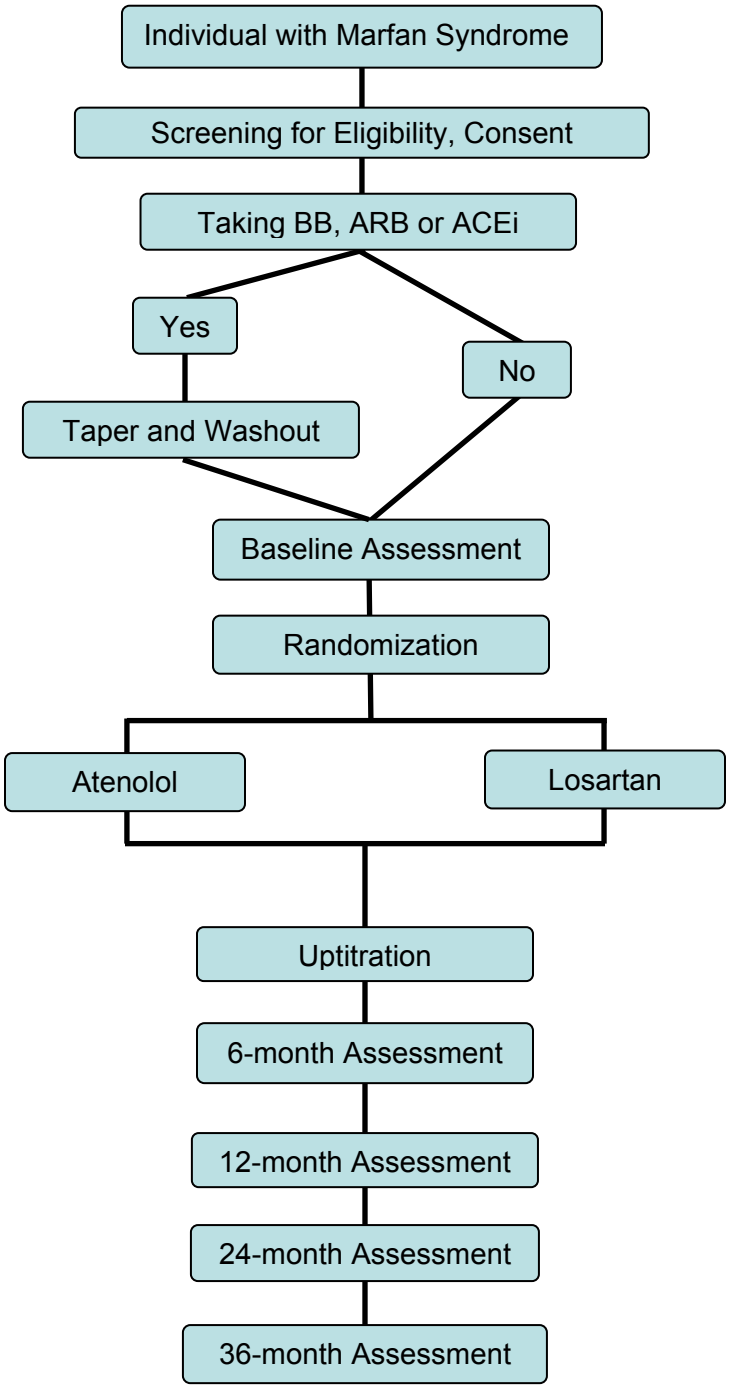
There are selected drug side effects that are common to both BBs and ARBs, prominently dizziness and syncope. BBs are more commonly associated with fatigue, impaired concentration, insomnia, and accentuation of reactive airway disease and/or depression. It is estimated that perceived drug side effects lead to cessation of treatment or impair patient compliance in 10-20% of individuals taking BBs [112]. We hypothesize that ARB therapy will be associated with a lower incidence of drug side effects. This will be monitored by questionnaire. Adverse Events (e.g., decreased renal function) will also be monitored and compared by treatment arm.

C. STUDY DESIGN AND METHODS

C.1 Overview

A randomized trial of beta blocker therapy (atenolol) vs. angiotensin II receptor blocker therapy (losartan) in individuals with MFS **(Fig. 10)**.

Figure 10. Trial Flow Outline



C.2 Human Subjects Considerations

This section includes information on the human subjects aspects of the research design and methods, as well as on methods to ensure the protection of human subjects.

C.2.1 Human Subjects Involvement and Characteristics

Infants, children, and young adults will be enrolled for this trial from among patients at up to 30 sites who meet Ghent criteria for MFS. All sites will be either PHN Clinical Centers or under contract to the PHN Data Coordinating Center (DCC), and will follow the same protocol and study procedures. The rationale for inclusion of infants and children, considered vulnerable populations from a research perspective, is that MFS can affect this age group, and that the animal data on losartan suggest a potential benefit in the growing organism.

C.2.1.a Inclusion Criteria

To be eligible for this trial the subjects must meet all of the following inclusion criteria at the time of enrollment:

1) Diagnosis of MFS according to Ghent criteria (Appendix D).
2) Age 6 months to 25 years.
3) Aortic root Z-score > 3.0.
4) Informed consent of parent(s) or legal guardian; informed consent or assent of subject as applicable.

C.2.1.b Exclusion Criteria

To be eligible for this trial, the subjects must meet none of the following exclusion criteria at the time of enrollment:

1) Prior aortic surgery.
2) Aortic root dimension at the sinuses of Valsalva > 5 cm.
3) Planned aortic surgery within 6 months of enrollment.
4) Aortic dissection.
5) Shprintzen-Goldberg syndrome (see Appendix D).
6) Loeys-Dietz syndrome (see Appendix D).
7) Therapeutic (e.g. for arrhythmia, ventricular dysfunction or valve regurgitation) rather than prophylactic use of ACE inhibitor, BB, or calcium channel blocker.
8) History of angioedema while taking an ACE inhibitor or BB.
9) Intolerance to losartan or other ARB that resulted in termination of therapy.
10) Intolerance to atenolol or other BB that resulted in termination of therapy.
11) Renal dysfunction (Creatinine >upper limit of age-related normal values).

12) Asthma of sufficient severity to preclude the use of a BB: Chronic use of steroids and/or beta-adrenergic agents with exacerbations of asthma that are frequent (averaging three or more per year) or severe (requiring hospitalization).
13) Diabetes mellitus.
14) Pregnancy or planned pregnancy within 36 months of enrollment.
15) Inability to complete study procedures including history of poor acoustic windows (inability to obtain accurate measurement of aortic root).

C.2.1.c Subject Availability

The estimated number of patients who meet trial eligibility criteria at the 7 PHN centers and Johns Hopkins is approximately 190. To capture the number of subjects required to fulfill four-year recruitment goals, several enrolling study centers will establish sub-sites at affiliated institutions, and the Network will recruit and add up to 15 auxiliary centers. In addition, all PHN centers will work to develop relationships with outside institutions that would be able to refer additional patients to the study centers. The estimated consent rate for this trial is 80%; therefore, 604 patients would be required to enroll the target sample size of 482 subjects. An inflation factor of 20% is also assumed to account for patient dropout, interim looks at the data, and potential crossover (estimated to be low) that may dilute the treatment effect. Therefore, approximately 750 eligible patients are required to obtain the target of 482 eligible, consenting patients who complete the 3-year follow-up. It is expected that the 310 known eligible patients can be screened for the trial over a period of 6 months. For the additional 440 total screenings required, to be comprised of newly diagnosed cases and newly referred cases that will come to the trial centers specifically to participate in the trial, about 42 additional months is estimated to be required. Therefore, up to 4 years is anticipated for subject enrollment, in conjunction with a three-year follow-up period for each randomized patient.

C.2.1.d Screening and Recruitment Protocol

The Principal Investigator or designee at each clinical center and the study coordinator will be responsible for case finding and subject recruitment.

Echocardiograms performed as part of routine care before enrollment will be reviewed to determine if the aortic root Z-score is > 3.0 (inclusion criterion #3). For the purpose of screening, an echocardiogram performed within 12 months of enrollment, at a study center or a non-study center, can be used. The echocardiographic images from the screening

eligibility study will be reviewed at the study center by the site investigator or designee. The study must adequately demonstrate the aortic root dimension at the sinuses of Valsalva. Height and weight measured within one month of the day of the echocardiogram must be available to calculate BSA and Z-score.

After consultation with the patient's cardiologist and/or geneticist, potential study participants (if over 18 years of age) or the parent(s) or legal guardian of potential participants, will be approached about participation. At this time, a final determination about study eligibility will be made, and eligible patients or parents will be asked to participate in the study.

A cardiologist or geneticist experienced in the treatment of patients with MFS will be involved in obtaining consent. Data (demographic, eligibility criteria and informed consent status) will be recorded on a screening form for all patients with MFS, regardless of their inclusion in the study, for definition of the study population. The screening data will be obtained and stored in a confidential manner, in compliance with HIPAA requirements or local national laws and regulations.

Each subject enrolled in the trial will be assigned a study identification (ID) number so that study information will be confidential. The link between subject name and ID number will be stored only at the site where the subject receives clinical care. Data that will be obtained on study participants includes demographic data and family history, information about their medical care, echocardiographic imaging data, and information on adverse drug reactions and adverse events. The echocardiographic images will be interpreted at a central echocardiographic laboratory, and may have the subjects' names on the images. The consent form will state this clearly. All Network procedures are conducted in accordance with local Institutional Review Board (IRB) or local national equivalent, NIH/DHHS, and HIPAA requirements or local national laws and regulations.

C.2.1.e Gender and Minority Inclusion

Based on current rosters of patients with MFS at the Network sites, it is estimated that 50% of patients will be female and 15-35% of the patients will be of minority race/ethnicity, depending on geographic location of the clinical center. The overall study population is expected to meet the NIH requirement of minority representation proportional to the population.

C.2.1.f Potential Risks

The possible risks or discomforts to the subjects include those listed below. Strategies for minimizing risk to the extent possible are summarized in C.2.2.

1. Treatment with atenolol may rarely induce adverse drug reactions including symptomatic bradycardia, dizziness, postural hypotension, fatigue, lethargy, mental depression, headache, nausea, diarrhea, sleep disturbances, and asthma exacerbation [113, 114].
2. As reviewed in Section B.1.5, treatment with losartan is generally safe and very well tolerated. Losartan has a negligible effect on resting blood pressure and heart rate in healthy volunteers [95-97] but may rarely induce adverse drug reactions [94] including dizziness and hypotension.
    a. Renal dysfunction has been reported after treatment with ARBs. Hyperkalemia has also been reported, most commonly in the presence of renal disease.
    b. Decreases in hemoglobin and hematocrit have been reported after treatment with ARBs, but the decreases are generally not of sufficient magnitude to warrant treatment discontinuation.
    c. Hepatitis has been reported rarely in conjunction with losartan use. Occasional elevations of liver enzymes have occurred but are usually of little or no clinical significance [88, 94].
    d. Losartan is contraindicated in pregnancy due to a risk of fetal renal failure during the second and third trimesters, rather than a direct teratogenic effect on the fetus. Exposure to losartan during the first trimester is not associated with significant risk to the fetus [115].
3. Angioedema has been reported rarely (<1/1000) with use of both atenolol and losartan [94, 113].
4. Sedation for echocardiographic studies may be necessary for some subjects and may cause transient irritability, vomiting, prolonged drowsiness, or unsteadiness. Respiratory depression and allergic or other drug reactions are expected to be very rare in this group of subjects.
5. Risks associated with drawing blood from a vein include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, and fainting are also possible, although unlikely.
6. Investigators from the clinical center will have access to the medical record for 5 years after each subject completes the study to review the results of follow-up clinical course, surgical intervention, and other relevant studies.

C 2.2 Protection Against Risks

Protection of study subjects is implicit in all PHN studies, and is achieved through sound study design, patient education, strict adherence to informed consent principles, careful monitoring of subjects, and compliance with International Conference on Harmonisation (ICH) Good Clinical Practices, including HIPAA regulations or local national laws and regulations.

C.2.2.a Informed Consent

Consent will be obtained from the parent(s) or legal guardian and consent or assent, as applicable, will be obtained from the subject. This will be facilitated by IRB-approved study brochures, and information about the trial that will be placed on the PHN's public Web site, www.PediatricHeartNetwork.org. The site study investigators, study coordinators, or assigned designees will obtain consents, documented by the subject, parent(s) or legal guardian's witnessed signature on an informed consent document that is compliant with HIPAA regulations or local national laws and regulations (Appendix A). If potential subjects or parents decline to participate, their or their child's medical care will not be adversely affected in any way. If they agree to participate, they are free to withdraw from the study at any time. Additionally, subjects who reach the age of consent, as determined by local Institutional Review Board (IRB) or local national equivalent, during their participation in the trial will be re-consented with the adult informed consent form.

C.2.2.b Protection Against Risks

To minimize the potential risks listed in Section C.2.1.f., the following steps will be taken:

1. The starting doses of both study drugs will be relatively low and will be up-titrated carefully (see Table 2). Subjects will be followed closely for adverse drug reactions as described in C.2.6.
2. Subjects with known renal dysfunction will be excluded from the study.
3. Blood draws, to the extent possible, will be performed after the application of a topical anesthetic cream.
4. Serum creatinine, potassium, hemoglobin, hematocrit, AST and ALT will be measured as described in Section C.5.1.
5. Pregnancy or a planned pregnancy during the trial are exclusion criteria. A statement regarding the potential risk of one of the study drugs will be given to female subjects of child-bearing potential and to the parents of female subjects less than 18 years of age. They will be asked questions about the subject's sexual activity. In addition, this risk is

listed in the consent and the assent. Written documentation of birth control measures will be required for female subjects who are sexually active.

6. Any sedation required for echocardiography will adhere to standard practices for sedation and analgesia [116], governed by the practice guidelines at each center.
7. The study sponsor will pay for all testing that is not part of routine care.
8. Confidentiality concerns are discussed in Section C.2.1.d

C.2.3 Potential Benefits of the Proposed Research

The possible benefits of participation in the proposed study to the subject are:

1. The subject's family, primary care provider, and cardiologist will receive extensive information regarding cardiac status.
2. For subjects randomized to the losartan treatment arm, potential benefits include decreased rate of aortic root dilation, decreased progression of mitral valve and aortic valve regurgitation, and improved vascular compliance.

C.2.4 Risk/Benefit Ratio

The risk/benefit ratio of the study is favorable. The risk of adverse drug reactions is low, and most are relatively minor in nature and reversible with a decrease in the dose of study drug or cessation of therapy. The risk of pregnancy while taking losartan will be emphasized and carefully monitored as described above. There is no identifiable risk of privacy being compromised in this study, given the PHN's standard procedures. There will be some unavoidable inconvenience to subjects and families because of study visits and blood draws. However, subjects and families will be compensated within the limits of the study budget and local IRB guidelines for travel and related expenses for study visits. All study costs not related to standard clinical care will be paid for by the sponsor, NHLBI. Given the anticipated benefits to subjects and others with MFS, the risks are reasonable.

C.2.5 Importance of Knowledge to be Gained

The results of this study and the wealth of systematic data collected will make an important contribution to the management of individuals with MFS. This contribution will occur by determining whether the rates of aortic growth and progression of aortic regurgitation are lower in those subjects receiving ARB therapy when compared to those receiving BB therapy, and by determining the effect of these two drugs on the secondary end points. The risk of not performing this study is that physicians will begin to prescribe losartan without collecting systematic data, and the opportunity to answer rigorously an important scientific question will be lost. Overall, the risks to subjects are reasonable in relation to the importance of the knowledge that reasonably may be gained.

C.2.6 Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan for this trial will follow standard PHN monitoring principles. Oversight of data and safety is provided by the PHN's standing, independent Data and Safety Monitoring Board (DSMB), appointed by NHLBI. The DSMB is composed of experts in pediatric cardiology, congenital cardiovascular surgery, biostatistics and clinical trial design, and ethics, as well as a member of the public. The DSMB meets at least twice a year. The DSMB reviews data on adverse events, adverse reactions, suspected adverse reactions, patient-reported outcomes, data quality, and study recruitment at regular intervals, and makes recommendations about study conduct to the Director, NHLBI. Minutes of DSMB meetings are posted on the secure DCC PHN Web site (not the public site). The DSMB and NHLBI are assisted by a Medical Monitor in reviewing serious adverse events in PHN trials. The PHN Medical Monitor will serve as the NHLBI's designee for determining causality and expectedness of all serious adverse events.

In this trial, Investigational New Drug (IND) applications will be filed with the FDA for atenolol and losartan. In addition, centers outside the United States will seek trial approval from the appropriate regulatory agency. The DCC and NHLBI will be responsible for compliance with FDA reporting requirements. Centers outside the United States will be responsible for compliance with local national reporting requirements. Local IRBs are also responsible for the safe conduct of research at each study site. No recruitment can begin at the clinical centers until the local IRB has approved the protocol. Per NHLBI policy, the consent form from each site, once approved, is reviewed again centrally to ensure that no changes inconsistent with Office of Human Research Protections policy or study design have occurred.

A major component of safety monitoring is ascertainment and reporting of adverse events, including adverse drug reactions. The approach to these activities for this trial is summarized in the sections that follow.

C.2.6.a Definition of Adverse Event, Suspected Adverse Reaction and Adverse Reaction

The FDA Final Rule on Investigational New Drug Safety Reporting Requirements [http://edocket.access.gpo.gov/2010/pdf/2010-24296.pdf] defines the following terms:

• Adverse Event	Any untoward (e.g. unfavorable, negative, or harmful) medical occurrence associated with the use of a drug in humans, whether or not the event is considered drug related. An event can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the product.
• Suspected Adverse Reaction	Any AE for which there is a reasonable possibility that the drug caused the event, meaning the event is possibly, probably, or definitely related to the study drug.
• Adverse Reaction	An AE for which there is a greater degree of certainty regarding causality; meaning the event is probably or definitely related to the study drug. Adverse reactions are a subset of Suspected Adverse Reactions.

C.2.6.b Classification of Adverse Events

Monitoring adverse events requires that they be classified as to seriousness, expectedness, and potential relationship to the study drugs, all of which drive the reporting process.

Seriousness

A serious adverse event is one that:

(a) Results in death,

(b) Is life-threatening (the subject was, in the view of the Principal Investigator, in immediate danger of death from the event as it occurred),

(c) Requires inpatient hospitalization or prolongation of existing hospitalization,

(d) Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,

(e) Is a congenital anomaly/birth defect in the offspring of a participant, or

(f) Is an Important Medical Event that may jeopardize the subject or may require medical/surgical intervention to prevent one of the serious adverse event outcomes.

The Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 (http://ctep.cancer.gov) provides a grading system that is used to categorize the severity of adverse events, as follows:

(a) Grade 1 Mild: transient, requires no special treatment or intervention, does not interfere with daily activities

(b) Grade 2 Moderate: alleviated with simple treatments, may limit daily activities

(c) Grade 3 Severe: requires therapeutic intervention and interrupts daily activities

(d) Grade 4 Life-threatening or disabling

(e) Grade 5 Death

A serious adverse event, as defined above, encompasses CTCAE grades 4 and 5, and any Grade 3 event that requires or prolongs hospitalization, or that substantially disrupts the ability of the subject to conduct normal life functions.

Expectedness

The purpose of reporting is to provide new, important information on serious reactions or events previously unobserved or undocumented. Therefore, all adverse events will be evaluated as to whether their occurrence was unexpected.

1. Unexpected: An unexpected adverse event or adverse reaction is one for which the nature or severity is not consistent with information in the protocol, consent form, or product brochure. An adverse event or adverse reaction also may be categorized as unexpected if the event has not previously been observed at the same specificity and/or severity.

2. Expected: An event is considered expected if it is known to be associated with the study drugs and/or the disease state. For this protocol, expected events include at least the following and may include other symptoms included in the product brochure but not listed below:

a. Bradycardia
b. Dizziness
c. Syncope
d. Hypotension
e. Palpitations
f. Fatigue
g. Lethargy
h. Mood changes
i. Behavior changes
j. Sleep disturbances
k. Nightmares
l. Headache
m. Nasal congestion
n. Gastrointestinal pain
o. Dysgeusia
p. Nausea
q. Emesis
r. Diarrhea
s. Constipation
t. Raynaud's phenomenon
u. Myalgias
v. Back pain
w. Peripheral edema
x. Wheezing
y. Shortness of breath
z. Cough

aa. Chest pain
bb. Renal dysfunction
cc. Hyperkalemia
dd. Anemia
ee. Hepatic dysfunction
ff. Expected events for the Marfan population

Causality

Causality assessment is required in clinical investigations to help determine which events require expedited reporting. The following criteria will be used to determine causality:

1. Not Related: The event is clearly related to other factors, such as the subject's clinical state, or non-study drugs or interventions.
2. Possibly Related: The event follows a compatible temporal sequence from the time of administration of the study drug, but could have been produced by other factors such as the subject's clinical state or non-study drugs or interventions.
3. Probably Related: The event follows a reasonable temporal sequence from the time of drug administration, and cannot be reasonably explained by other factors such as the subject's clinical state, or non-study drugs or interventions.

C.2.6.c Identification of and Data Collection Procedures for Adverse Events

In this trial, a primary safety concern as well as one of the secondary end points is to capture and compare suspected adverse reactions and adverse reactions between the two study groups. In addition, following standard clinical trial procedures, information on other adverse events and patient-reported symptoms will be collected.

Adverse Events will be assessed at baseline, at the end of each up-titration cycle, and at each study visit. Subjects and families will be encouraged to report adverse events to study personnel as soon as the event occurs, rather than waiting for scheduled visits. Patient-reported symptoms will be captured at each of these time points through a standardized questionnaire that will be administered by the study coordinators or investigators. (See Table 3, Section C.5.1). Assessments at baseline will be obtained while the subject is not taking any study drug and is essential to accurate assessment of drug effects. *Adverse event*s that are not considered adverse reactions or suspected adverse reactions will be identified when they are reported to the clinical center or during scheduled study visits by study coordinators and investigators.

Laboratory monitoring will be conducted to assess drug safety as outlined in section C.4.2.c. Abnormal values will be used to adjust study drug dosage, and will not be reported separately as adverse reactions or adverse events. However, analysis of safety at the end of the trial will encompass adverse reactions, other adverse events, and these laboratory abnormalities, for a comprehensive assessment of the safety profile of the two study drugs.

C.2.6.d Reporting Procedures (Table 1 and Table 2)

1. Marfan Investigators will report all adverse events, regardless of expectedness and relationship to study drug, to the DCC according to the following timeframes:

Table 1. Reporting Timeline for Clinical Centers:

Classification	Reporting Timeframe
Serious	Within 24 hours of first knowledge of the event
Important Medical Event (Pregnancy)	Within 24 hours of first knowledge of the event
Not Serious	Within 7 calendar days of first knowledge of the event

The site Investigator or designee will report all serious adverse events to the local IRB according to local IRB policies.

2. For the Marfan Trial, the DCC will fulfill safety reporting requirements to the FDA, the Belgian Competent Authority (BCA), the DSMB Chair, the Medical Monitor, the NHLBI, and all Marfan Trial Investigators. The DCC will adhere to the timeframes listed below in Table 2 for all safety reporting:

Table 2. Reporting Timeline for the DCC:

Classification	Expectedness	Relatedness	Reporting Timeframe
Serious: Fatal life-Threatening	Unexpected	Related	Within 7 calendar days of first knowledge of event
Serious: Fatal life-threatening	Unexpected or Expected	Unrelated	Semi-annually to DSMB and NHLBI; Annually to FDA and BCA
Serious (Suspected Adverse Reactions)	Unexpected	Related	Within 15 calendar days of first knowledge of event
Serious	Unexpected or Expected	Unrelated	Semi-annually to DSMB and NHLBI; Annually to FDA and BCA

Table 2. Reporting Timeline for the DCC: (continued)

Classification	Expectedness	Relatedness	Reporting Timeframe
Important Medical Event (Pregnancy)			Within 15 calendar days of first knowledge of event
Not Serious	Unexpected or Expected	Unrelated or Related	Semi-annually to DSMB and NHLBI; Annually to FDA and BCA

C.2.6.e Reporting Adverse Events to Institutional Review Boards for NIH-Supported Multi-Center Clinical Trials

After each DSMB meeting, a Summary Report of Adverse Events will be prepared within 30 days and will be distributed by NHLBI staff to each Principal Investigator and Study Coordinator with instructions that each Principal Investigator forward the Summary Report to the local IRB. The Summary Report will contain the following information:

- A statement that a DSMB review of outcome data, adverse events, and information relating to study performance across all centers took place on a given date
- A statement as to whether or not the frequency of adverse events exceeded what was expected and indicated in the informed consent
- A statement that a review of recent literature relevant to the research took place
- The DSMB's recommendation with respect to progress or need for modification of the protocol or informed consent. If the DSMB recommends changes to the protocols or informed consent, the rationale for such changes and any relevant data will be provided
- A statement that if safety concerns are identified, the NHLBI Program Official will communicate these promptly to the investigators.

C.2.6.f Post-Study Procedures for Adverse Events

All adverse events unresolved at the time of the subject's termination from the study will be followed by the investigators until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained or has stabilized. At the last scheduled contact, the investigator will instruct each subject or parent/guardian to report any event(s) occurring in the next 30 days. Any death or other clinically serious adverse event that may be related to the study drugs and that occurs at any time after a subject has discontinued study drug or terminated study participation will be reported as in C.2.5.d.

C.3 Trial Enrollment

C.3.1 Stratification

The study design is a multicenter, randomized trial. The distributions of BSA-adjusted Z-score change rates differ based on attainment of maximum height. Also, investigators have postulated based on clinical experience that the treatment effect will be greatest in patients with higher BSA-adjusted Z-score at baseline. Therefore, participating patients will be randomized to treatment groups using randomly permuted blocks within strata defined by attainment of maximum height and BSA-adjusted Z-score at baseline. Analysis of preliminary data indicates that approximately 50% of the study population will have a Z-score of ≥ 4.5 at baseline. This Z-score also corresponds to an aortic root dimension of 4.2 cm in an average adult, which approaches the 5 cm threshold for surgery. Z-score at baseline stratification groups will then be defined by the cut-off of 4.5. Erkula *et al* [111] show that patients with Marfan syndrome attain skeletal maturity at 15.8 years and 14.8 years for males and females, respectively. Because it may be difficult to determine at the time of enrollment if a subject has attained maximum height, the cut-off of 16 years for males and 15 years for females will be used as an approximation. Due to potential differences between centers in patient population and clinical practice, dynamic allocation by center will be used to ensure that treatment arm totals are balanced within center. Because center will be used as a dynamic rather than explicit stratification factor, this design will not result in excessively small stratum sizes.

C.3.2 Masking of Treatment Group Assignment

The primary endpoint and most of the secondary endpoints will be measured by the Echocardiography Core Laboratory, whose personnel will be blinded to each subject's treatment group assignment. Given the difference in heart rate response between the two therapies and the consequent differences in uptitration protocol (to heart rate response for atenolol but not for losartan), the study coordinator and study physician supervising uptitration will be aware of the subject's treatment assignment. Depending upon the policies and procedures at the individual

clinical centers, treatment assignment (Treatment A or Treatment B) will be obtained over the internet from the randomization computer at the DCC either by an investigator (or by his/her delegate) or by the responsible pharmacist.

No one else including subjects, their families, and primary care providers will be informed of the subject's treatment assignment. However, many subjects will have prior experience taking a beta blocker and might be able to guess their drug assignment. Furthermore, there are some differences between the two suspensions (e.g., taste, color) and between the two tablet preparations (e.g., markings on atenolol tablets denoting the manufacturer and dosage versus unmarked clinical images of losartan); hence, subjects and families may be able to determine which drug they are taking.

The DSMB will determine their preference for receipt of blinded or unblinded trial results prior to the interim look. No clinical investigators will review trial outcomes, by arm or in aggregate, until the end of the trial.

C.3.3 Randomization

After eligibility has been confirmed as described in Section C.2.1 and the trial consent has been signed, an echocardiogram will be performed on all subjects at the study center to confirm that the aortic root Z-score is > 3. It is anticipated that very few subjects will be deemed eligible by a screening echocardiogram but ineligible by the baseline study echocardiogram. Subjects confirmed to be eligible will be randomly assigned in a 1:1 ratio to receive atenolol or losartan using randomly permuted blocks. All eligibility criteria must be confirmed, and written informed consent obtained, before randomization. Randomization will be accomplished over the Internet using the randomization computer at the DCC (available 24 hours a day, 7 days a week). There will be a backup sealed envelope system in the event that technical problems prevent computer randomization. After verifying key eligibility criteria and supplying stratification information (see Section C.3.1), the randomization computer will return the treatment assignment as well as the letter code (Treatment A or Treatment B) corresponding to either atenolol or losartan. Investigators at each institution will maintain a log containing the subject's study I.D. and name, date of enrollment, and treatment assignment.

C.4 Treatment

C.4.1 Pre-Study Drug Taper and Washout

For subjects on prophylactic therapy with atenolol or other BB, ARB, ACEi, or calcium-channel blocker, before the study, the drug will be weaned over a 14-day period as follows:

Day 1: 50% of regular dose

Day 7: 25 % of regular dose

Day 14: Stop medication

After the 14-day drug taper, a drug washout period of 14–21 days will take place before baseline assessment and randomization.

C.4.2 Study Drugs

C.4.2.a Uptitration Period

After the baseline clinical evaluation, all subjects will be entered into the uptitration period, during which they will receive atenolol or losartan in addition to their usual medications. The goal of the uptitration period is to reach the effective dose (defined below) that will then be continued throughout the maintenance phase. Based upon the subject's body weight, the pharmacist will determine the appropriate initial dose of atenolol or losartan. Starting doses and the up-titration/down-titration protocols are shown in Table 2. Dose ranges are provided but actual doses will be rounded up or down according to the available pill sizes. Each drug will be administered once a day, at about the same time of day. Each cycle in the uptitration schedule will last 21 – 28 days (+7 day window).

Table 2. Uptitration Schedule

Atenolol							
	Cycle 1 mg/kg			Cycle 2 mg/kg	Cycle 3 mg/kg	Cycle 4 mg/kg	Down-Titration Increment
Days 1-6 Days 7-21	0.4-0.6 0.9-1.1		Days 1-21	1.9-2.1	2.9-3.1	to 4 (max 250 mg)	BID dosing first, then 0.5 mg/kg/d (see text)
Losartan							
	Cycle 1 mg/kg	Cycle 2 mg/kg	Cycle 3 mg/kg		Down-Titration Increment		
Days 1-21	0.3-0.5	0.7-0.9	1.0-1.4 (max 100 mg)		1st BID dosing then 0.2 mg/kg/d		

Cycle 1: Initiation of study drug

For patients randomized to atenolol, ~ 0.5 mg/kg will be given on days 1-6. The subjects will be instructed to call the study coordinator or the study physician if any adverse drug reactions occur. On day 6, 7 or 8, the nurse coordinator will contact each subject (or parent) and verify the absence of adverse drug reactions. Once verified, the dose of the study drug will be increased to ~1 mg/kg through day 21.

For patients randomized to losartan, ~ 0.4 mg/kg will be given on days 1-21. The subjects will be instructed to call the study coordinator or the study physician if any adverse drug reactions occur. On day 6, 7 or 8, the nurse coordinator will contact each subject (or parent) and verify the absence of adverse drug reactions.

After each subject has taken the study drug for at least 21 days but less than 28 days (+7 day window), the study coordinator will verify the absence of adverse drug reactions and obtain a 24-hour ambulatory electrocardiogram (24-hour ECG). For subjects less than 18 months of age, blood pressure will be measured at the study center or locally and reported to the study coordinator before increasing the dose in the next cycle. If the blood pressure is <2%ile for age, the subject will be recalled to the study center for evaluation.

Subjects receiving losartan will proceed to Cycle 2 (see below). For subjects receiving atenolol, the dose will be increased if the 24-hour ECG shows less than 20% decrease in average heart rate compared to baseline.

Subsequent Cycles: Up-titration

Uptitration will proceed according to the schedule outlined in Table 2.

For subjects receiving atenolol, the dose will be increased in each subsequent cycle by ~ 1 mg/kg to a maximum dose of 4 mg/kg, not to exceed 250 mg, daily. Uptitration will continue in this manner until the 24-hour ECG shows at least a 20% decrease in average heart rate compared to baseline. This dose will continue through the maintenance phase.

For subjects receiving losartan, the dose will be increased as tolerated to a dose of ~0.8 mg/kg in cycle 2, and ~1.2 mg/kg, not to exceed 100 mg, in cycle 3. The maximum dose will continue through the maintenance phase.

For each cycle, after subjects have taken the drug for at least 21 days but less than 28 days (+7 day window), the study coordinator will verify the absence of adverse drug reactions and obtain a 24-hour ECG. For subjects less than 18 months of age, blood pressure will be measured at the study center or locally and reported to the study coordinator prior to increasing the dose in the next cycle. If the blood pressure is <2%ile for age, the subject will be recalled to the study center for evaluation.

C.4.2.b Assessment of Beta-Blockade

Assessment of beta-blockade is challenging. First, since BBs are competitive antagonists of sympathetic agonists, *complete* beta-blockade is not possible. Beta-blockade must be assessed in terms of the amount of an agonist or the strength of a stimulus necessary to overcome blockade [117]. The *degree* of beta-blockade is assessed by determining the decrease in a beta-receptor mediated response such as heart rate to an adrenergic agonist. For this trial, any technique chosen needs to be done at multiple points during uptitration of the atenolol and during follow-up. The technique chosen should be reasonably reproducible, cause little or no discomfort, and involve minimal to no risk.

The most robust method involves use of an *exogenous* agonist as this is easiest to quantitate [117]. For example, the dose of a medication such as isoproterenol (administered intravenously or inhaled) required to increase the heart rate by 25 beats/min both before and after blockade can be determined. This extent of beta-blockade is then expressed as the "dose-ratio", that is, the ratio of the dose of isoproterenol required before to that required after beta-blockade. Administration of beta-agonists often causes discomfort such as nausea and vomiting. In addition, placing an intravenous line is invasive and inhalation of medications is not always reproducible. This is not appropriate for the subjects in this trial. Another approach is pupillometry, since beta-blockade blunts the pupillary dilator response to a topical beta-agonist. However, this is likely difficult or impossible in young children, and is of questionable reliability because of the known ophthalmologic abnormalities in patients with MFS.

Other techniques use evaluation of the heart rate response to an *endogenous* adrenergic agonist. One possible stimulus involves the heart rate response to upright posture. This maneuver is not suitable for this trial for several reasons: 1) it is not possible to perform reliably in younger subjects (such as those less than 2 years of age); 2) it will be influenced by the subject's anxiety regarding the testing, and 3) the response depends on the subject's

previous sodium intake [118]. Another technique involves measuring the inhibition of the adrenergic component of exercise-induced tachycardia. Unfortunately, the response obtained may depend on effort, and formal exercise testing is logistically cumbersome. Furthermore, exercise is very difficult and therefore not reliable for young patients with MFS and for those patients with MFS who have important musculoskeletal disability.

Beta blockade will be assessed in this trial by monitoring the average heart rate over 24 hours as measured by an ambulatory electrocardiographic recording (24-hour ECG). A 24-hour ECG is non-invasive and the recording instrument can be mailed to the study subject thus obviating the need for repeated visits to the study center. The average heart rate over the entire 24 hour recording period (or a minimum of 20 hours) will be used as this will allow for variation in activity levels over the course of the day among the subjects.

The goal of treatment with atenolol will be a decrease in the average heart rate of 20%. This goal has been documented to indicate an adequate degree of beta-blockade in a number of previous studies [119-121]. A decrease in average heart rate of 15-30% corresponds to a similar decrease in heart rate response to submaximal exercise.

C.4.2.c Safety Assessment

For subjects less than 18 months of age, serum creatinine, serum potassium, complete blood count, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) will be obtained at baseline, at the end of cycle 1 (21-28 days (+7 day window) after starting study drug), and at the end of uptitration. For subjects over 18 months of age, these safety labs will be obtained at baseline and at the end of uptitration. End of uptitration safety labs for all subjects may be obtained at any point from 21 days after achieving maximum dose until the day of the Month-6 Follow-up Study Visit. A pregnancy test will be obtained at baseline on all female subjects of child-bearing potential (all female subjects ≥ 12 years of age OR girls < 12 years of age who have experienced menarche).

Study drug will be decreased if the serum creatinine, serum potassium, AST, or ALT are increased such that they would be classified as a moderate or more severe adverse event according to schema in the Common Terminology Criteria for Adverse Events [122]. For serum creatinine, this is greater than 1.5 times the upper limits of normal for age; for potassium this is 5.6 – 6 mEq/L in a non-hemolyzed sample; and for AST and ALT, this is

greater than 2.5 times the upper limit of normal for age. Study drug will also be decreased if the hemoglobin concentration decreases by more than 2 gm/dl.

For the purposes of this study, the 24-hour ECG output will consist only of average daily heart rate. If the average heart rate decreases below 60 beats per minute for infants under one year of age, below 55 for children one to two years of age, or below 50 for children over the age of two and adults, the 24-hour ECG will be scanned and symptoms will be assessed, and an appropriate clinical response will be documented.

C.4.2.d Decrease of Study Drug Dose for Adverse Reactions or Suspected Adverse Reactions

If a subject develops certain adverse reactions or suspected adverse drug reactions such as fatigue or dizziness, the study drug can be changed to night-time administration or b.i.d. administration at the discretion of the study cardiologist. If adverse reactions or suspected adverse reactions persist or abnormalities are noted in the safety labs, the dose of study drug will be decreased by the down-titration increments listed in Table 2. Abnormal laboratory values will be re-evaluated after down-titration of the study drug.

C.4.2.e Down-titration of Study Drug

If it is necessary for a subject to stop the study drug, the subject should optimally be weaned off the drug as follows:

Day 1: 50% of regular dose
Day 14: 25 % of regular dose
Day 21: Stop drug

In the event of severe clinical instability or inability to take the study drug (i.e., severe vomiting), weaning may need to occur over a shorter period of time, at the discretion of the study physician and care providers.

C.4.2.f Re-titration during Maintenance Period

A 24-hour ECG will be obtained at the 6-, 12-, 24-, and 36-month visits. For subjects taking atenolol, the daily dose will be increased by 0.5 mg/kg up to a maximum of 25 mg increment if the 24-hour ECG shows less than a 20% decrease in average heart rate from the baseline 24-hour ECG. The subjects will be instructed to call the study coordinator or the study physician if any suspected adverse reactions or adverse reactions occur on the new dose.

After the subject has taken the uptitration dose of drug for 7 days and before s/he has taken the drug for 14 days, the study coordinator will verify the absence of adverse drug reactions and obtain a 24-hour ECG. Uptitration will continue in this manner until the heart rate response is achieved OR maximum dose of 4 mg/kg or 250 mg daily is reached. For subjects taking losartan, the daily dose will be adjusted to account for changes in weight, to maintain the mg/kg dosage (maximum dose 100 mg daily).

C.4.2.g Protocol Deviations in Treatment

All deviations from the above schedules will be noted and described on a study report form. In addition, for all non-study drugs, the dosage, treatment period, and reason for use will be recorded. For modified and interrupted therapy, the length of and reason for the modification/interruption will be recorded. Every attempt will be made to keep protocol deviations and non-protocol treatment to a minimum.

C.4.2.h Dispensing of Study Drug and Compliance Monitoring

Suspension and Pill Dispensing: The family will receive a medication bottle and, if applicable, a marked syringe to draw up the correct dose of the study drug. Study drug in the form of pills will be dispensed by the pharmacy every 3 months. Study drug in the form of liquid will be dispensed by the pharmacy every 3 weeks.

Unused study drug will be returned to the pharmacy to monitor compliance. Study drug compliance will be assessed by comparing the expected to the measured residual volume of the suspension or the number of doses in pill form of study drug returned.

Once measurement of residual volume or the pill count by the pharmacy is complete, the pharmacy may then destroy the returned study medication according to standard operating procedure.

C.4.2.i Subject Monitoring

Subjects will be monitored for adverse events at each study visit and through quarterly phone contact by the study coordinators. If, during the study, the study investigator determines that the subject has developed an adverse reaction, further monitoring will be performed as clinically indicated.

C.4.3 Indications for Permanent Discontinuation of Study Drug

- Intolerance to study drug
- If the serum creatinine, serum potassium, or liver function tests increase and fail to decrease below the thresholds given in C.4.2.c despite 3 attempts at dose adjustment
- Continued administration of study drug felt to be not indicated by the attending cardiologist or study investigator (i.e., because of the need for therapeutic ACEi or calcium channel blocker therapy)
- Pregnancy or planned pregnancy
- Other adverse events that require discontinuation of study drug in the judgment of the study investigator

The reason and the circumstances for permanent discontinuation of study drug will be documented. If study drug is permanently discontinued, the subject will continue to be followed and undergo expected tests and measurements through the planned completion date (36 months after randomization), unless an indication for withdrawal from trial is met.

C.4.4 Indications for Withdrawal from the Trial

- Subject/guardian refusal to continue in the trial, including refusal of the subject to sign the adult informed consent form upon reaching the age of consent
- Aortic root surgery, aortic dissection, and/or death
- Subject failure to return for scheduled appointments

The reason for withdrawal and the circumstances of withdrawal will be documented for all subjects withdrawn from the study.

C.4.5 Trial Completion

Subjects will be considered to have completed the study if they have completed the assessment scheduled for 36 $\pm$ 1 months post-randomization. Down-titration of the study drug, as described in C.4.2.e, is recommended. Further treatment of each study subject will be directed by his/her cardiologist and/or geneticist.

C.4.6 Other Treatments/Interventions

Subjects will receive non-study medical care as recommended by their cardiologist and other care providers. The need for additional procedures and all prescription medications will be recorded on study forms.

C.5 Measurements

C.5.1 Schedule of Measurements

Table 3. Schedule of Trial Measurements

	Baseline	Follow-up (time after randomization)				
Measurement	Study entry	Up-titration	6 months	12 months	24 months	36 months
Medical History	X		X	X	X	X
Height, Weight, Body Mass Index, Upper-to-lower segment ratio	X		X	X	X	X
Echocardiogram	X		X	X	X	X
24-hour ECG	X	X*	X	X	X	X
Aortic dissection or aortic root surgery			X	X	X	X
Patient-Reported Symptoms Questionnaire	X	X	X	X	X	X
Blood pressure	X	X	X	X	X	X
Safety Labs	X	X**				

* 24-hour ECGs will be performed on all subjects at the end of each uptitration cycle

**For subjects less than 18 months of age, safety labs will be obtained at baseline, after Cycle 1 of up-titration, and at the end of up-titration. For subjects more than 18 months of age, safety labs will be obtained at baseline and at the end of up-titration.

C.5.1.a Baseline Data

Baseline data will be collected on demographic variables (age, gender, race), past medical and surgical history, family and social history, and physical examination. Particular emphasis will be placed on documenting the diagnosis of MFS by Ghent criteria. Any procedures or significant events will be noted.

- Medical history will be reviewed at baseline.
- Height, weight, upper-to-lower segment ratio, and blood pressure will be recorded at the time of the echocardiogram.
- Echocardiographic images will be obtained for analysis at study entry (see Table 2 and Appendix C). Although an echocardiogram performed before enrollment and

reviewed by the site investigator or his/her designee can be used to determine eligibility for enrollment, the baseline study echocardiogram will be used to confirm study eligibility (aortic root Z-score > 3.0) before randomization of the subject.

C.5.1.b Follow-Up Visits

Follow-up data will be obtained at 4 time points: 6, 12, 24, and 36 months after randomization. Should a subject withdraw from the study for any reason, every attempt will be made to obtain these data at the time of withdrawal.

- Medical history will be reviewed at each study visit.
- Height, weight, upper-to-lower segment ratio, and blood pressure will be recorded at the time of each echocardiogram.
- Echocardiographic images will be obtained for analysis at 6, 12, 24, and 36 months after randomization (see Table 2 and Appendix C). Studies obtained at 12, 24 and 36 months after randomization are part of routine care. Additional echocardiograms performed for clinical indications will not be included in the study.
- A 24-hour ECG will be obtained at each study visit.
- Date of aortic dissection, aortic surgery, and/or death will be recorded.
- A symptoms questionnaire will be administered at each study visit to gather patient-reported outcomes.

C.5.1.c Windows for Visits

- The 6-, 12-, 24-, and 36-month visits will occur with a window of ± 1 month.

C.5.2 Outcome Variables

Outcome variables have been chosen that will reflect a clinically-relevant inhibition of TGFβ signaling associated with ARB therapy but not with BB therapy (Table 4). The primary outcome is rate of change in aortic root (sinuses of Valsalva) BSA-adjusted Z-score. Details regarding the secondary outcome measures are provided in Sections C.6.3.c.

Table 4. Outcome Variables

Primary Outcome

Rate of change in aortic root (sinuses of Valsalva) BSA-adjusted Z-score

Secondary Outcomes

- Rate of change in aortic root (sinuses of Valsalva) absolute dimension
- Rate of change in ascending aorta absolute dimension and BSA-adjusted Z-score
- Rate of change in aortic annulus absolute dimension and BSA-adjusted Z-score
- Rate of change of aortic regurgitation, measured as change in vena contracta area indexed for BSA
- Aortic dissection, aortic root surgery, or death at 36 months after randomization
- Time to first occurrence of aortic dissection, aortic root surgery, or death up to 36 months after randomization
- Rate of change of mitral regurgitation, measured as change in vena contracta area indexed for BSA
- Rate of change of left ventricular mass, volume, mass to volume ratio, and ejection fraction by two-dimensional echocardiography
- Rate of change of left ventricular end-diastolic and end-systolic dimensions, diastolic septal and posterior wall thickness, left ventricular mass and shortening fraction by M-mode
- Rate of change of ascending aortic elastic modulus and stiffness index
- Rate of change in Z-scores for weight, height, BMI corrected for age in subjects as determined by availability of Z-scores (see text)
- Rate of change in weight and BMI with covariate adjustment for age in all subjects
- Incidence of adverse reactions and suspected adverse reactions reported during routine surveillance

C.5.2.a Echocardiography

Study echocardiograms will be performed at baseline and at 6, 12, 24, and 36 months after randomization, or at study withdrawal, if applicable. The echocardiogram will consist of a complete two-dimensional echocardiogram, Doppler evaluation, and measures of central aortic stiffness as outlined in Appendix C. Height and weight will be measured at the time of each echocardiogram as outlined in Appendix B. Systolic, diastolic, and mean blood pressure will be measured using an automatic vital signs monitor. The use of sedation will be managed according to the practice at the individual center.

Collection of echocardiographic data at 12, 24, and 36 months corresponds with current clinical practice for most patients with MFS. The 6-month echocardiogram will be obtained for safety reasons. Additional echocardiograms may be performed for clinical indications but will not be included in the study.

In addition to the raw measurements and derived indices, data will be expressed as Z-scores relative to body surface area or age in normal subjects. Z-scores indicate the position of each measurement relative to the normal population expressed as the number of standard deviations from the population mean. Reporting the data as Z-scores adjusts for the effects of variation in body size or age.

C.5.2.b Anthropometric Measurements

Weight, height, BMI, arm span-to-height ratio and upper-to-lower segment ratio will be obtained at baseline and at 6, 12, 24, and 36 months after randomization, and at study withdrawal, if applicable. The following analyses will be restricted by age based on availability of normative data.

- Rate of change in Z-scores for weight corrected for age in subjects up to 20 years
- Rate of change in Z-scores for BMI corrected for age in subjects 2-20 years of age
- Rate of change in Z-scores for recumbent length corrected for age in subjects from birth up to 2 years
- Rate of change in Z-scores for height corrected for age in subjects from 2-20 years
- Rate of change in weight and BMI with covariate adjustment for age in all subjects

C.5.2.c Adverse Drug Reactions

Adverse drug reactions will be screened with a standard questionnaire that will be developed as a study form. Subjects (or parents) will complete this form as part of the baseline assessment, at the end of up-titration, and at each study visit (6, 12, 24, 36 months, and at study withdrawal, when applicable).

C.6 Statistical Methods

C.6.1 Background Data for the Primary Endpoint

Because the primary outcome measure for the trial is a rate of change over time, a review of previous data from two participating centers was performed to calculate estimates of the rate of change and the covariance structure of the primary outcome. Echocardiographic measurements, BSA, and age of patients meeting Ghent criteria were provided by the centers. Patients were

selected for analysis if they met the following criteria: 1) at least three consecutive measurements on beta blockers only; 2) age at first measurement between 6 months and 25 years; 3) aortic root BSA-adjusted Z-score at first measurement greater than 3; and 4) aortic root dimension less than 5 cm. For each center, BSA-adjusted Z-scores were modeled against age in a longitudinal analysis, assuming a linear trend over time and exponential covariance structure. Estimates of between-subject variance and within-subject correlation were calculated using restricted maximum likelihood (REML). Estimates of the between-subject standard deviation of BSA adjusted aortic root Z-score were 2.29 and 1.62, while the within-subject correlation for observations one year apart was estimated to be 0.82 for both centers. For sample size calculations, the between-subject standard deviation of BSA adjusted aortic root Z-score was assumed to be 2.29 and the within-subject correlation for observations one year apart was assumed to be 0.82.

C.6.2 Sample Size and Power

C.6.2.a Primary Endpoint

This study will be powered to detect a difference in the primary outcome (rate of change in aortic root BSA-adjusted Z-score) between the treatment groups within three years of randomization. The distribution of Z-score change rates may vary based on attainment of maximum height. For subjects who have not attained maximum height ('children'), a negative Z-score change rate implies that the aortic root is growing more slowly than expected given the rate of change of BSA, a desirable outcome of treatment for a child with an enlarged aortic root. Subjects who have attained maximum height ('adults') will on average maintain stable BSA; thus a negative aortic root Z-score change rate is difficult to achieve. Therefore, the minimum clinically significant difference (MCSD) between the treatment groups is identified separately for adults and children. The overall MCSD is then a weighted average of these two quantities. Erkula *et al*. [111] showed that on average patients with Marfan syndrome achieve skeletal maturity at approximately 16 years of age, so this is the age cutoff for these two groups.

Analysis of the feasibility data indicates that the 'average' child in this study population is nine years old, with BSA of 1.1 M^2 and Z-score of 4.5. Analysis of the background data for the primary outcome (C.6.1) indicates that the Z-score annual change rate in children on BB is -0.1. The MCSD is based on the viewpoint of some clinicians that a management goal is to have Marfan children reach adulthood (i.e., 16 years) with an aortic root dimension (ARD) that is minimally dilated, defined here as an aortic root BSA-adjusted Z-score of 2. Calculations show that achieving this goal means that the average child needs to have a Z-

score change rate of -0.35, resulting in a Z-score of 2.05 and ARD of 3.31 cm (assuming BSA = 1.70 M^2) at 16 years of age. Examples are provided in Table 4 for selected ages and Z-scores at enrollment, assuming that these patients maintain an annual Z-score change rate of -0.35 sd/year. The MCSD in children is therefore -0.1 – (-0.35) = 0.25.

Table 5. Aortic Root BSA-adjusted Z-score and ARD attained if Z-score change rates of -0.35 for losartan and -0.1 for atenolol are maintained until end of growth, for selected ages and Z-scores at start of trial

Age at Entry (years)	BSA at Entry (M^2)	Z-score at Entry	ARD at Entry (cm)	Z-score at 16 years on Losartan	ARD at 16 years (cm) on Losartan	Z-score at 16 years on Atenolol	ARD at 16 years (cm) on Atenolol
9*	**1.1**	**4.5**	**3.19**	**2.05**	**3.31**	**3.8**	**3.78**
1	0.5	4.5	2.17	-0.75	2.47	3.0	3.59
6	0.9	4.5	2.88	1.00	2.99	3.5	3.74
11	1.3	4.5	3.49	2.75	3.52	4.0	3.89
1	0.5	7.0	2.56	1.75	3.22	5.5	4.34
6	0.9	7.0	3.39	3.50	3.74	6.0	4.49
11	1.3	7.0	4.12	5.25	4.26	6.5	4.64

* Average patient based on our feasibility data is a 9 year old with a BSA of 1.1 M^2 and a Z-score of 4.5.

For adults, the MCSD is defined as an effect that would delay surgery by 10 years. The surgery is typically performed when ARD crosses the threshold of 5 cm. Analysis of the feasibility data indicates that the average adult in this study population is 20 years old and has a Z-score of 4.3 and BSA of 1.8 M^2. Because the preliminary datasets contained extremely few adults who met study eligibility criteria, we have estimated a Z-score annual change rate based on the mean age at death of 41 years reported by Silverman *et al* [3]. Death at 41 years implies that on average patients will reach the surgical threshold at 35 to 40 years of age. The average adult in the study population will then progress to the surgical threshold in 15 to 20 years, (Z-score = 7.3, ARD = 5.04 cm, assuming no change in BSA), corresponding to a Z-score annual change rate of 0.20. With a Z-score change rate of 0.12, the average adult will reach this threshold in 25 years (i.e. 10 years later than the adult with a change rate of 0.20), so the MCSD in adults is 0.20 – 0.12 = 0.08.

In the feasibility dataset, 67% of the subjects were children, so the overall MCSD is calculated as 0.25(0.67) + 0.08(0.33) = 0.194.

We would like to have 85% power to detect this difference. Using a likelihood ratio test (LRT) of the null hypothesis that the treatment group by time interaction effect in the longitudinal model is zero, conducted at the 0.05 level of significance, a sample of size 482, or 241 subjects per treatment arm, is required. To account for patient dropout, three interim looks at the data, and potential crossover, an inflation rate of 20% was chosen. This rate is conservative as patient dropout and crossover rates are anticipated to be low in this highly motivated population, and the power loss due to dropouts will be mitigated by use of data collected prior to dropout, which can be incorporated into the longitudinal analysis for patients who withdraw from the trial. It is also anticipated that dropout and crossover due to side effects will be low because patients who have been on BB will be used to the side effects and fewer side effects are expected on ARB. A total of 604 subjects will be enrolled into the trial (302 per treatment arm).

It is of interest to determine whether there is a differential treatment effect for pediatric and adult Marfan patients (defined here as < 16 vs. ≥ 16 years of age). However, this trial will be unable to sufficiently power a formal test of interaction. Instead, exploratory within-subgroup analyses of the primary endpoint have been specified for the subgroups defined by attainment of maximum height. These within-subgroup analyses will be based on an effective total sample size of 322 children and 160 adults, respectively. At a 0.025 level of significance (after Bonferroni correction), the power to detect an effect size of 0.25 for children is 82% and the power to detect an effect size of 0.08 for adults is 6%. We have 80% power to detect an effect size of 0.32 in the 160 adults. These data, despite less than desirable power particularly for the adult analysis, will provide valuable effect size estimates which can be used in future clinical trials.

Because the power to detect the MCSD is much greater in children than in adults, the power of the primary analysis of the primary endpoint will be compromised if a large proportion of adults are enrolled in the trial. To prevent this, adult enrollment will be capped at 33%, i.e. no more than 200 adults will be enrolled in the trial.

C.6.2.b Secondary Endpoints

Estimates of the minimum mean detectable difference between groups for selected secondary outcomes demonstrate that with these continuous endpoints and relatively large sample size, small group differences can be detected. The algorithms assume an effective sample size (i.e., the number of expected evaluable patients) of 241 subjects per group and a two-sided test conducted at a 0.05 significance level.

Aortic Growth

Analysis of the preliminary datasets indicates that the between-subject standard deviation of aortic root dimension is 0.58 cm and the within-subject correlation for observations one year apart is 0.90. With 241 subjects per treatment arm, we will have 85% power to detect a difference in aortic root dimension annual growth rate of 0.038 cm/yr. Power ranges from 75% to detect a mean growth rate difference of 0.034 cm/yr to 95% power to detect a mean growth rate difference of 0.045 cm/yr.

Central Aortic Stiffness

Vitarelli *et al*. report stiffness index standard deviation to be 1.7 [123]. Assuming that within-subject correlation for observations one year apart ranges from 0.60 to 0.90, with 241 subjects in each treatment arm and specified 85% power, the minimum detectable difference in stiffness index annual change rate ranges from 0.19 to 0.11.

Skeletal and Somatic Growth

The covariance structure of body mass measurements taken longitudinally was estimated based on the growth curve data for Marfan patients reported by Erkula *et al*. [111]. The standard deviation of body mass in a cohort with the age distribution observed in the feasibility dataset, based on reported standard deviations of body mass for age, is estimated to be 26 kg. The correlation of body mass measurements taken one year apart, based on reported standard deviations of body mass growth velocity, is estimated to be 0.90. With 241 evaluable subjects per group and desired power of 85%, the minimum detectable difference in body mass annual growth rate is 1.7 kg/yr.

C.6.3 Analysis Plan

C.6.3.a Primary Analysis of the Primary Endpoint

All primary analyses will be performed on an intention-to-treat basis. All subjects will be analyzed according to their treatment group assignment regardless of actual treatment received. The primary outcome will be modeled using the parametric curves longitudinal model described by Fitzmaurice, Laird, and Ware [124]. Fit of the mean model and covariance structure will be assessed according to the strategy suggested by these authors. The rate of change in aortic root BSA-adjusted Z-score of the two treatment groups will be compared by a LRT of the null hypothesis that the treatment group by time interaction effect is zero. A strength of the parametric curves longitudinal model is that available data from subjects who have any missing observations during follow-up (including those who do not complete the study follow-up period) can be included in analysis. Note that this requires an assumption that the data are missing at random (MAR), i.e., the probability of missingness is independent of outcome, conditional on observed data. If there are significant missing data, a sensitivity analysis of the MAR assumption will be conducted and, if indicated, values may be imputed for the missing observations using multiple imputation.

C.6.3.b Secondary Analysis of the Primary Endpoint

Three classes of secondary analyses will be conducted to compare the two trial arms with respect to the primary trial endpoint (rate of change in aortic root BSA-adjusted Z-score):

1) covariate-adjusted analysis;
2) comparison of groups according to treatment actually received (non-intention-to-treat); and
3) comparison of groups after exclusion of any subjects randomized but found after randomization to have been trial ineligible at the time of enrollment.

Potential confounders of the relationship between treatment and aortic root dimension will be included in the longitudinal model for the primary endpoint, retained if significant at the 0.10 level, and the covariate-adjusted expected difference in the rate of change will be reported. This approach will provide a more precise estimate of treatment efficacy and also removes the effect of any imbalance in the data.

The second class of secondary analysis is a non-intention-to-treat approach to account for subjects who are not compliant or are permanently withdrawn from study drug. Compliance will be estimated at each follow-up time point. If compliance cannot be estimated or estimated total dose does not fit a secondary analysis definition of treated, then that

observation and all subsequent observations will be excluded from analysis. Crossovers will be reclassified according to the treatment actually received. If significant non-adherence to study drug assignment and/or crossovers occurs during the trial, then this analysis may provide a clearer understanding of the efficacy of BB vs. ARB therapy, with the caveat that there may be unmeasured differences between the two newly constructed treatment arms. Therefore bias in the treatment group comparison may be present due to subjects not being analyzed within the groups to which they were randomly assigned.

The second secondary analysis of the primary endpoint will effectively provide an estimate of treatment efficacy after a) correctly classifying subjects to received drug (outside of the randomization scheme) and b) removing subjects who did not receive a sufficient total dose of either drug. The third secondary analysis will provide an estimate of treatment efficacy in the target study population.

C.6.3.c Analyses of the Secondary Endpoints

Analytic methods for the secondary endpoints will be varied and depend on the form of the secondary endpoint. For the echo and growth measurements that compare change over time, analysis will proceed as described for the primary outcome in C.6.3.a. For the dichotomous composite outcome of aortic dissection, aortic root surgery, or death by 36 months after randomization, Fisher's exact test will be employed. A more powerful analysis of this endpoint compares the time to event in the treatment groups, so the Kaplan-Meier method will be used to estimate the event-free survival curves and a logrank test will be conducted to compare the event-free survival curves of the two treatment groups. All data will be censored at 36 months after randomization. Incidence rates (number of events per 100 subjects) of reported side effects during 36 months of follow-up will be compared using Poisson regression. Because subjects who experience multiple events are of interest and may unduly influence incidence rates, number of events experienced during follow-up will be categorized into an ordered multinomial variable and treatment groups will be compared using the Mantel-Haenszel test for linear trend. For all secondary analyses, a sensitivity analysis may also be conducted with imputed values based on multiple imputation. All primary analyses of secondary endpoints will be according to the intention-to-treat principle. Secondary analyses of secondary endpoints will be conducted as described for the primary trial endpoint in Section C.6.3.b. For the time to event analysis, a Cox model will be used to adjust for covariates.

C.6.3.d Interim Monitoring and Early Stopping

A Data and Safety Monitoring Board (DSMB) has been established by NHLBI to monitor this trial. The DSMB will meet at least twice a year.

Efficacy. In addition to routine data reviews a formal early stopping procedure will be used to monitor the trial for large treatment differences. An O'Brien-Fleming stopping boundary, allowing for flexibility in the exact times of the interim analysis, will be used for this purpose [125, 126]. The O'Brien-Fleming plan is conservative in the sense that it is difficult to reach the boundary during the trial. Therefore, most of the Type I error is conserved for the final analysis and the effect on statistical power is minimal. Three formal interim analyses are planned, timed in terms of the fraction of post-baseline measurements collected, as an approximation of the information fraction. With 604 subjects potentially contributing four post-baseline measurements each, 2416 measurements post-baseline are expected. The interim analyses will take place when one-third, one-half, and three-quarters of these measurements are expected to have taken place. The information fraction will be calculated as suggested by Jennison and Turnbull [127] and the Lan-DeMets methodology [128] will be used to adjust the boundary appropriately. The primary endpoint will be analyzed and the nominal p-value compared with the stopping boundary to judge the significance of the treatment effect while adjusting for multiple testing. For the assumed information increments, the nominal p-values to reject the null hypothesis at the first to third interim looks are 0.0002, 0.003, 0.018, respectively, with a p-value of 0.044 required at the final analysis to declare the primary trial result significant, with an overall experiment-wise alpha of.0.05 (Table 6).

Table 6. Early Stopping Rule

Analysis	**First**	**Second**	**Third**	**Final**
Fraction of total information	1/3	1/2	3/4	1
Nominal p-value to reject null	0.0002	0.0030	0.0183	0.0440

Even if there is a statistically significant treatment difference at the interim analysis, the DSMB may decide that an ethical imperative to stop the trial is not present.

Safety. It is possible that the DSMB may consider it necessary to stop the trial early due to safety concerns. To monitor safety, adverse event rates will be compared between the two trial arms and reported to the DSMB.

Early stopping rules are only guidelines; the DSMB may take a more global view of the trial during data monitoring [129-131]. To provide this broader perspective, the DSMB reports will include summaries of accrual, patient characteristics, adverse events, information related to increased occurrences of serious, suspected adverse reactions and new risks, compliance rates with therapy, frequency of protocol violations, data quality, primary and secondary endpoints, other information as requested by the DSMB, and any unanticipated special problems that arise during the conduct of the trial. Furthermore, crossover and withdrawal rates will be monitored and the potential impact on power assessed.

Interim evaluation of sample size assumptions. Unforeseen inaccuracies in design assumptions, in particular having to do with within- and between-subject variation in measurements, could lead to decreases in the trial's statistical power to detect treatment effects. Accordingly, a blinded analysis will be conducted to estimate the variance of the estimated difference, between treatment arms, in within-subject rate of change in aortic root Z-score. This analysis will be conducted within the intention-to-treat framework under which the primary analysis of the primary endpoint will be conducted (Section C.6.3.a).

To allow time for corrective action if necessary, this analysis will be performed approximately 2.5 years after trial start date. Computations will be performed using the method proposed by Zucker and Denne [132], which accommodates the planned parametric curves longitudinal regression model (C.6.3.a) and allows information to be used from all subjects, including those who have not yet completed all study visits. As these analyses are limited to consideration of variance measures and do not address the primary or secondary trial hypotheses (which involve differences in means by treatment), they do not constitute interim analyses in the classic sense, and do not influence the trial's type-I error rate [132]. If necessary, this method will be used to obtain a recommended increase in sample size commensurate with the maintenance of adequate power to detect the minimum clinically significant difference in aortic root Z-score rate of change by treatment arm.

C.6.3.e Subgroup Analyses

To determine whether the effect of BB therapy vs. ARB therapy differs across subgroups, separate treatment comparisons will be made within the following subgroups:

- Attainment of maximum height at baseline: patients with no change in height after the baseline visit vs. patients with increased height after the baseline visit
- Age at baseline as a continuous variable

- Baseline aortic root BSA-adjusted Z-score: <4.5 vs. ≥ 4.5
- Prior use of BB (yes/no)

Covariate by treatment group interaction tests will be performed to test whether the treatment effect is homogenous across subgroups. Statistical testing within subgroup will not be conducted unless the interaction test p-value is ≤ 0.10.

C.6.3.f Site and Cohort Differences

During the ongoing trial, analyses will be conducted on a periodic basis to assess site differences in protocol violation rates, enrollment rates, subject characteristics and adverse event rates. Differences identified may lead to a site visit to review subject data. The characteristics of patients who are screened for but do not participate in the trial will be compared with randomized subjects. This analysis will allow assessment of the generalizability of trial findings and whether the enrolled subject cohort is representative of the entire patient population.

C.7 Data Management

C.7.1 Information Flow

Data will be received from several sources, including the clinical sites and Core Laboratories. The flow of data among the units is illustrated in **Figure 11**.

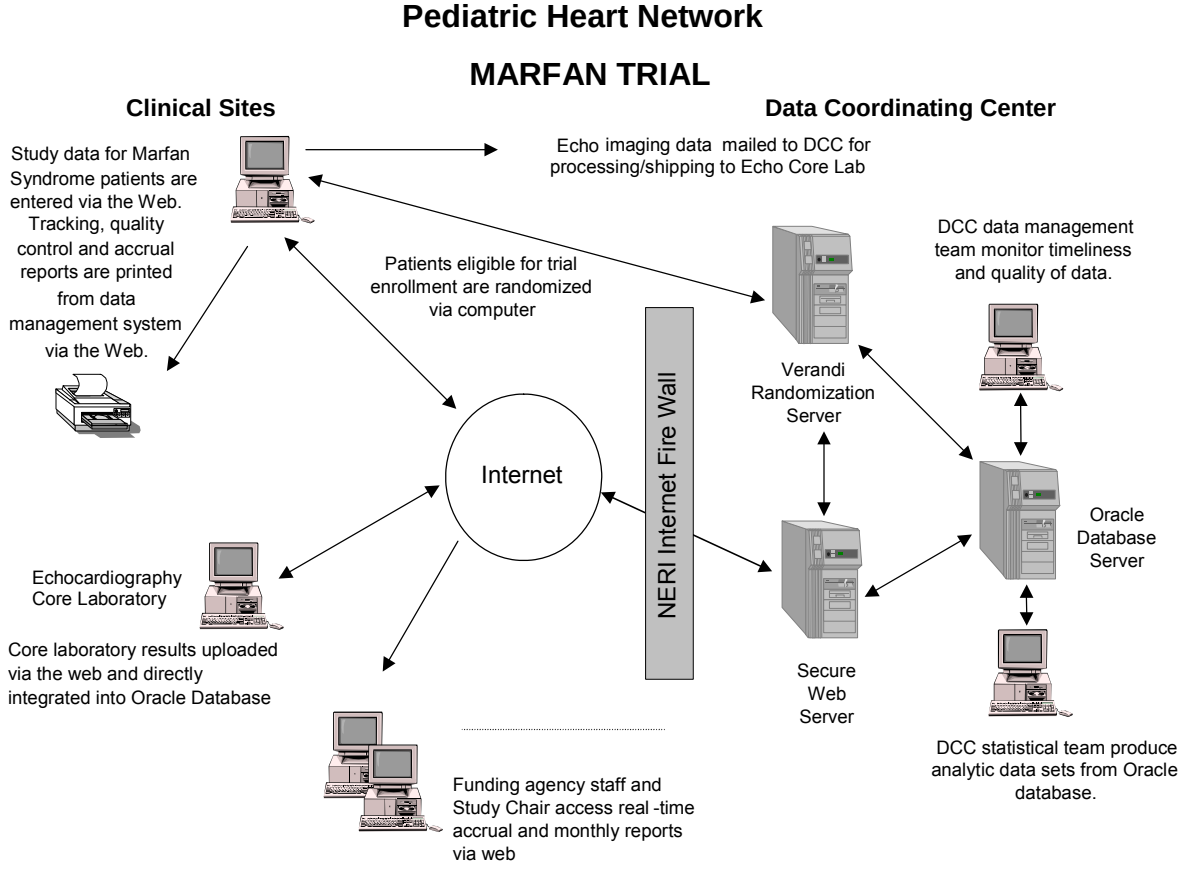


Figure 11. Data Management System and Information Flow

Entry and Protocol Tracking (ADEPT) software, a customized and secure Web application (see Section C.7.2). Echocardiogram files from clinical sites may be transmitted to the DCC via the Web using the PHN File Transfer Program (FTP) site at the New England Research Institutes (NERI) or submitted on other storage media, such as optical disk or CD-ROM. The DCC will forward the echocardiogram data to the Echocardiography Core Laboratory, either electronically or by FedEx. Results of studies performed by the Echocardiography Core Laboratory will be directly uploaded to an Oracle database at the DCC or entered electronically using the ADEPT DMS.

C.7.2 Overview of Data Management System

ADEPT uses a "browser-based" user interface together with an Oracle relational database engine which allows direct data entry from multiple study sites or at NERI, and then stores these data centrally at the DCC. Information entered into the data entry system will be by subject study I.D. number; names will not be linked with subject data in the database. Clinical sites will maintain

records linking the subject name with the I.D. number assigned for the study in secure areas. Sites will have full access to their own data and be able to view these data remotely, over the Internet.

The ADEPT data entry system will include real-time field level validations and context sensitive help. Electronic data entry forms will be formatted using HTML to resemble closely the paper-based study instruments. These forms will be enhanced with client side JavaScript code to ensure rapid data entry, proper validations of all data fields, and proper skip patterns within study data forms. Data will be saved at regular intervals during data entry to prevent loss of information in the event of a disruption of the Internet connection. In the unlikely event of a major disruption of the Internet infrastructure, the ADEPT system has a dial-in backup system to allow for dial-up access to the DMS.

NERI's proprietary VERANDI randomization system, which supports a number of different allocation methodologies, will be used for random assignment of subjects to treatment arms. Site personnel will perform randomization over the Internet as described in Section C.3.3.

Key capabilities of the ADEPT system are described below.

C.7.2.a Data Entry and Editing

The data entry system will include a number of standard features designed to ensure consistently high quality data. Each question on a study form will be associated with a validation, and validations will be executed in real-time during data entry. If the response to a particular question falls outside the range of allowable values specified in the validation for that question, the user will be alerted so that the error can be corrected immediately. Validations will include both inter- and intra-instrument data checks. In addition to alerting the user to invalid entry of items, edit reports will be automatically generated at the completion of data entry for a form. These edit reports will provide the information necessary to investigate any data entry errors or resolve questions regarding out-of-range or questionable values. Edit reports will list the subject I.D. number, instrument name, and a detailed description of why each specific data item was flagged. These edit reports can be printed out and reviewed by a supervisor, or returned to the data collector for resolution.

The ADEPT system will track expected, partially completed, and missing data entry forms by instrument and data collector. Data entry quality will be monitored through a sample-based, double data entry quality control system. This quality control system utilizes a self-adjusting

algorithm to enforce higher double data entry rates on data entry staff that have higher error rates. This system also allows for a minimum double data entry rate to be specified for each individual study instrument. This minimum rate of double data entry is adhered to regardless of a data entry staff's error rate.

C.7.2.b Reporting

The ADEPT system will produce visit schedules to assist Clinical Site staff in scheduling of appointments, and visit control sheets that will list all of the forms and procedures for a scheduled visit. In addition, the system will produce a variety of reports in both graphical and tabular format, as applicable, for the Study Chairs, Program Officer, clinical site and Core Laboratory staff. These will include:

- Study Instruments pending entry;
- Study Instruments pending edit resolution;
- Missing data rates;
- Time between collection and entry of data;
- Time to physically key each study instrument;
- Audit logs for all edits to study data;
- Subjects with overdue visits;
- Reimbursement information for sites and Core Laboratories;
- Other customized reports will be developed within the ADEPT system as needed.

C.7.2.c Data Security and Integrity

The Web-based components of the data management system utilize several levels of security to ensure privacy and integrity of the study data as noted below:

- Web access to ADEPT requires use of assigned user names and passwords;
- Passwords are changed every 90 days;
- Web-based data entry uses secure socket layer (SSL) data encryption;
- Access to any study-specific system features are controlled by Oracle database rights and privileges;
- A full Oracle back up is done on a daily basis and stored offsite;
- Duplicate NT servers are available to replace the Oracle or Web Server;
- Primary Identification is via study I.D.;
- Access to electronic linkage limited by Oracle Database Administrator;
- Access to hard copies of linkage kept in locked cabinets by Clinical Center Coordinators;

- NERI firewall limits which internet protocols are allowed to access the Web server;
- No direct access is allowed to the Oracle server from the Internet;
- NERI's firewall monitors for unusual (hacker) activity and automatically notifies NERI IS staff.

All study data will be stored on NERI's Microsoft NT-based, Oracle server. Access to data on this server (from both inside and outside the data center) is controlled by Oracle's extensive security features. The Oracle archiving and back-up system ensures minimal data loss, even in the most catastrophic system failure.

C.8 Quality Control

This section describes the quality control program that will be implemented as part of the study to ensure standard implementation of the protocol, protocol compliance, and data integrity. The DCC will develop and update the Manual of Operations in collaboration with study investigators and Core Laboratory directors. In addition, an ADEPT Manual will be developed for clinical site and Core Laboratory personnel who will be using the ADEPT data management system. The two manuals will serve as both training and reference manuals and will be accessible on NERI's password-protected PHN Web site.

C.8.1 Clinical Center Coordinator Training

The DCC, Study Chairs, and the Marfan Trial Subcommittee will provide central training of clinical center staff in the areas of protocol implementation, data collection and management, collection and handling of imaging data, medical records abstraction, anthropometric measurement techniques, and quality control expectations. Training manuals will be prepared that reflect clearly and succinctly the learning goals for clinical coordinators and represent the skills and protocol components required to collect quality data. Training follow-up and training of new staff will be completed through conference calls and site visits.

C.8.2 Certification of Personnel

Before the study is started, each center must complete certification requirements, which include demonstration of familiarity with study procedures, methods for endpoint measurement, use of the database management system, and designation of staff to conduct the study. The DCC will work with each center on certification and notify NHLBI when it is achieved.

Echocardiography personnel at each center will undergo sessions on standardization of technique as required by the Core Laboratory. All echocardiograms will be read in a Core Laboratory. Poor quality studies may necessitate site visits.

C.8.3 Data Monitoring/Site Visits

Each clinical center will be visited once by representatives from the DCC and the NHLBI during the study period. The primary roles of the site visit team will be to evaluate general protocol compliance and adherence to IRB requirements, review site data files for correct filing of copies of consent forms and study forms, audit a random sample of records to assess data integrity, and identify and resolve general problems with study progress. At each site visit, a random sample of adverse event reports will be reviewed in order to determine whether reporting of data has been accurate and complete. Follow-up actions by the site coordinator or investigator and a schedule for completion will be identified at each site visit. An evaluation checklist will be completed at each site visit for inclusion in a Site Visit Report to the investigators. New staff will be trained and existing staff will be retrained, if necessary. Site coordinators will be expected to provide materials and answer questions prior to and during these visits.

The DCC may conduct site visits to the Core Laboratory during the first year to review in-house quality assurance (QA) and quality control (QC) procedures and data transfer to the DCC. Review of central laboratory-related reports will be conducted at least monthly to identify overall or site-specific problems in data or specimen acquisition and reporting of results.

D. STUDY LIMITATIONS

Study limitations include the following:

- Although the study will be a prospective, randomized trial, it will not be possible to blind all of the subjects' care providers to the study drug therapy. The study coordinator and the cardiologist involved in uptitration will need to know which medication each subject is receiving since the atenolol dose will be titrated to heart rate response. The subject's primary cardiologist may be able to determine whether the subject is on atenolol because of expected heart rate response to BB therapy. However, the primary trial endpoint and many of the secondary endpoints will be evaluated at the Echocardiography Core Laboratory by physicians who will be unaware which study drug a subject is taking.
- Although every effort will be made to prevent the study subjects and their families from learning their treatment assignment, many subjects will have prior experience taking a

beta blocker and might be able to guess their drug assignment. Furthermore, there are some differences between the two suspensions and between the two tablet preparations; hence, subjects and families may be able to determine which drug they are taking. Given the inherent differences in the physiologic effects of the two study drugs (e.g., heart rate response), the unavailability of unmarked clinical images of atenolol, the fact that over-encapsulated tablets may be difficult to swallow for many subjects, that subjects cannot be prevented from cutting open the gel cap to see the actual over-encapsulated tablet, and the inability to obtain the required tablets for a double-dummy design, the study design outlined in the protocol was adopted as most practical. Withdrawal and crossover rates, as well as the reasons for withdrawal and crossover, will be monitored very closely.

- The study may not detect an effect that is smaller than that for which the study is powered.
- The study may be underpowered for subgroup analyses and some secondary endpoints.
- The study results may not be generalizable to individuals with MFS who have aortic root *Z*-scores ≤ 3.0 or to those individuals who carry the diagnosis of “MFS” but who do not meet the Ghent diagnostic criteria for MFS.

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TRIAL OF BETA BLOCKER THERAPY (ATENOLOL) VS. ANGIOTENSIN II RECEPTOR BLOCKER THERAPY (LOSARTAN) IN INDIVIDUALS WITH MARFAN SYNDROME

Date: October 10, 2006

Amendment: October 19, 2006

Amendment: January 14, 2008

Amendment: November 5, 2008

Amendment: September 26, 2011

F. Appendices

APPENDIX A

SAMPLE INFORMED CONSENT FORMS FOR TRIAL

Informed Consent Template for Research

Pediatric Heart Network

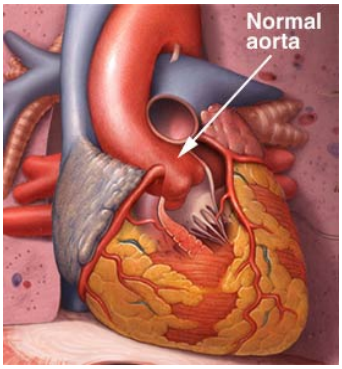
Study Title: Trial of Beta Blocker Therapy (Atenolol) vs. Angiotensin II Receptor Blocker Therapy (Losartan) in Individuals with Marfan Syndrome

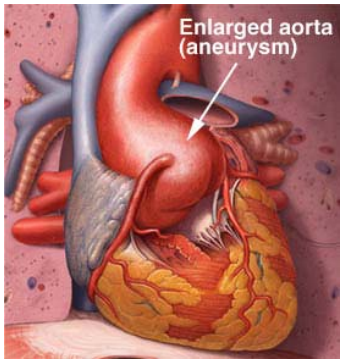
Parental Consent

IRB Study Number:

Why is this study being done?

The aorta is the large blood vessel that takes blood from the heart out to the body. People with Marfan syndrome may have enlargement of their aorta in the area where it leaves the heart. This area is called the aortic root (see diagram below).





This study is being done to compare two drugs to see which one is better at slowing the rate of aortic root enlargement. One drug is called losartan (an angiotensin II receptor blocker or ARB) and the other drug is atenolol (a beta blocker). The study will also find out which drug may have fewer drug side-effects.

Losartan is a drug that slows the rate of aortic root enlargement in mice with Marfan syndrome. Losartan is approved by the United States Food and Drug Administration (FDA) for use in adults and children over 6 years of age who have high blood pressure. This medication has not been studied in people with Marfan syndrome. On the other hand, studies have shown that atenolol slows the rate of aortic root enlargement in people with Marfan syndrome. Atenolol is approved by the FDA for use in adults with high blood pressure. Although atenolol is not officially approved by the FDA for use in children, this drug is commonly used to treat children with a variety of conditions including Marfan syndrome.

Approximately XX patients will be studied at <*site/institution*>. This study is being conducted at all Pediatric Heart Network sites as well as additional sites in the United States. It is planned that a total of XXX patients will be enrolled from all of the sites. This study is being funded by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health. Portions of Dr. <*site PI*> and his/her research team's salaries are being paid by this grant.

Why is your child being asked to participate in this research study?

Your child has been asked to participate in this research study because your child has Marfan syndrome and an aortic root that is enlarged. In order for your child to participate in this study certain criteria must be met and the Consent Form must be signed. Your child's geneticist or

cardiologist has reviewed the medical information and has determined that your child may be eligible for this study.

How long will your child be in the study and what will happen during the study?

1. We will review your child's medical chart from time to time to get data about your child's heart problem, results of an echocardiogram performed in the past 12 months, how your child has been treated, and other medical problems your child may have.

2. If your child is currently taking a beta-blocker (for example, atenolol or propranolol), an angiotensin converting enzyme inhibitor (for example, enalapril, lisinopril, ramapril) or an angiotensin receptor blocker (for example losartan, candesartan, valsartan), the medication will need to be decreased over a period of 2 weeks under medical supervision and then stopped for 2 to 3 weeks. It is safe for your child to be off any of these medications during this brief period of time. Following this period of time, you will bring your child back to the clinic to have an echocardiogram.

3. If you agree to allow your child to participate in this study, your child will have an echocardiogram to evaluate the heart and aortic root. An echocardiogram is a painless test, lasting about 30 - 60 minutes, that uses sound waves to make a moving picture of the heart.

4. Your child will receive either losartan or atenolol by random assignment (like flipping a coin). At the beginning of the study, a computer will decide which group your child will be in and neither your doctor nor you will know which drug your child is taking. One cardiologist, one research nurse and the pharmacist will know which drug your child is taking. This information is easy to find if it is necessary for medical care or in an emergency.

5. Your child will start the study drug (either losartan or atenolol) at a low dose. It will be increased over time until the best tolerated or target dose is reached. If your child is less than 18 months of age, blood pressure and blood tests will be measured between 21 and 28 days after starting the medicine. Each time that we increase the medicine your child will be asked to wear a Holter monitor for 24 hours to check your child's heart rate. If your child is less than 18 months old, a blood pressure measurement will be taken each time that we increase the medicine.

   The dose of study drug will be increased depending on your child's weight and how well he/she tolerates each dose. It may be adjusted upwards or downwards a number of times during the study according to the heart rate and if there are any side effects. Your child will swallow a pill or liquid once or twice a day.

6. Your child will take the study drug for up to 3 years. You will bring your child for study visits at 6 months, 12 months, 24 months and 36 months after entry into the study. Your doctor may want to see you for routine visits in addition to the study visits. If you move during the time that you are in the study, we will make every attempt to find a participating center closer to you so that your child can stay in the study.

   After your child finishes the study, the study doctors and nurses will continue to review the medical record for up to 5 years. They will review the results of your child's follow-up health history, any surgery, and other relevant studies. You may be contacted during that 5-year period to add medical events and test results related to your child's heart condition to the research record.

In addition to taking the study drug, there will be several tests done during the study. The tests are:

Echocardiogram: An echocardiogram is considered a painless test that uses sound waves to take pictures of the heart. Your child will need to lie quietly on a table for about 30 - 60 minutes while the test is being done. The test will measure aortic root size, valve function, and heart function. Echocardiograms are a usual test done in people with Marfan syndrome. Some extra echocardiograms will be done only for the study. The first study echocardiogram will be done before starting the study drug. A second one will be done at 6 months after starting the drug. We will collect data from your child's normally scheduled echocardiograms for the 12, 24, and 36 month visits.

Your child may need a medication to help him/her lie still during the echocardiogram. If so, a small band to monitor your child's pulse and blood oxygen content will be placed on a toe or finger. Breathing, heart rate, and blood pressure will also be monitored to be sure there are no side effects from the sedation medicine. The sedation medicines are standard medications routinely used to sedate children less than about 2 years of age or those who are unable to lie quietly during the echocardiogram.

Blood Samples: Blood tests are done as part of this study to check how well your child's kidneys and liver are working. The amount of blood taken at each visit is about 3 milliliters or about half (1/2) of a teaspoon. We will take blood before the study drug is started and once when the target dose is reached. For children less than 18 months of age, a blood test will also be taken between 21 and 28 days after starting the study drug. We will talk with you about options to reduce your child's discomfort during the blood draw. If your child is a female who could possibly become pregnant, a blood pregnancy test will be performed once. The test must be negative before your child can begin the study.

Holter Monitoring: A Holter monitor is a 24 hour electrocardiogram (ECG). Your child will wear patches applied to the skin; the patches will be connected to a small box which will record your child's heart rate and rhythm. Your child will be able to have regular daily activity during this time. After a 24 hour recording, you will mail the Holter monitor back to the study coordinator using a pre-paid envelope. Based on the heart rate, the dose of study drug may be adjusted upwards or downwards. Your child will need to wear a Holter monitor after each increase in the dose of the study drug and also at the 6-, 12-, 24-, and 36- month visits.

Questionnaire: You and your child will be asked to fill out a questionnaire before starting the study drug, once the target dose of study drug is reached and at each study visit (6, 12, 24, and 36 months). This will help us to see how well your child is doing while taking the study drug and if your child is having any side-effects. The study coordinator will be in contact with you regularly to see how your child is doing. You can let the coordinator know if your child is having side-effects when he/she calls. You may also contact the study coordinator or doctor at any time.

7. During the study, you should tell either < site PI > or < study coordinator > your child's medical history, all of the medicines your child is taking (including over-the-counter medicines and herbal supplements) and any pain or signs of illness your child has during the study. It is very important that you keep all of the visits with the study doctor.

8. If you or your child's doctor decides that your child should stop taking the study drug for any reason, it is still important for the research study to measure how your child is growing and

to obtain the echocardiograms at the study visits (6, 12, 24 and 36 months). With your permission, your child will remain in the study but no additional blood tests or Holter monitors will be done for the study.

WHAT ARE THE RISKS AND DISCOMFORTS OF THE RESEARCH STUDY?

People usually tolerate losartan well. There is a small risk that the kidneys or liver may not work as well as they should. We will watch carefully to see how your child's kidneys and liver are working by checking the blood. Losartan may lower blood pressure, so your child's blood pressure and heart rate will be monitored during the study.

People generally tolerate atenolol well. Atenolol may cause drug side-effects including a slow heart rate, dizziness, low blood pressure, tiredness, depression, headache, nausea, diarrhea, sleep disturbances, and worsening of asthma (wheezing and shortness of breath). Your child's heart rate and blood pressure will be monitored throughout the study.

Angioedema, (swelling of face, hands or feet, eyes, lips, tongue, difficulty in swallowing or breathing) is an extremely uncommon event (about 1 in 1,000) but is serious and may occur at any time during treatment. If your child develops any signs or symptoms suggesting angioedema, stop the drug immediately and seek medical attention.

Risks associated with drawing blood from a vein include brief discomfort and/or bruising. Infection, excess bleeding, clotting, and fainting are also possible, although unlikely. Every effort will be made to obtain the blood samples needed for all research tests at the same time as other blood tests that your child's doctor might order.

There are no known risks with having an echocardiogram.

If sedation is required for the echocardiogram(s), we will monitor your child's vital bodily functions during the procedure and recovery. Risks associated with the sedation include, but are not limited to, conditions such as respiratory (breathing) problems, allergic or other unexpected drug reactions, irritability, vomiting, prolonged drowsiness, unsteadiness or lack of balance, minor pain and discomfort, failure to achieve adequate sedation and/or possible awareness or memory of the procedure. Severe drug reactions may occur but are extremely rare.

The only risk when using the Holter monitor may be brief skin irritation from the patches placed on the skin.

There may be risks that we don't know about yet.

Pregnancy/Birth Control:
If your child becomes pregnant while taking losartan there may be risks to her or to the baby which we don't currently know about or cannot predict. We know that when used in pregnancy during the second and third trimesters, ARB medications (like losartan) can cause injury and even death to the developing fetus.

There is no way to guarantee that the study drug will not have a harmful effect on a developing baby (embryo, fetus, or infant). Therefore, your child will not be able to take part in this study if there is any possibility that she is pregnant, or is planning to become pregnant in the next 3 years. If she is sexually active, she may participate in this study if she uses an effective form of contraception. If she suspects that she might be pregnant during the study (such as a missed or

late menstrual period or change in the usual menstrual cycle), notify the study doctor immediately. If she is pregnant, the study drug will be stopped and she will not have any more blood tests or Holter monitors. However, she will continue to have the study echocardiograms at the scheduled visits (6, 12, 24 and 36 months). The study doctor or nurse will help find appropriate counseling or obstetrical care.

Are there benefits to taking part in the research study?

Your child may or may not benefit by being in this study. However, taking part in this study may help other people with Marfan syndrome in the future.

What other choices are there?

Instead of being in this research study, your child has the option to receive standard treatment as determined by your child's cardiologist. There may be other drugs or treatments that could be given for your child's condition. Talk with your doctor about what these other choices might be.

What are your rights as a participant?

Participation in this study is completely **voluntary**. You may choose not to allow your child to be in this study. If you agree for your child to be in the study, he/she may leave at any time. This will not affect your child's regular care or cause your child to lose any benefits. If you do decide to withdraw your child from the study, it is very important that you contact Dr. < site PI > and let him/her know because it could be harmful to just stop taking the study drug.

If you withdraw your child from the study, no new data will be collected for study purposes unless the data concern an adverse event (a bad effect) related to the study. If such an adverse event occurs, we may need to review your child's entire medical record. All data that have already been collected for study purposes, and any new information about an adverse event related to the study, will be sent to the study sponsor.

If you decide to withdraw your child from the study, we will ask you to return any unused study drug.

We will tell you and your child about new things we learn during this study that may affect your or your child's willingness to stay in the study. If you decide to leave the study, your research doctor will work with you on the best way to continue your child's care. Also, for health, safety or other reasons, your research doctor might think it is best to withdraw your child from the study. He/she will explain the reasons and arrange for your child's care to continue.

A signed copy of this consent form will be given to you for your records.

How will your information be kept confidential and private?

Every effort will be made to keep your child's medical and research information private. <site/institution> and/or <site PI> will do the following things to maintain your child's privacy:

- Study records that identify your child will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of <site/institution>.
- All study information sent outside of <site/institution> will be linked to your child through a study identification (ID) number and through a combination of initials. The link between the study ID and your child will be kept in locked files in Dr. <site PI's>office.

- Echocardiogram studies will be sent to New England Research Institutes (NERI), the Pediatric Heart Network Data Coordinating Center, for submission to a laboratory outside of <site/institution> for reading. The Echo data files, which may have your child's name on them, will be kept in locked files and stored on secure servers at this laboratory. Your child's name will not be recorded in any other records kept outside of <site/institution>.
- Information gathered during this study and your child's medical records may be inspected and verified by staff of the study sponsor (the National Institutes of Health), <site/institution> Institutional Review Board, or the Pediatric Heart Network Data Coordinating Center. Medical records for this study and medical records from other institutions that contain your child's identity will be treated as confidential by the National Institutes of Health and will be shared only with these agencies, or as required by law.
- The results of this study may be published for all the subjects as a group but will not identify your child individually.

Will it cost you anything to be in this study?

There will be no additional costs to you if your child takes part in this study. Tests required only for the study and not part of your child's standard care will be provided free of charge. You will be responsible for all other costs related to your child's normal medical care such as hospitalization, surgery, drugs, laboratory tests, diagnostic procedures and physician fees which are considered standard medical care.

Will you be paid to join this study?

The study investigators will pay for <insert center language here related to parking, travel, meals, stipend, etc>.

What happens if you believe you are injured during this study?

Immediate necessary medical care is available at <site/institution> in the event that your child is injured as a result of his/her participation in this research study. However, there is no commitment by <site/institution>, Dr. <site PI> or your <site> physicians to provide monetary compensation or free medical care to your child in the event of a study-related injury. Further information concerning this and your child's rights as a research subject can be obtained from the <site/institution> Institutional Review Board (IRB) Office at: <phone number>. (Or insert institutional language)

Who do you call if you have questions about this study?

If you have questions about this study, you should contact:
<Site PI>, MD Telephone Number: <telephone number>
Pager Number: <pager number

If you have questions or want more information about the Pediatric Heart Network or about being in a study, you may go to www.PediatricHeartNetwork.org. You will also find information about this study on the website.

If you have questions concerning your child's rights as a subject in this study, you should contact:
<Site> Institutional Review Board (IRB) Office at:
Telephone Number: <phone number>

If you have any questions concerning the study, drug side effects, or to report a research related injury, you should contact:

<name> Telephone Number: <phone number>
Pager Number: <pager number>

CONSENT:

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask the questions I have, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I have read this consent form and agree to allow my child to be in this study with the understanding that I may withdraw him/her at any time. I have been told that I will be given a signed copy of this consent form."

_____ _____

Signature of child if over age 12 Date

_____ _____

Signature of Parent or Guardian Date

_____ _____

Signature of person obtaining consent Date

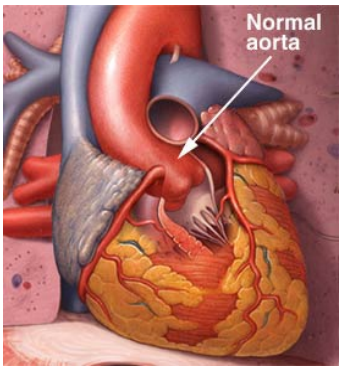
Informed Consent Template for Research

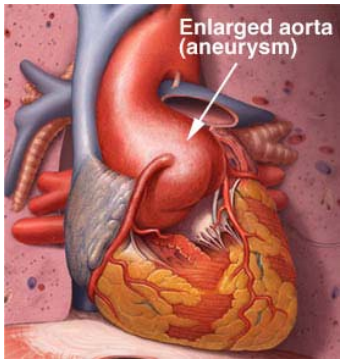
Pediatric Heart Network

Study Title: Trial of Beta Blocker Therapy (Atenolol) vs. Angiotensin II Receptor Blocker Therapy (Losartan) in Individuals with Marfan Syndrome

Subject Consent
IRB Study Number:

Why is this study being done?
The aorta is the large blood vessel that takes blood from the heart out to the body. People with Marfan syndrome may have enlargement of their aorta in the area where it leaves the heart. This area is called the aortic root (see diagram below).





This study is being done to compare two drugs to see which one is better at slowing the rate of aortic root enlargement. One drug is called losartan (an angiotensin II receptor blocker or ARB) and the other drug is atenolol (a beta blocker). The study will also find out which drug may have fewer drug side-effects.

Losartan is a drug that slows the rate of aortic root enlargement in mice with Marfan syndrome. Losartan is approved by the United States Food and Drug Administration (FDA) for use in adults with high blood pressure. This medication has not been studied in people with Marfan syndrome. On the other hand, studies have shown that atenolol slows the rate of aortic root enlargement in people with Marfan syndrome. Atenolol is approved by the FDA for use in adults with high blood pressure. Although atenolol is not officially approved by the FDA for use in children, this drug is commonly used to treat children with a variety of conditions including Marfan syndrome.

Approximately XX patients will be studied at <*site/institution*>. This study is being conducted at all Pediatric Heart Network sites as well as additional sites in the United States. It is planned that a total of XXX patients will be enrolled from all of the sites. This study is being funded by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health. Portions of Dr. <*site PI*> and his/her research team's salaries are being paid by this grant.

Why are you being asked to participate in this research study?
You have been asked to participate in this research study because you have Marfan syndrome and your aortic root is enlarged. In order for you to participate in this study you must meet certain criteria and you must sign this Consent Form. Your geneticist or cardiologist has reviewed your medical information and has determined that you may be eligible for this study.

How long will you be in the study and what will happen during the study?

1. We will review your medical chart from time to time to get data about your heart problem, the results of an echocardiogram performed in the past 12 months, how you have been treated, and other medical problems you may have.

2. If you are currently taking a beta-blocker (for example, atenolol or propranolol), an angiotensin converting enzyme inhibitor (for example, enalapril, lisinopril, ramapril) or an angiotensin receptor blocker (for example losartan, candesartan, valsartan), your medication will need to be decreased over a period of 2 weeks under medical supervision and then stopped for 2 to 3 weeks. It is safe for you to be off any of these medications during this brief period of time. Following this period of time, you will come back to the clinic and have an echocardiogram.

3. If you agree to participate in this study, an echocardiogram will be done to evaluate your heart and aortic root. An echocardiogram is a painless test lasting 30-60 minutes that uses sound waves to make a moving picture of your heart.

4. You will receive either losartan or atenolol by random assignment (like flipping a coin). At the beginning of the study, a computer will decide which group you will be in and neither your doctor nor you will know which drug you are taking. One cardiologist, one research nurse and the pharmacist will know which drug you are taking. This information is easy to find if it is necessary for your medical care or in an emergency.

5. You will start the study drug (either losartan or atenolol) at a low dose which will be increased over time until you reach the best tolerated or target dose. Each time that we increase your medicine you will be asked to wear a Holter monitor for 24 hours to check your heart rate. The dose will be increased depending on your heart rate and whether you have any side effects. The study drug may be adjusted upwards or downwards a number of times during the study according to your heart rate and how well you tolerate the study drug. You will swallow a pill once or twice a day. All doses of the study drug will be based on your weight and how well you tolerate each dose.

6. You will take the study drug for up to 3 years. You will come for study visits at 6 months, 12 months, 24 months and 36 months after entry into the study. Your doctor may want to see you for routine visits in addition to the study visits. If you move during the time that you are in the study, we will make every attempt to find a participating center closer to you so that you can stay in the study.

   After you finish taking the study drug, the study doctors and nurses will continue to review your medical record for up to 5 years. They will review the results of your follow-up health history, any surgery, and other relevant studies. You may be contacted during that 5-year period to add medical events and test results related to your heart condition to the research record.

In addition to taking the study drug, you will have several tests during the study. The tests are:

Echocardiogram: An echocardiogram is considered a painless test that uses sound waves to take pictures of the heart. You will need to lie quietly on a table for about 30 – 60 minutes while the test is being done. The test will measure aortic root size, valve function, and heart function. Echocardiograms are a usual test done in people with Marfan syndrome. Some extra echocardiograms will be done only for the study. The first study echocardiogram will be done

before starting the study drug. A second one will be done at 6 months after starting the drug. We will collect data from your normally scheduled echocardiograms for the 12, 24, and 36 month visits.

Blood Samples: You will have blood tests done as part of this study to check how well your kidneys and liver are working. The amount of blood taken at each visit is about 3 milliliters or about half (1/2) of a teaspoon. We will take blood before you start the study drug and once when you reach the target dose. If you are a woman who could possibly become pregnant, a blood pregnancy test will be performed. The test must be negative before you begin the study.

Holter Monitoring: A Holter monitor is a 24 hour electrocardiogram (ECG). You will wear patches applied to your skin; the patches will be connected to a small box which will record your heart rate and rhythm. You will be able to have regular daily activity during this time. After a 24 hour recording, you will mail the Holter monitor back to the study coordinator using a pre-paid envelope. Based on your heart rate, the dosage of study drug may be adjusted upwards or downwards. You will need to have a Holter monitor after each increase in the dose of study drug and also at the 6-, 12-, 24-, and 36- month visits.

Questionnaire: You will be asked to fill out a questionnaire before you start the study drug, once you reach the target dose of study drug and at each study visit (6, 12, 24, and 36 months). This will help us to see how well you are doing while taking the study drug and if you are having any side-effects. The study coordinator will keep in contact with you regularly during the study to see how you are doing. You can let him/her know if you are having any side effects at that time. You may also call the study coordinator or doctor at any time.

7. During the study, you should tell either <site PI> or <study coordinator> your medical history, all of the medicines you are taking (including over-the-counter medicines and herbal supplements) and any pain or signs of illness you have during the study. It is very important that you keep all of your visits with the study doctor.

8. If you or your doctor decides that you should stop taking the study drug for any reason, it is still important for the research study to measure your growth and to obtain the echocardiograms at the study visits (6, 12, 24 and 36 months). With your permission, you will remain in the study but no additional blood tests or Holter monitors will be done for the study.

WHAT ARE THE RISKS AND DISCOMFORTS OF THE RESEARCH STUDY?

People usually tolerate losartan well. There is a small risk that the kidneys or liver may not work as well as they should. We will watch carefully to see how your kidneys and liver are working by checking your blood. Losartan may lower your blood pressure enough to cause dizziness. You will be monitored for these side effects throughout the study.

Atenolol may cause drug side-effects including a slow heart rate, dizziness, low blood pressure, tiredness, depression, headache, nausea, diarrhea, sleep disturbances, and worsening of asthma (wheezing and shortness of breath). You will be monitored closely for these side effects throughout the study.

Angioedema, (swelling of face, hands or feet, eyes, lips, tongue, difficulty in swallowing or breathing) is an extremely uncommon event (about 1 in 1,000) but is serious and may occur at any

time during treatment. If you develop any signs or symptoms suggesting angioedema, you should stop the drug immediately and seek medical attention.

Risks associated with drawing blood from a vein include brief discomfort and/or bruising. Infection, excess bleeding, clotting, and fainting are also possible, although unlikely. Every effort will be made to obtain the blood samples needed for all research tests at the same time as other blood tests that your doctor might order.

There are no known risks with having an echocardiogram.

The only risk when using the Holter monitor may be brief skin irritation from the patches placed on the skin.

There may be risks that we don't know about yet.

Pregnancy/Birth Control:
If you become pregnant while taking losartan there may be risks to you or to the baby which we don't currently know about or cannot predict. We know that when used in pregnancy during the second and third trimesters, ARB medications (like losartan) can cause injury and even death to the developing fetus.

There is no way to guarantee that the study drug will not have a harmful effect on a developing baby (embryo, fetus, or infant). Therefore, you will not be able to take part in this study if there is any possibility that you are pregnant, or are planning to become pregnant in the next 3 years. If you are sexually active, you may participate in this study if you use an effective form of contraception. If you suspect that you might be pregnant during the study (such as a missed or late menstrual period or change in your usual menstrual cycle), you must notify the study doctor immediately. If you are pregnant, the study drug will be stopped and you will continue to have the study echocardiograms at the scheduled visits (6, 12, 24 and 36 months). The study doctor or nurse will help you to find appropriate counseling or obstetrical care.

Are there benefits to taking part in the research study?
You may or may not benefit by being in this study. However, taking part in this study may help other people with Marfan syndrome in the future.

What other choices are there?
Instead of being in this research study, you have the option to receive standard treatment as determined by your cardiologist. There may be other drugs or treatments that could be given for your condition. Talk with your doctor about what these other choices might be.

What are your rights as a participant?
Your participation in this study is completely voluntary. You may choose not to be in this study. If you agree to be in the study, you may leave at any time. This will not affect your regular care or cause you to lose any benefits to which you are entitled. If you do decide to withdraw, it is very important that you contact Dr. <site PI> and let him/her know because it could be harmful to just stop taking your study drug.

If you withdraw from the study, no new data about you will be collected for study purposes unless the data concern an adverse event (a bad effect) related to the study. If such an adverse event occurs, we may need to review your entire medical record. All data that have already been

collected for study purposes, and any new information about an adverse event related to the study, will be sent to the study sponsor.

If you decide to withdraw from the study, we will ask you to return any unused study drug.

We will tell you about new things we learn during this study that may affect your willingness to stay in the study. If you decide to leave the study, your research doctor will work with you on the best way to continue your care. Also, for health, safety or other reasons, your research doctor might think it is best to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

A signed copy of this consent form will be given to you for your records.

How will your information be kept confidential and private?
Every effort will be made to keep your medical and research information private. <site/institution> and/or <site PI> will do the following things to maintain your privacy:

- Study records that identify you will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of <site/institution>.
- All study information sent outside of <site/institution> will be linked to you through a study identification (ID) number and through a combination of your initials. The link between the study ID and you will be kept in locked files in Dr. <site PI's >office.
- Echocardiogram studies will be sent to New England Research Institutes (NERI), the Pediatric Heart Network Data Coordinating Center, for submission to a laboratory outside of <site/institution> for reading. The Echo data files, which may have your child's name on them, will be kept in locked files and stored on secure servers at this laboratory. Your child's name will not be recorded in any other records kept outside of <site/institution>.
- Information gathered during this study and your medical records may be inspected and verified by staff of the study sponsor (the National Institutes of Health), <site/institution> Institutional Review Board, or the Pediatric Heart Network Data Coordinating Center. Medical records for this study and medical records from other institutions that contain your identity will be treated as confidential by the National Institutes of Health and will be shared only with these agencies, or as required by law.
- The results of this study may be published for all the subjects as a group but will not identify you individually.

Will it cost you anything to be in this study?
There will be no additional costs to you when you participate in this study. Tests required only for the study and not part of your standard care will be provided to you free of charge. You will be responsible for all other costs related to your normal medical care such as hospitalization, surgery, drugs, laboratory tests, diagnostic procedures and physician fees which are considered standard medical care for patients with your condition.

Will you be paid to join this study?
The study investigators will pay for <insert center language here related to parking, travel, meals, stipend, etc>.

What happens if you believe you are injured during this study?
Immediate necessary medical care is available at <site/institution> in the event that you are injured as a result of your participation in this research study. However, there is no commitment by <site/institution>, Dr. <site PI> or your <site> physicians to provide monetary compensation or free medical care to you in the event of a study-related injury. Further information concerning this and your rights as a research subject can be obtained from the <site/institution> Institutional Review Board (IRB) Office at: <phone number>. (Or insert institutional language)

Who do you call if you have questions about this study?
If you have questions about this study, you should contact:
<Site PI>, MD Telephone Number: <telephone number>
Pager Number: <pager number

If you have questions or want more information about the Pediatric Heart Network or about being in a study, you may go to www.PediatricHeartNetwork.org. You will also find information about this study on the website.

If you have questions concerning your rights as a subject in this study, you should contact: <Site> Institutional Review Board (IRB) Office at:
Telephone Number: <phone number>

If you have any questions concerning the study, drug side effects, or to report a research related injury, you should contact:

<name> Telephone Number: <phone number>
Pager Number: <pager number>

CONSENT:

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I have read this consent form and agree to be in this study with the understanding that I may withdraw at any time. I have been told that I will be given a signed copy of this consent form."

_____ _____
Signature of Subject Date

_____ _____
Signature of person obtaining consent Date

Assent Template for Research

Study Title: Trial of Beta Blocker Therapy (Atenolol) vs. Angiotensin II Receptor Blocker Therapy (Losartan) in Individuals with Marfan Syndrome

Assent for children ~7 to 17 yrs of age

IRB Study Number:

These are some things we want you to know about research studies:
We are asking you to be in a research study. Research is a way to test new ideas. Research helps us learn new things.

You can decide if you want to be in this research study or not. You can say Yes or No. Whatever you choose is OK. We will still take good care of you whether you say Yes or No.

Why am I being asked to be in this research study?
You are being asked to be in this study because you have Marfan syndrome. The big blood vessel that comes out from your heart and goes to your body is called the aorta. The aorta can sometimes get too big in people who have Marfan syndrome.

What is the study about?
The doctors need to learn more about the aorta in people with Marfan syndrome and they need to come up with the safest and best medicines to prevent the aorta from getting big in people with Marfan syndrome.

What will happen during this study?
If you agree to be in this study, you will:

- Take medicine every day. There are 2 different kinds of medicine that are used in this study. The doctors will pick one for you (like flipping a coin) because they want to find out which medication works better.
- Come and see the research doctors and nurses in the clinic.
- Get echocardiograms done in the clinic. An echocardiogram takes pictures of your heart and does not hurt at all.
- Wear a heart rate monitor at home which will measure how fast your heart is beating.
- Get blood taken.
- Answer some questions about how you are feeling on the medication.

Will the study hurt?
The doctor will need some of your blood. The needle stick hurts for a little bit as the blood is taken. We will tell you about things that might help to make the stick hurt less.

The echocardiogram and heart rate monitor tests do not hurt.

What else should I know about the study?
If you feel sick or afraid that something is wrong with you, tell an adult at once.

What are the good things that might happen?
The medications in this study will be given to you to try to keep your aorta from getting big. The doctors hope to learn about the best and safest way to help people with Marfan syndrome so that they can find something that will help other people like you.

What if I don't want to be in this study?
You do not have to be in the study if you do not want to. The doctors will still take care of you. If you don't want to be in this study, you can still get your medical care at <site/institution>.

Even if you say yes now, you can change your mind later. It is up to you. No one will be mad at you if you don't want to do this. The doctors will also be talking to your parents about this study.

Who should I ask if I have any questions?
If you have any questions about this study, you or your parents can call Dr. < site PI> at <telephone number>.

Now that I have asked my questions and think I know about the study and what it means, here is what I decided:

_____OK, I'll be in the study. _____ No, I do not want to be in the study.

The researchers have told me about the research. I had a chance to ask questions. I know I can ask questions at any time. I want to be in the research.

If you sign your name below, it means that you agree to take part in this research study.

_____	____	_____
Your Name (Printed)	Age	Date
_____		_____
Your Signature		Date
_____		_____
Signature of Witness		Date
_____		_____
Signature of Person Obtaining Consent		Date

APPENDIX B

MEASUREMENTS OF SOMATIC GROWTH

APPENDIX B. MEASUREMENTS OF SOMATIC GROWTH

Measurements of weight, recumbent length, stature (height), and body mass index will be performed according to the guidelines developed by the U.S. Department of Health and Human Services, Health Resources and Services Administration, Maternal and Child Health Bureau (MCHB) and the Centers for Disease Control and Prevention (CDC). All study personnel who will obtain height and weight measurements will complete the Growth Charts Training provided on the HRSA website:
http://128.248.232.56/mchbgrowthcharts/module4/text/mainintro.htm
and
http://www.cdc.gov/nchs/products/subject/video.html

Equipment to determine accurate and reliable weight, recumbent length, stature (height), and body mass index will conform to the MCH/CDC specifications described on the HRSA website:
http://128.248.232.56/mchbgrowthcharts/module4/text/page1a.htm

Weight, recumbent length, stature (height), and body mass index will be normalized to the patient's age using data from the National Health and Nutrition Examination Survey available on the CDC website:
http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/zscore/zscore.htm
and
http://www.cdc.gov/nchs/about/major/nhanes/Anthropometric%20Measures.html

The following additional measurements will be obtained to assess disproportionate growth and stature:

Upper-to-lower segment ratio: The lower segment is defined as the distance from the top of the symphysis pubis to the floor. For non-ambulatory infants and children, the lower segment can be measured while supine. The upper segment is derived from height minus lower segment. Upper-to-lower segment ratio varies with age. Abnormal values are as follows [1, 2, 3]:

Age	Abormal Ratio
0-1 yr	< 1.5
1-2 yrs	< 1.4
2-3 yrs	< 1.3
3-4 yrs	< 1.2
4-5 yrs	< 1.1
5-6 yrs	< 1.0
6-7 yrs	< 0.95
7-8 yrs	< 0.90
> 8 yrs	< 0.85

Arm span-to-height ratio: Maximum span from fingertip to fingertip divided by the stature (height) or recumbent length. A ratio of > 1.05 is considered abnormal. [4]

REFERENCES

1. Dietz, H.C., Personal communication, 2006.
2, Headings, D.L. (ed.) The Harriet Lane handbook. (7th edn). Year Book Medical Publishers, Chicago, 1975.
3. McKusick, V.A. Heritable disorders of connective tissue. (4th edn). The C.V. Mosby Company, St. Louis, 1972.
4. DePaepe, A., Devereux, R.B., Dietz, H.C., Hennekam, R.C., Pyeritz, R.E., *Revised diagnostic criteria for the Marfan syndrome.* Am J Med Genet, 1996. **62**: p. 417-426.

APPENDIX C

ECHOCARDIOGRAPHIC STUDIES

APPENDIX C: ECHOCARDIOGRAPHIC STUDIES

A. STUDY EQUIPMENT

A.1 Echocardiographic imaging system equipped with transthoracic transducers appropriate to patient size.

A.2 Studies will be recorded in either sVHS format with standard 1/2" sVHS videotape or in any of the DICOM standard digital formats with an optimal clip length of 10 seconds (minimal clip length 3 beats). If feasible, de-identification prior to shipment to the Data Coordinating Center (DCC) is preferred. Studies saved in digital format will be archived to a CD or DVD. Sites with analog image storage will send original sVHS videotapes to the DCC for forwarding to the Core Laboratory. The Network is currently exploring the option of image transfer using FTP services and this may become an alternative image transfer option. All informed consents should state that patient identifiers could potentially be sent outside the institution, but that the echocardiogram will be kept in secure locations at the DCC and Core Laboratory. CD and DVD disks and sVHS videotapes are to be labeled on the exterior with labels supplied by the DCC and transferred to the DCC at least semi-monthly. If FTP image transfer is implemented, specific instructions regarding study labeling will be distributed.

B. TIMING OF STUDIES

Five echocardiograms per subject will be evaluated by the Core Laboratory. One study will be obtained at randomization. Four additional echocardiograms will be required, performed 6 months, one year, two years and three years following enrollment. Should a patient exit the study, all attempts should be made at that time to obtain an echocardiogram that includes all study parameters below. All studies will be performed according to the acquisition protocol as described herein.

C. STUDY ACQUISITION

C.1 Height and weight: Patient length in centimeters and weight in kilograms will be measured by the Marfan Syndrome Trial Study Coordinator at the time of echocardiography. Height and weight will be used to calculate body surface area (BSA) [1].

C.2 Blood pressure: An automated blood pressure device (e.g. Dinamap) will be used to record the average of 3 right arm brachial blood pressures (systolic, diastolic, and mean) and heart rate. These measurements are used to calculate aortic root and ascending aortic stiffness indices and

therefore should be obtained in close temporal proximity to the recording of these dimensions and should be obtained in the supine position.

C.3 Sedation may be required to obtain high quality echocardiographic images that are adequate for analysis. Sedation will be performed according to the Sedation Policy at each study center and will conform to the "Practice guidelines for sedation and analgesia by non-anesthesiologist" [2].

C.4 Echocardiography Protocol

C.4.1 Subxiphoid view (Distal thoracic aorta)

C.4.1.a 2D: The sagittal view is used to image the distal thoracic aorta along its long axis. Zoom mode is activated over the distal thoracic aorta to maximize.

C.4.1.b Pulsed wave Doppler: Color Doppler mode is used to guide placement of the pulsed Doppler sample site, and recording of the flow signal in the distal thoracic aorta is at or right above the level of the diaphragm. The scale is adjusted to position the zero velocity such that both positive and negative velocities are displayed correctly positioned relative to each other. The wall filter is turned down to enable visualization of velocity baseline crossing.

C.4.2 Suprasternal Notch View (Proximal thoracic aorta)

C.4.2.a 2D: The sagittal view of the aortic arch is used to image the proximal thoracic aorta along its long axis.

C.4.2.b Continuous wave Doppler: Color Doppler mode is activated and is used to guide positioning of the pulsed wave Doppler sample site in the proximal thoracic aorta. The scale is adjusted to position the zero velocity such that both positive and negative velocities are displayed correctly positioned relative to each other. The wall filter is turned down to enable visualization of velocity baseline crossing.

C.4.3 Parasternal short axis view (LV dimensions and wall thicknesses, main pulmonary artery dimension, and aortic valve regurgitation)

C.4.3.a 2D

- Image in the plane of the main pulmonary artery including the bifurcation is obtained. Zoom mode is activated over the main pulmonary artery to optimize resolution.
- The short axis image of the LV is obtained at the position of the largest short axis cross-sectional area in a plane orthogonal to the long axis of the left ventricle. Depth of the image is set to maximize the size of the recorded image with inclusion of the posterior wall throughout the cardiac cycle.

C.4.3.b M-mode: 2D sweeps through the ventricle should be recorded to permit the off-line reviewer to confirm correct positioning of the M-mode sample. Zoom mode is activated to provide maximum resolution and adjusted until the endocardium and epicardium of the left ventricle as well as the endocardium of the right ventricular surface of the septum are within the echocardiographic sector and visible throughout the cardiac cycle. The position of the M-mode cursor is positioned to sample the maximum diameter of the circular cross-section, and M-mode is activated at maximum sweep speed. When a clear image of endocardium and epicardium is obtained, the M-mode image is switched to full screen.

C.4.3.c Color Doppler: Color Doppler sweep of the aortic valve will be recorded. Aortic regurgitation with the color sector centered just below the aortic valve will be recorded with zoom mode activated to maximize resolution.

C.4.4 Parasternal long axis view (Aortic "annulus", aortic root at sinuses of Valsalva, sinotubular junction, ascending aorta, tricuspid and mitral valve prolapse, **aortic and mitral valve regurgitation**)

C.4.4.a 2-D

- A 2-D image of the aortic root aligned to the long-axis plane of the aortic root is recorded, with zoom mode activated to maximize resolution. Sweeps of the aortic root should be performed and recorded for optimal visualization of the aortic root.
- Ascending aorta will be recorded in the long axis plane of the aorta at the level of the right pulmonary artery from the high parasternal long-axis view.
- A 2-D image of the mitral valve is recorded, with zoom mode activated to maximize resolution.
- A 2-D image of the tricuspid valve is recorded, with zoom mode activated to maximize resolution.

C.4.4.b Color Doppler

- Color Doppler sweep of the aortic valve will be recorded. Aortic regurgitation with the color sector centered just below the aortic valve will be recorded with zoom mode activated to maximize resolution.
- Color Doppler sweep of the mitral valve will be recorded. Mitral valve regurgitation with the color sector centered to include the mitral valve jet in the area near the valve closure plane will be recorded with zoom mode activated to maximize resolution.

C.4.5 Apical 4-chamber view (LV cross-sectional areas and mitral valve regurgitation)

C.4.5.a 2-D: Left ventricle from the apical 4-chamber view, where the long-axis image will be recorded in the plane transecting both atrioventricular valves and intersecting the true apex of the ventricle. Optimize the settings to obtain a clear image of the endocardial and epicardial borders, using lateral gain if needed. Dual focus mode should be activated with the far focus near the base of the heart and the near-field focus near the apex of the ventricle to enable visualization of the apical endocardium.

C.4.5.b Color Doppler: Color Doppler sweep of the mitral valve will be recorded. Mitral valve regurgitation with the color sector centered just above the mitral valve will be recorded with zoom mode activated to maximize resolution.

C.5 Core Laboratory Data Processing

C.5.1 Digital images will be transferred from the DCC to the Core Laboratory on CD or DVD, or by FTP. Sites with analog image storage will FedEx the original sVHS videotape to the DCC for forwarding to the Core Laboratory to archive. The Core Laboratory will return the original videotape to the DCC to return to the site. The turn-around time between receipt of the videotape at the DCC and its return to the originating site will be no more than 7 working days.

C.5.2 The Core Laboratory administrator will create a log of study identifier and date of receipt at the Core Laboratory. Digital images will be transferred to the Core Laboratory image server using a file system appropriate to segregate the exams by study and by Core Laboratory personnel. If FTP image transfer is implemented for this study, the Core Laboratory administrator will assume responsibility for image management including transfer from the FTP server to the Core Laboratory server. The Core Laboratory administrator will also create the study event in the analysis program database (EchoTrace) and enter the study-related data such as date of study, date of study transfer, height, weight, study identifier, blood pressure, etc.

C.5.3 Measurements will be performed on a microcomputer-based workstation custom programmed for electronic caliper overlay of captured digital images for recording.

C.5.4 The Core Laboratory server will be backed up daily with incremental tape backups and weekly with complete backups for archiving purposes. At study completion, a separate permanent archive digital tape backup will be made of the data for this study prior to deletion of the image data from the Core Laboratory server.

C.6 CORE LABORATORY ANALYSIS: MEASURED PARAMETERS

2D measurements of the anatomic structures will be performed in systole and diastole on 2D images using the inner edge to inner edge technique unless otherwise specified.

C.6.1 Subxiphoid view (Distal thoracic aorta)

C.6.1.a The anteroposterior diameter of the distal thoracic aorta is measured at the level of the diaphragm (maximum dimension).

C.6.1.b Duration of diastolic flow and duration of diastolic reversal of flow in the distal thoracic aorta are measured.

C.6.2 Suprasternal Notch View (Proximal thoracic aorta)

C.6.2.a Duration of diastolic flow and duration of diastolic reversal of flow in the proximal thoracic aorta are measured.

C.6.3 Parasternal short axis view (LV dimensions and wall thicknesses, main pulmonary artery dimension and aortic valve regurgitation)

C.6.3.a Main pulmonary artery is measured at a level midway between the pulmonary valve annulus and pulmonary artery bifurcation (maximum dimension).

C.6.3.b M-mode of the left ventricle: End-diastole is taken at maximum dimension (during or just after the QRS), end-systole at minimum dimension (usually the position of maximum anterior excursion of posterior wall). Septal thickness, posterior wall thickness, and dimension are measured at end-diastole and end-systole.

C.6.3.c Areas of the left ventricle: End-diastole is defined as maximum area or dimension (the frame at which the mitral valve closes) and end-systole is defined as minimum area or dimension (the frame immediately preceding mitral valve opening). Using these end-diastolic and end-systolic 2-dimensional images, cross-sectional areas are measured by tracing the endocardial and epicardial borders at end-diastole and the endocardial border at end-systole.

C.6.3.d Proximal jet width of the regurgitant jet of the aortic valve is measured.

C.6.4 Parasternal long axis view (Aortic "annulus", aortic root at sinuses of Valsalva, sinotubular junction, ascending aorta, tricuspid and mitral valve prolapse, **aortic and mitral valve regurgitation)**

C.6.4.a The aortic diameters will be measured at their maximum and minimum dimensions in systole and diastole, respectively, from inner-edge to inner-edge at the levels indicated in figure 1 below [3]. Each of these 4 measurements will be performed in triplicate by two independent observers.

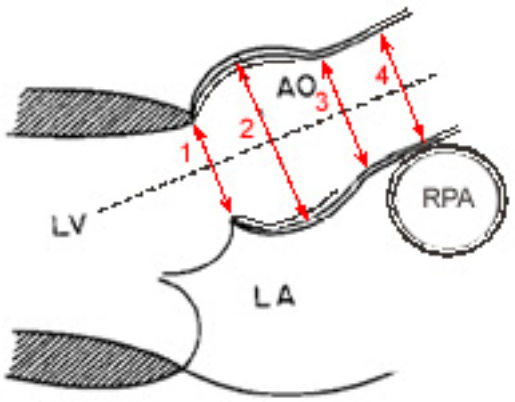


Figure 1 Aortic root in parasternal long axis view.

Maximum and minimum measurements will be taken from inner-edge to inner edge:

1= aortic valve "annulus" at the inferior points of attachment of the leaflets;

2= aortic root at the largest diameter within the sinuses of Valsalva;

3= sinotubular junction at the transition points from sinus to tubular aorta;

4= ascending aorta as it crosses the right pulmonary artery.

C.6.4 b Presence or absence of mitral and tricuspid valve prolapse is documented. Mitral and tricuspid valve prolapse are classified as present if all or part of a valve leaflet is observed to pass through the plane of the valve annulus on parasternal long axis 2D images [4]. Prolapse will be classified as borderline if one of the valve leaflets is observed to manifest posterior motion relative to the other leaflet over the course of systole but the leaflet does not actually move through the plane of the valve annulus.

C.6.4.c Proximal jet widths of the regurgitant jet(s) of the mitral and the aortic valves are measured.

C.6.5 Apical 4-chamber view (LV cross-sectional areas and mitral valve regurgitation)

C.6.5.a End-diastole is taken as the frame at which the mitral valve closes and end-systole is taken at the frame preceding mitral valve opening. The endocardial and epicardial borders are traced at end-diastole and the endocardial border is traced at end-systole.

C.6.5 b Proximal jet width of the regurgitant jet(s) of the mitral valve is measured.

C.7 CORE LABORATORY ANALYSIS: DERIVED PARAMETERS

C.7.1 Diameters at the level of the aortic annulus, aortic root at sinuses of Valsalva, sinotubular junction, and ascending aorta

The triplicate measurements for each of these dimensions will be averaged and the averaged values as well as the Z-scores for each of the averaged values will be reported for each of the 2 independent observers.

C.7.2 Left ventricular volumes, mass, and function (2-D)

C.7.1.a The end-diastolic (frame at which atrioventricular valve closure occurs) and end-systolic (frame preceding atrioventricular valve opening) endocardial and epicardial borders of the ventricle on long- and short-axis images, excluding the papillary muscles, will be used to compute volumes using a modified Simpson's rule algorithm [5] to provide end-diastolic (LVEDV) and end-systolic volumes (LVESV), left ventricular ejection fraction (LVEF), LV mass, and LV mass to volume ratio. Raw values and Z-scores for each of the volumes and derived parameters will be reported.

C.7.3 Left ventricular dimensions, wall thicknesses, mass and function (M-mode) The left ventricular end-diastolic (LVEDD) and end-systolic (LVESD), diastolic septal (IVSD) and posterior wall thickness (LVPWD) dimensions will be obtained and LV mass and shortening fraction (LVSF) will be calculated according to the following formulas [6]:

LV mass (g) $= 0.83 \times [(\text{LVEDD} + \text{IVSD} + \text{LVPWD})^3 - (\text{LVEDD})^3] + 0.6$

(1.05 = relative density of the heart muscle in g/ml)

LV SF (%) $= [(\text{LVEDD} - \text{LVESD}) / \text{LVEDD}] \times 100$

Raw values and Z-scores for each of the dimensions and the derived parameters will be reported.

C.7.4 Aortic valve regurgitation jet area indexed to BSA

The proximal aortic regurgitant jet widths from the 2 orthogonal planes will be used to calculate the proximal jet area using the formula for an ellipse:

> Area = pi × (proximal color jet width in parasternal short /2) × (proximal color jet width in parasternal long/2)

The dimensions, regurgitant area, and area/BSA will be reported.

Percent duration of diastolic flow reversal in the proximal and distal thoracic aorta will be calculated. The duration of diastole, duration of flow reversal, and percent duration of flow reversal will be reported.

C.7.5 Mitral valve regurgitation jet area indexed to BSA

The proximal jet widths from the 2 orthogonal planes will be used to calculate the proximal jet area using the formula for an ellipse [7-9].

Area = pi × (proximal color jet width in apical/2) × (proximal color jet width in parasternal long/2)

If there are multiple jets of mitral regurgitation, they will be measured separately and the total regurgitant orifice area will be calculated as the sum of these jets. The dimensions, total regurgitant orifice area, and area/BSA will be reported.

C.7.6 Central aortic stiffness indices

The ascending aortic maximum dimension (AA_{max}) and minimum (AA_{min}) dimensions, the aortic root maximum and minimum dimensions, and the brachial systolic (SBP) and diastolic BP (DBP) will be measured. The arterial pressure-strain elastic modulus (Ep) [10] and stiffness index (β_{index}) [11] will be calculated for the ascending aorta according to the following formulas:

$$Ep = (SBP - DBP) / [(AA_{max} - AA_{min}) / AA_{min}] \text{ [mmHg]}$$

$$\beta_{index} = [\ln (SBP / DBP)] / [(AA_{max} - AA_{min}) / AA_{min}]$$

Ep and β_{index} will also be calculated for the aortic root. The dimensions, blood pressures, Ep and β_{index} will be reported.

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APPENDIX D

DIAGNOSTIC EVALUATION FOR MARFAN SYNDROME: "GHENT CRITERIA"

APPENDIX D. DIAGNOSTIC EVALUATION FOR MARFAN SYNDROME: "GHENT CRITERIA"

Introduction: The diagnosis of MFS can be complex and is complicated by significant overlap between MFS and other connective tissue disorders. In this light, a set of rules were established regarding the clinical and molecular observations that contribute to diagnosis. These rules, often referred to as the "Ghent criteria" for diagnosis of MFS, were developed by a panel of clinician-scientists with extensive experience with MFS [1]. Certain findings are relatively specific for MFS and are given extra weight in the diagnostic evaluation (termed **Major Manifestations**). In selected organ systems a single major manifestation is sufficient to determine that a **Major Criterion** for the diagnosis of MFS has been satisfied (e.g. eye, cardiovascular system, and dura). In the skeleton, one must observe at least 4 of 8 specific major manifestations to determine that a major criterion has been satisfied (see below). If findings in an organ system are insufficient to meet a major criterion, a separate set of rules can be applied to determine if that organ system can be considered "**Involved**" for diagnostic purposes. This can include consideration of **Minor Manifestations** that are less specific for MFS.

A major criterion can be established in any (or all) of four organ systems:

Skeletal:	- 4 of 8 specific major manifestations
Ocular:	- ectopia lentis
Cardiovascular:	- dilatation of the ascending aorta involving the sinuses of Valsalva and/or dissection of the ascending aorta
Dura	- dural ectasia

A **single** major criterion can also be established in family or genetic history by any 1 of the following 3 observations:

- a first degree relative who independently meets the diagnostic criteria
- presence of a mutation in *FBN1* that is known to cause MFS or that involves a highly conserved amino acid
- presence of a haplotype around *FBN1* that is inherited by descent and unequivocally associated with MFS in the family

Minor Manifestations: The minimal requirements for designation of a system as "involved" are detailed by system in the diagnostic worksheet below.

The diagnostic rules are summarized below.

For the index case:

- If family or genetic history is not contributory, major criteria in at least 2 different organ systems AND involvement of a third organ system.
- If a mutation known to cause MFS in others or a mutation involving a highly conserved amino acid is detected, one major criterion in an organ system AND involvement of a second organ system.

For a relative of an index case:

- Presence of a major criterion in the family or genetic history AND one major criterion in an organ system AND involvement in second organ system.

Of note, if an organ system satisfies a major criterion, it can also be considered "involved" for diagnostic purposes. **Any organ system can only be used once.** Thus, if a major criterion is satisfied in 3 organ systems, 2 can be used for major criteria and the 3rd can be considered as "involved". Finally, there are two syndromes with phenotypes that overlap with MFS, Shprintzen-Goldberg syndrome (SGS) [2] and Loeys-Dietz syndrome (LDS) [3], which need to be excluded. These syndromes are described in more detail in the worksheet below.

Diagnostic Worksheet for Marfan syndrome by Ghent Criteria

Identifier _____

Date _____

Examiner _____

Age (years/months)	__ __ / __ __	Arm span (cm)	__ __ __ . __
Height (cm)	__ __ __ . __	Lower segment[1] (cm)	__ __ __ . __
Weight (kg)	__ __ __ . __	Upper segment[2] (cm)	__ __ __ . __
BSA (m^2)	__ . __	Upper-to-lower segment ratio	__ . __ __
		Arm span-to-height ratio	__ . __ __

1. Skeletal system

Major manifestations:

Pectus carinatum	yes / no / unknown
Pectus excavatum (subjectively judged as moderate-severe)	yes / no / unknown
Reduced upper-to-lower segment ratio for age[3] OR arm span-to-height ratio greater than 1.05	yes / no / unknown
Wrist[4] AND thumb[5] signs	yes / no / unknown
Scoliosis > 20^o OR spondylolisthesis	yes / no / unknown
Reduced extension at the elbow (<170^o)	yes / no / unknown
Medial rotation of the medial malleolus causing pes planus	yes / no / unknown
Protrusio acetabuli of any degree (ascertained on radiographs)	yes / no / unknown

Minor manifestations:

Pectus excavatum (mild)	yes / no / unknown
Joint hypermobility	yes / no / unknown
Highly arched palate	yes / no / unknown
Facial	
Dolichocephaly	yes / no / unknown
Malar hypoplasia	yes / no / unknown
Enophthalmos	yes / no / unknown
Retrognathia	yes / no / unknown
Down-slanting palpebral fissures	yes / no / unknown

Major criterion in skeletal system (at least 4 major manifestations)? **yes / no**

If no, criteria met for skeletal system involvement (at least 2 major manifestations OR 1 major manifestation and at least 2 minor)? **yes / no**

[1] Top of symphysis pubis to floor (standing); estimation in supine position for infants

[2] Height minus lower segment

[3]

Age	Abnormal Ratio
0-1 yr	< 1.5
1-2 yrs	< 1.4
2-3 yrs	< 1.3
3-4 yrs	< 1.2
4-5 yrs	< 1.1
5-6 yrs	< 1.0
6-7 yrs	< 0.95
7-8 yrs	< 0.90
> 8 yrs	< 0.85

[4] Wrist sign - thumb overlaps the entire distal phalynx of the 5th digit when wrapped around the contralateral wrist

[5] Thumb sign – entire thumb nail protrudes beyond the ulnar border when the thumb is closed within a fist (without assistance)

2. Ocular system

Major manifestation

Ectopia lentis — yes / no / unknown

Minor manifestations:

Flat cornea — yes / no / unknown

Increased axial length of the globe — yes / no / unknown

Hypoplastic iris OR hypoplastic ciliary muscle causing decreased miosis — yes / no / unknown

Major criterion in ocular system system (1 major manifestation)? **yes / no**

If no, criteria met for ocular system involvement (at least 2 minor)? **yes / no**

3. Cardiovascular system

Aortic root (cm)	_._ _	Aortic root Z-score	_._ _
Pulmonary root (cm)	_._ _	Pulmonary root Z-score	_._ _

Major manifestations:

Dilation of the ascending aorta at the Sinuses of Valsalva (Z-score > 2.0)	yes / no / unknown
Dissection of the ascending aorta	yes / no / unknown

Minor manifestations:

Mitral valve prolapse	yes / no / unknown
Dilatation of the main pulmonary artery (Z-score > 2.0) (in the absence of valvular or peripheral stenosis)	yes / no / unknown
Calcification of the mitral annulus	yes / no / unknown
Dilatation or dissection of the descending aorta	yes / no / unknown

Major criterion in cardiovascular system (at least 1 major manifestations)? yes / no

If no, criteria met for cardiovascular system involvement (at least 1 minor manifestation)? yes / no

4. Pulmonary system

Minor manifestations:

Spontaneous pneumothorax	yes / no / unknown
Apical blebs (by chest X-ray CT or MRI)	yes / no / unknown

Criteria met for pulmonary system involvement (at least 1 manifestation)? yes / no

5. Skin and Integument

Minor manifestations:

Striae distensae	yes / no / unknown
Recurrent or incisional hernia	yes / no / unknown

Criteria met for skin/integument involvement (at least 1 manifestation)? yes / no

6. Dura

Major manifestation:

Lumbrosacral dural ectasia (by CT or MRI)	yes / no / unknown

Major criterion in dura (1 major manifestation)? yes / no

7. Family and genetic history

Major findings:

First degree relative who independently meets the diagnostic criteria?	yes / no / unknown
Presence of a mutation in *FBN1* known to cause Marfan syndrome?	yes / no / unknown
Presence of a haplotype around *FBN1* that is inherited by descent and unequivocally associated with Marfan syndrome in the family?	yes / no / unknown

Major criterion in family/genetic history (at least 1 major finding)? yes / no

8. Exclusion of other disorders:

While the differential diagnosis of Marfan syndrome is extensive, there are only two disorders that can show sufficient manifestations to meet diagnostic criteria for Marfan syndrome (Shprintzen-Goldberg syndrome or SGS [2]; Loeys-Dietz syndrome or LDS [3]). They show a very different natural history and need to be excluded from this study. Fortunately, both conditions show manifestations that are not seen in Marfan syndrome. Observation of any of the following findings will preclude participation, unless the site investigators can provide information to the Study Chairs that would support a diagnosis of MFS rather than SGS or LDS (e.g., a history of ectopia lentis, *FBN1* mutation status, etc.).

Craniosynostosis (SGS and LDS)	yes / no
Hypertelorism (SGS and LDS)	yes / no
Mental retardation (SGS and LDS)	yes / no
Cleft palate (LDS)	yes / no
Bifid uvula (split or central raphe) (LDS)	yes / no
Club foot (SGS and LDS)	yes / no
Documented arterial tortuosity (prominently of neck and head vessels) (LDS)	yes / no
Chiari malformation (SGS and LDS)	yes / no
Cervical spine instability (LDS)	yes / no

If any of these manifestations has been observed, the patient is not eligible for the study. The diagnostic evaluation is complete. Proceed to number 10. If none are observed, proceed to number 9.

9. Decision tree:

Major criteria in:		***Involvement of:***	
Skeletal system	yes / no	Skeletal system	yes / no
Ocular system	yes / no	Ocular system	yes / no
Cardiovascular system	yes / no	Cardiovascular system	yes / no
Dura	yes/ no	Pulmonary system	yes / no
Family/genetic history	yes / no	Skin and integument	yes / no

For index case:

Major criteria in 3 organ systems **yes / no**

Major criteria in 2 organ systems AND involvement of a 3rd system **yes / no**

Presence of an FBN1 mutation known to cause Marfan syndrome AND **yes / no**

Major criteria in 2 organ systems OR

Major criterion in 1 organ system with involvement of a 2nd system

If the answer for any of these questions in "yes", the patient satisfies diagnostic criteria for Marfan syndrome and is eligible for participation in this study. Proceed to number 10.

Relative of index case:

A major criterion in family/genetic history AND

Major criteria in 2 organ systems OR

Major criterion in 1 organ system with involvement of a 2nd system **yes / no**

If the answer is "yes", the patient satisfies diagnostic criteria for Marfan syndrome and is eligible for participation in this study. Proceed to number 10.

10. Patient is eligible for study **yes / no**

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APPENDIX E

DRUG PREPARATIONS

APPENDIX E. DRUG PREPARATIONS

Atenolol

Commercially available atenolol tablets, 25 mg, 50, and 100 mg, will be used in this trial. The 25 mg tablet will be cut in half to provide 12.5 mg doses. For young children and older patients who cannot or prefer not to take tablets, a 2 mg/mL suspension will be used.

Preparation of Atenolol Suspension (for 200 mL of a 2 mg/mL suspension) [1, 2]

Ingredients:

Atenolol tablets 50 mg		#8
Ora-Sweet Sugar Free SF™	QS	200 mL

Directions:

1) Crush and grind tablets in a mortar to a fine powder.
2) Once a fine powder consistency is achieved, add a small amount glycerine and triturate the drug and glycerine. After a fine slurry is achieved, Ora-Sweet Sugar Free™ will be added and mixed to a pouring consistency. This new pourable slurry will be transferred to a graduated cylinder.
3) Add more Ora-Sweet Sugar Free™ to the mortar and triturate any remaining drug/slurry to rinse the drug from the mortar and pestle. Add this new slurry to the graduated cylinder. Repeat this process two more times until the mortar and pestle are free from drug/slurry residue. Be sure to add the rinsing material to the graduated cylinder..
4) Add more Ora-Sweet Sugar Free™ to the mortar and triturate any remaining drug/slurry to rinse the drug from the mortar and pestle. Add this new slurry to the graduated cylinder. Repeat this process two more times until the mortar and pestle are free from drug/slurry residue. Be sure to add the rinsing material to the graduated cylinder.
5) QS to the final volume using Ora-Sweet Sugar Free™. Pour suspension from the graduated cylinder into an amber vial and shake well.
6) Rinse the sides of the graduated cylinder with this mixture several times until the consistency of the suspension on the sides of the cylinder is the same as in the amber bottle.

7) Place in amber polyethylene terephthalate (PET) bottle and label. Labeling: Refrigerate. Shake well before each use. Stable for 28 days.

Both suspensions will be dispensed in amber PET bottles, and amber syringes will be provided by the local pharmacy in an effort to maintain subject blinding.

Losartan

Clinical images (unmarked, plain white versions) of COZAAR® (Losartan Potassium Tablets), 12.5 mg, 25 mg, and 50 mg, will be used in this trial. For young children and older patients who cannot or prefer not to take tablets, a 2.5 mg/mL suspension will be used.

Preparation of Losartan Suspension (for 200 mL of a 2.5 mg/mL suspension)

Ingredients:

Losartan tablets 50 mg	#10
Purified Water, USP	10 mL
*Ora-Plus™	95 mL
*Ora-Sweet Sugar Free SF™	95 mL

*Ora-Blend SF™ is a 50/50 mixture of Ora-Plus™ and Ora-Sweet Sugar Free SF™ and may be substituted.

Directions:

1) Add 10 mL of Purified Water USP to an 8 ounce (240 mL) amber polyethylene terephthalate (PET) bottle containing ten 50 mg COZAAR® tablets.
2) Immediately shake for at least 2 minutes.
3) Let the concentrate stand for 1 hour and then shake for 1 minute to disperse the tablet contents.
4) Separately prepare a 50/50 volumetric mixture of Ora-Plus™ and Ora-Sweet SF™.
5) Add 190 mL of the 50/50 Ora-Plus™/Ora-Sweet SF™ mixture to the tablet and water slurry in the PET bottle and shake for 1 minute to disperse the ingredients.
6) The suspension should be refrigerated at 2-8˚C (36-46˚F) and can be stored for up to 4 weeks.
7) Shake the suspension prior to each use and return promptly to the refrigerator.
8) Labeling: Refrigerate. Shake well before each use. [3].

REFERENCES

1. Nahata, M.C., Hipple, T.F., *Pediatric Drug Formulations*. 2000, Cincinnati, OH: Harvey Whitney Books.
2. Patel D, Doshi DH, Desai A. *Short Term Stability of Atenolol in Oral Liquid Formulations*. IJPC; Nov/Dec 1997;1(6): p. 437-9.
3. Merck & Co., I., *COZAAR® package insert*, Merck & Co., Inc.