Mechanisms of aortic aneurysm formation: translating preclinical studies into clinical therapies

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ABSTRACT
Aneurysms are common in the abdominal and thoracic regions of the aorta. While generally asymptomatic, progression of aneurysms is associated with the devastating consequences of aortic rupture. Current therapeutic options to prevent rupture are restricted to surgical repair, as there remains a lack of validated pharmaceutical approaches. Absence of proven medical therapies may be a consequence of the paucity of knowledge on mechanisms of aneurysmal initiation, progression and rupture. Many potential therapeutic targets have been identified in several widely used animal models of these diseases. A small number of these targets are currently under clinical evaluation, while many more are in preclinical stages of evaluation. The purpose of this review is to: (1) overview current understanding of mechanisms of aneurysmal initiation and progression and (2) summarise medical therapies that have been investigated clinically, as well as highlight future therapeutic targets.

INTRODUCTION
Aortic aneurysms are a relatively common disease characterised by permanent dilation of the aorta that have region specificity. Abdominal aortic aneurysms (AAA) are the most common form of this disease with dilation typically presenting in the infrarenal region. Population ultrasound screening studies have determined that the prevalence of AAAs is 4–7% in males over the age of 65, and 1–2% in females, with some studies indicating AAA incidence is declining. For AAAs, the incidence increases with age, male gender and smoking. Aneurysms also occur in the thoracic region (thoracic aortic aneurysms; TAA), and can be present at a much earlier age than AAAs, while having similar incidence in both genders. TAAs are less common than AAAs, occurring at a rate of 4.5–5.9/100 000 person years. Aortic dilation in either region is largely asymptomatic, but increased size is associated with increased risk of rupture. Mortality following rupture is high; approximately 80% of those who reach the hospital and 50% of those who undergo surgery for a rupture will die.

Currently, the treatment of diagnosed aortic aneurysms is to temporally monitor the aortic dimensions and perform open or endovascular surgical repair when the diameter has attained sufficient expansion that portends a high likelihood of rupture. To overcome this reliance on surgical approaches, it would be preferable to have medical therapy directed at limiting the progressive aneurysm expansion. While there are guidelines for medical approaches to treat individuals with aneurysms, these guidelines are not based on substantial evidence of decreased expansion or rupture. The lack of effective therapies is associated with an inadequate understanding of the mechanisms that form AAAs and TAAs. Over the past decade, substantial work has been devoted to uncovering mechanisms underlying aneurysm formation. Through characterisation of surgical samples and animal models, a complex picture of cellular processes have been shown to play pivotal roles in aneurysmal disease. The current review focuses on recent scientific advances that provide mechanistic insights into AAAs and TAAs. Additionally, we will highlight preclinical and ongoing clinical trials that seek to uncover novel therapeutic strategies for the treatment of these conditions.

AAAS AND TAAS: SAME VESSEL, DIFFERENT PATHOPHYSIOLOGY
AAAs and TAAs are two vascular diseases that are distinguished by their different anatomical locations, and also by differing aetiologies. For example, AAAs are associated with the aged population, male gender and lifestyle-related risk factors, such as smoking, hypertension and hypercholesterolemia. At the pathological level, AAAs display destructive extracellular matrix remodelling, vascular smooth muscle cell (VSMC) apoptosis, and marked inflammatory cell infiltration. There is evidence of multifactorial, genetic determinants of AAAs with several genetic loci potentially implicated in different populations.

Conversely, TAAs occur frequently at younger ages without overt gender propensity, and have strong hereditary influence. Hereditary studies estimate that 20% of TAAs have a positive family history. Many presentations show classic Mendelian inheritance patterns, as exemplified by Marfan syndrome with mutations in fibrillin-1. Pathologically, inherited TAAs demonstrate elastin fragmentation, proliferation of VSMCs, and a less prominent leukocytic infiltration. Inherited TAAs can be subdivided into syndromic presentations that exhibit prominent features of systemic connective tissue disease (such as Marfan syndrome and Loeys-Dietz syndrome (LDS)) and non-syndromic presentations, such as bicuspid aortic valve with TAA and isolated familial TAA.

The aetiological differences of AAAs and TAAs may be partially attributable to region-specific diversity in embryological origins of VSMCs. The abdominal aorta originates from the paraxial mesoderm-derived somites, while the ascending aorta arises from neural ectoderm-derived progenitors of the cardiac neural crest. Other contributing factors may also relate to the differing haemodynamic forces placed on regions of the aorta, or the progressive decrease in collagen to elastin ratio.
from proximal to distal along the aorta. Given the aetiological differences between AAAs and TAAs, underlying mechanisms that contribute to disease development and subsequent progression vary in a region-specific manner.

**CELLULAR AND MOLECULAR MECHANISMS OF AAAs**

Histological analyses of human AAA surgical samples have revealed leukocytic infiltration, degradation of extracellular matrix, and depletion of VSMCs as three pathological hallmarks of AAAs. Despite the importance of these initial human studies in understanding characteristics of aneurysmal disease, surgically excised AAA tissue is only acquired at an advanced stage, which does not provide information related to initiation or maturation.

Given the difficulty of defining mechanisms from human AAAs, research has relied heavily on the use of animal models. The three most commonly used mouse AAA models are: adventitial exposure to calcium chloride, transient perfusion of elastase into the infrarenal aorta, and chronic subcutaneous infusion of angiotensin II (AngII). Lessons learned from these animal models have indicated that aneurysm development involves local inflammatory responses leading to infiltration of macrophages, neutrophils, mast cells, and T and B lymphocytes. This inflammatory response is further augmented by various cytokines and extracellular proteases which, in combination, lead to VSMC apoptosis and extracellular matrix degradation (figure 1). Many of these mechanisms have been reviewed previously. In this section, we will focus primarily on recent findings regarding mechanisms of AAA formation.

### Leukocytes and mast cells

Many different types of leukocytes have been detected in AAA tissues. One of the most prominent cell types present in aortic media and adventitia of AAAs is macrophages. C-C chemokine receptor type 2 (CCR2) and myeloid differentiation factor 88 (MyD88) are important for macrophage-mediated response to inflammation. CCR2 deficiency in mice attenuates AngII-induced and calcium chloride-induced AAAs. Likewise, deficiency of MyD88 in macrophages reduced AAA formation in the AngII mouse model. Although there is compelling evidence that the presence and function of macrophages are critical for AAA development, their contribution to the disease has not been fully defined. It is currently unclear whether specific targeting of macrophage function attenuates AAAs in humans.

Neutrophils are also present in experimental and human AAA tissue. Trafficking of neutrophils from plasma into the aortic wall is mediated through the L-selectin adhesion molecule. Pharmacological and genetic inhibition of L-selectin-attenuated AAA formation in the elastase mouse model.

In addition to leukocytes, mast cells, which are known to synthesise and release multiple inflammatory factors and extracellular proteases, have been identified in human AAA tissue. Genetic depletion or pharmacological inhibition of mast cells prevented AAA formation in mouse and rat models.

### Chemokines and cytokines

A number of proinflammatory and anti-inflammatory cytokines controlling leukocyte recruitment and functions have been implicated in AAAs. Among the cytokines upregulated in plasma of patients with AAAs is tumour necrosis factor (TNF-α). Effects of TNF-α on experimental AAA differs between mouse models with genetic deficiency and pharmacological inhibition of TNF-α attenuating calcium chloride-induced AAAs; while p55 TNF-α receptor deficiency had no significant effect on AngII-induced AAA formation in LDL-receptor deficient mice.

Another cytokine that plays a critical role in inflammatory processes is transforming growth factor (TGF)-β. Although it has been most extensively studied in TAAs, TGF-β may also have a mechanistic role in AAAs. Systemic blockade of TGF-β activity was found to augment AngII-induced AAAs and increase VSMC death, elastin fragmentation and aortic rupture in mice. Furthermore, in a rat model with xenograft transplant aneurysms located in the infrarenal aorta, TGF-β overexpression via endovascular delivery of an adenoviral construct stabilised pre-existing aneurysms. In addition to directly intervening on TGF-β activity, several drugs are known to affect TGF-β expression. For example, cyclosporine induces TGF-β expression and its administration attenuated AAA formation in rat elastase and mouse calcium chloride models of AAA.

Finally, interleukins (IL) are known to be important regulators of inflammation and cellular apoptosis during chronic inflammatory diseases. Within human AAAs, many ILs, including IL-1β, IL-6, IL-17, and IL-23, have increased abundance in comparison with healthy aortic tissue. Furthermore, plasma concentrations of IL-1β are also increased nearly 10-fold in AAA patients. Genetic depletion of IL-17 and IL-23 in mice markedly attenuated elastase-induced AAA. Likewise, genetic depletion of IL-1β or pharmacological inhibition of IL-1β receptor prevented elastase-induced AAA formation.

### Proteases

Elastin and collagen are key structural proteins that maintain integrity of the aortic wall. Numerous studies suggest that degradation of these proteins in the medial layer leads to weakening and dilation of the aortic lumen. A wide array of proteases and their inhibitors have been implicated within AAA formation and progression, including: matrix metalloproteinases (MMP), serine proteases and cysteine proteases. The most extensively researched class of proteases are MMPs, a zinc endopeptidase family. In rodent models, the most prominently researched MMP is MMP-9. Mice deficient in this specific protease had attenuation of elastase-induced and calcium chloride-induced AAAs. Furthermore, infusion of macrophages from wild-type mice reversed the protection of MMP-9 deficiency in the calcium chloride model, indicating that macrophage-derived MMP-9 is required for AAA development. Additionally, several other MMPs have been studied in genetically deficient mice. Deficiency of macrophage-derived MMP-2 attenuated calcium chloride-induced AAAs; while MMP-12 deficiency in mice reduced calcium chloride-induced AAAs, but had no affect on elastase-induced AAAs in mice.

The validation of MMPs as critical components of AAA pathology is compromised by the lack of a specific inhibitor. Doxycycline, a broad spectrum MMP inhibitor, has been investigated extensively as a potential medical approach to prevent AAA progression. In animal models, doxycycline prevented initiation of elastase-induced; calcium chloride-induced, and AngII-induced AAAs. However, not all studies have demonstrated doxycycline prevented AngII-induced AAAs. Also, doxycycline failed to attenuate aneurysm growth or risk of rupture in mice with established AAAs.

### Renin-angiotensin system

Animal experiments have reported consistently a relationship between the renin-angiotensin system (RAS) and aneurysm development. The most direct implication of a role of RAS is the demonstration of AAA formation during subcutaneous...
infusion of AngII into normo- and hypercholesterolemic mice. AngII-induced aneurysms exhibit a regional and temporal heterogeneity characterised by initial inflammation, followed by elastin degradation with focal and transmural medial rupture leading to lumen expansion. To further define mechanism, mice with cell-specific depletion of AT1a receptors have been created. However, depletion of AT1a receptors in two major cell types of the aortic wall, endothelium and SMCs, failed to have any effect on AngII-induced AAAs. Other mouse models have also supported a mechanistic role of RAS, as deficiency of AT1a receptor attenuates elastase-induced aortic expansion. Inhibition of the RAS has been investigated extensively as a potential treatment of AAAs. In rodent models of AAA, including those relying on Ang II or employing chemical agents, AT1 receptor blockers (ARBs) inhibit aneurysm development. Additionally, captopril, an ACE inhibitor, prevents aneurysm formation in a rat elastase model.  

**CURRENT AND EVOLVING MEDICAL THERAPY FOR HUMAN AAAS**

The current management of AAAs is primary determined by the absolute size or percent dilation of the aorta. However, normal aortic dimensions have many variables, such as physical size, age and gender that compromise the designation of an aneurysm based on arbitrary size or percentage. Even after accounts of these caveats, management of most people with aneurysms in the diameter range of 3–5.5 cm involves watchful waiting. Once the risk of rupture exceeds the risk of surgery, patients may undergo surgical repair. The hope of medical treatment is that it would delay, or even arrest AAA growth, to prevent the need for invasive operations. Over the years, a number of cardiovascular medications have attracted interest as potential therapeutic strategies. Yet, the current level of evidence for medical treatment to prevent expansion or rupture of AAAs remains minimal (see online supplementary table S1). Currently, there have been relatively few completed double-blinded clinical trials to determine the efficacy of medical intervention on AAAs in humans. A detailed description of many of these prior clinical trials on β blockade, statin therapy, and antiplatelet medication have been reviewed previously. To date, no medical intervention has been shown to positively alter the course of AAA progression.

In this section, we will focus on ongoing clinical trials that have sought to translate preclinical findings into clinical therapies. In humans, MMP-9 is the most frequently studied protease. A recent meta-analysis revealed significantly higher plasma

**Figure 1** Pathophysiology and therapeutic targets of AAA. Progressive dilation of the aortic wall is associated with recruitment of leukocytes, macrophage activation, and production of proinflammatory cytokines. Over a period of years, apoptosis and cellular senescence of smooth muscle cells occurs in conjunction with infiltration of lymphocytes, mast cells and neutrophils. Macrophages and SMCs also produce proenzyme forms of proteases that are activated in the extracellular space and degrade extracellular matrix proteins (elastin and interstitial collagens). Adventitial fibroblasts are presumed to promote structural repair, however, the interstitial collagen becomes disorganised. The table legend illustrates major cell types involved in AAA pathogenesis, as well as selected examples of future therapeutic targets that are involved in AAA pathogenesis. VSMC, vascular smooth muscle cell; TGF-β, transforming growth factor-β; TNF, tumour necrosis factor; IL, interleukin; IFN, interferon; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; miR, microRNA; RAS, renin-angiotensin system; AT1R, angiotensin type 1 receptor; AAA, abdominal aortic aneurysm.
MMP-9 concentrations in patients with AAAs. Clinical studies investigating MMP inhibition demonstrated that patients receiving doxycycline for 2 weeks prior to surgical intervention had reduced inflammation in AAA biopsies than patients receiving placebo. A small randomised trial of 32 patients with small AAAs found a reduction in aneurysm expansion with doxycycline, but only after exclusion of the first 6 months of follow-up. However, the small size of this study, combined with the doxycycline group having fewer smokers, failed to justify potential benefits of doxycycline therapy. Recently, a much larger randomised trial of 286 patients surprisingly found that doxycycline treatment significantly increased AA expansion. Although further validation of doxycycline as a treatment for AAAs is ongoing in an additional randomised control trial (Non-Invasive Treatment of Abdominal Aortic Aneurysm Clinical Trial (NCT01756833)), the most recently completed trial firmly challenges its therapeutic potential (table 1).

A number of retrospective clinical studies have investigated the efficacy of ACE inhibitors or ARBs in limiting AAA progression. Within these studies, ACE inhibitors have yielded conflicting data. Hackam et al demonstrated a reduced likelihood of rupture in patients who had a prescription for ACE inhibitors; while the UK small AAA group reported an increased AAA growth rate in patients with ACE inhibition. Retrospective ARB studies have been associated with a reduction in AAA progression. Currently, there are several large randomised control trials ongoing that are designed to investigate the effects of ACE inhibition (AARDVARK (NCT01118520)) or ARBs (TEDY (NCT01683084); and NCT01904981) on AAA growth rate (table 1).

**POTENTIAL FUTURE THERAPEUTIC STRATEGIES FOR AAAS**

Numerous pharmacological therapies are being investigated as potential novel treatments for AAAs in preclinical studies (table 2). Given the predominance of inflammation in AAAs, several studies have attempted to attenuate aneurysm formation by targeting inflammatory cell types including macrophages and T lymphocytes. Other studies have targeted inflammatory regulators, including ILs, TNF-α, and TGF-β. However, many of the therapies used in these animals are not approved for human use. In an attempt to expedite the translation of preclinical findings, several studies examined the effects of clinically approved immunosuppressive medications including cyclosporine, infliximab and azathioprine on AAA formation. Despite promising results from these studies in attenuating experimental AAA formation, potential translation of these anti-inflammatory medications to treatment of human AAAs should be approached with caution, and considered in the context of their broader immunosuppressive capabilities. As an illustration, despite cyclosporine attenuation of experimental AAAs, a recent case report demonstrated rapid dilation of an AAA in a patient receiving immunosuppressive dosages of cyclosporine.

Traditional therapeutic approaches, such as those detailed above, typically interact with specific cellular targets. Given that AAA formation involves a complex interaction of inflammation and extracellular matrix degradation, a novel therapeutic approach to management of AAAs would modulate extensive functional networks. This has been demonstrated recently through use of microRNAs. MicroRNAs, which are small, non-coding RNAs, have emerged as key post-transcriptional regulators for a large number of genes and physiological processes occurring during AAA development. Two microRNAs implicated in aneurysmal disease are miR-21, which is highly expressed in VSMCs, and miR-29b, which targets gene transcripts of extracellular matrix proteins. Overexpression of miR-21 decreased AAAs induced by either elastase or AngII as well as prevented smooth muscle cell apoptosis. By contrast, inhibition of miR-29b reduced aortic dilation in elastase and AngII-induced aortic aneurysms. The varying effects of overexpression or inhibition of microRNAs on AAA formation relate to the downstream gene targets of each specific microRNA. This is highlighted further in miR-712, which is derived from vascular endothelium, and contributes to AAA formation by targeting and preventing translation of endogenous inhibitors of MMPs. This subsequently produces overactivation of proteases. Silencing of miR-712 in AngII-infused mice decreased MMP activity, leukocyte accumulation and aneurysm formation. Currently, it is unclear if manipulation of microRNAs represent a viable therapeutic strategy for human AAAs. Challenges remain in microRNA manipulation, such as

### Table 1 Ongoing clinical trials investigating medical management of AAAs

<table>
<thead>
<tr>
<th>Trial registration</th>
<th>Study phase</th>
<th>Study size</th>
<th>Intervention</th>
<th>Primary outcome</th>
<th>Secondary outcome</th>
<th>Estimated completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflammatory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02007252</td>
<td>Phase 2</td>
<td>100</td>
<td>Subcutaneous ACZ885 vs placebo</td>
<td>Growth rate of AAA by U/S</td>
<td>Adverse events of ACZ885</td>
<td>2015</td>
</tr>
<tr>
<td>Renin angiotensin system</td>
<td>Phase 4</td>
<td>40</td>
<td>Telmisartan (40 mg daily) vs placebo</td>
<td>Growth rate of AAA by CTA</td>
<td>AAA biomarkers, quality of life, change in distensibility</td>
<td>2015</td>
</tr>
<tr>
<td>NCT01683084</td>
<td>Phase 4</td>
<td>225</td>
<td>Perindopril (10 mg daily) vs amlodipine (5 mg daily) vs placebo</td>
<td>Growth rate of AAA by U/S</td>
<td>Mortality, quality of life</td>
<td>2014</td>
</tr>
<tr>
<td>NCT01118520</td>
<td>Phase 4</td>
<td>400</td>
<td>Valsartan (80 mg daily) vs atenolol (50 mg daily)</td>
<td>Growth rate of AAA by CT</td>
<td>Mortality, quality of life</td>
<td>2015</td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>Phase 2</td>
<td>140</td>
<td>Ticagrelor (90 mg twice daily) vs placebo</td>
<td>AAA volume growth by MRI</td>
<td>Mean reduction in diameter growth rate, operation rate</td>
<td>2015</td>
</tr>
<tr>
<td>Protease inhibition</td>
<td>Phase 2</td>
<td>248</td>
<td>Doxycycline (100 mg twice daily) vs placebo</td>
<td>Growth of AAA by CT</td>
<td>MMP plasma levels, quality of life, AAA biomarker</td>
<td>2016</td>
</tr>
</tbody>
</table>

Trials listed above are in compliance with time frame for updating registration records as specified by the FDA. AAA, Abdominal aortic aneurysm; CTA, CT angiography; MMP, matrix metalloproteinases; U/S, ultrasound.
potential off-target effects and a need for local or cell type-specific delivery mechanisms prior to deployment of a clinically feasible therapy.\textsuperscript{5,50}

In an attempt to understand mechanisms underlying AAs, many preclinical studies have focused on therapeutic options that prevent AAA formation. However, in the clinical setting, therapies are only initiated in patients with established AAs. Given the recent failure of doxycycline to prevent AAA progression in humans,\textsuperscript{17} it is unclear whether continued AAA expan-

### Cellular signalling

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Target</th>
<th>Intervention: mechanism</th>
<th>Response to AAA in rodent model</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-JNK</td>
<td>TNF</td>
<td>Infliximab: monoclonal antibody against TNF-α\textsuperscript{21}</td>
<td>↓</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Polyclonal anti-TGF-β neutralising antibody\textsuperscript{11}</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>PPARy</td>
<td>Cyclosporine: immunosuppressive that also induces TGF-β signalling\textsuperscript{524}</td>
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### ECM degradation

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<th>Pathway</th>
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<th>Response to AAA in rodent model</th>
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</thead>
<tbody>
<tr>
<td>MMP</td>
<td>Doxycycline: non-selective MMP antagonist.</td>
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</table>

### MicoRNAs

<table>
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<th>Pathway</th>
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<th>Response to AAA in rodent model</th>
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</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>Anti-miR-21: miR-21 antagonist\textsuperscript{49}</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>miR-29b</td>
<td>Lentiviral vector of miR-29b: systemic viral vector that overexpresses miR-29b\textsuperscript{571}</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>miR-712</td>
<td>Anti-miR-712: miR-712 antagonist\textsuperscript{22}</td>
<td>↑</td>
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</table>

### Phospholipid components

<table>
<thead>
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<th>Pathway</th>
<th>Target</th>
<th>Intervention: mechanism</th>
<th>Response to AAA in rodent model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclooxygenase-2</td>
<td>Celecoxib: selective COX-2 inhibitor\textsuperscript{23}</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Prostanoid Receptor EP4</td>
<td>ONO-AE-208: a prostanoid receptor antagonist\textsuperscript{274}</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>5-lipoxygenase</td>
<td>LP105: pirenixic acid derivative; inhibitor of 5-LOX\textsuperscript{275}</td>
<td>↓</td>
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</tr>
</tbody>
</table>

AAA, Abdominal aortic aneurysm; CCLS, C-C motif ligand 5; COX-2, cyclooxygenase-2; CXCL4, chemokine (C-X-C motif) ligand 4; CXCR4, chemokine (C-X-C motif) receptor 4; ECM, extracellular matrix; IL, interleukins; JNK, Jun N-terminal kinase; miR, microRNA; MMP, matrix metalloproteinases; mTOR, mammalian target of rapamycin; PPAR, Peroxisome proliferator-activated receptor; TNF, tumour necrosis factor; 5-LOX, 5-lipoxygenase.

### Cellular and Molecular Mechanisms of TAA S

TAA S can be classified into four main categories: ascending aortic aneurysms (60%), aortic arch aneurysms (40%), and thoracoabdominal aortic aneurysms (10%), with some aneurysms developing in multiple locations.\textsuperscript{24} TAA S can also result from aneurysmal degeneration of chronic aortic dissection for which a detailed account on mechanisms of formation and therapeutic options has been reviewed.\textsuperscript{51} Given that TAA S are predominantly located in the ascending aorta, this section will focus on mechanisms within this region.

The aetiologies underlying ascending aortic aneurysms are diverse and range from degenerative or hypertensive associated aortic enlargement to less common genetic disorders, such as Marfan syndrome, Ehlers-Danlos, and other syndromic connective tissue diseases. TAA tissue samples are characterised by...
degradation of the extracellular matrix in conjunction with smooth muscle cell apoptosis and a less prominent inflammatory component. National registries, such as Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC), have provided important resources for identifying many of the pathways that contribute to thoracic aortic aneurysm formation. Over the past two decades, a variety of genetically engineered and chemically produced animal models of TAAs have provided mechanistic insight into aberrant signalling pathways that contribute to ascending aortic aneurysm pathogenesis.

**Transforming growth factor-β signalling and the extracellular matrix**

TGF-β is a multifunctional cytokine that is essential for tissue morphogenesis and homeostasis. It is secreted from a variety of cell types as a large latent complex that includes fibrillin-1. Upon release from the latent complex and activation, TGF-β binds to its receptors and signal transduction is mediated through phosphorylation of Smad 2/3 proteins (Smad is an abbreviation of mothers against decapentaplegic), leading to activation of numerous genes involved in extracellular matrix homeostasis. Additionally, multiple non-classical pathways (Smad independent) have also been implicated in TGF-β signalling, specifically the mitogen-activated protein kinase (MAPK) cascades (p38 and extracellular-signal regulated kinase (ERK)).

The most frequently used ascending aortic aneurysm mouse models have manipulations in fibrillin-1, the genetic determinant of Marfan syndrome. These manipulations in mice include hypomorphic expression of fibrillin-1 (Fbn1<sup>1mgR<sub>1mg</sub>β<sub>1mg</sub>β<sub>1mg</sub>β</sup>) or a mutant form of this protein derived from a C to G substitution at position 1039 (Fbn1<sup>1C1039G</sup>). Alterations in fibrillin-1 lead to an inability to effectively sequester latent TGF-β, thereby resulting in overactivation of TGF-β signalling. Notably, this is in conflict with experimental AAA formation where decreased, rather than overactivation of TGF-β signalling increase disease. Within ascending aortic aneurysms, TGF-β activation of the classical Smad signalling pathway typically produces a profibrotic response; however, activation of the ERK1/2 pathway has been shown to be responsible for aortic dilation through enhancing synthesis of t-PA, MMP-2, MMP-9, and collagen propeptides, thereby inducing a proteolytic response.

The implication of TGF-β in ascending aneurysm pathogenesis is demonstrated further in LDS, which presents with a more aggressive form of ascending aortic dilation. Mutations in genes encoding either TGF-β receptor type I or type II are the genetic basis for LDS. Although TGF-β receptor mutations result in impaired function, detection of enhanced TGF-β driven gene products are observed in mouse models and surgical samples from LDS patients implying that TGF-β signalling is actually increased. The mechanism underlying this paradoxical effect remains unknown. Several other aneurysmal disorders have also been linked to alterations in the TGF-β pathway, including familial TAA and bicuspid aortic valve, with surgical samples demonstrating increased presence of downstream TGF-β signalling molecules.

**Renin-angiotensin system**

Another pathway that has been invoked as a major mediator of experimental ascending aortic aneurysms is the RAS. Chronic infusion of AngII in rodent studies promotes pathological dilation localised to the ascending portion of the thoracic aorta. Progressive dilation continues throughout AngII infusion and ruptures of the ascending aorta occur within the first 6–10 days. Unlike AngII-induced AAA pathology, changes in the ascending aorta are characterised by concentric medial thickening with elastin fragmentation and limited inflammatory cell infiltration.

AngII mediates its effects mainly through AT1 and AT2 receptors. It has been reported that AngII can activate intracellular Smad2 and MAPK signalling cascades producing upregulation of proteases and disruptions of extracellular matrix in a TGF-β independent manner. Direct evidence for a role of AngII in TAA development was gleaned from multiple ascending aortic animal models, where administration of losartan, an AT1 receptor antagonist, led to a complete attenuation of aortic aneurysm and preservation of vessel wall architecture. Clinical genetic association studies have also implicated the RAS within thoracic aneurysms, as polymorphisms in ACE have been shown to confer a risk of ascending aortic aneurysm.

**CURRENT AND EVOLVING MEDICAL THERAPY FOR HUMAN TAAS**

Current recommended therapy for TAAs is dependent on aneurysm-specific factors (size, growth rate) and patient-specific factors (comorbidities, presence of aneurysmal complications). Surgical intervention remains a definitive treatment to prevent rupture, with recent guidelines emphasising aortic size and aneurysmal aetiology as criteria for surgery. If aortic dilation does not meet criteria for intervention, medical management for TAAs revolves around stringent blood pressure control. Anti-hypertensives have been most extensively studied in the Marfan population, with findings of these investigations serving as prototypes for other ascending aortic aneurysms. β-blocker and ACE inhibitors have recently generated interest for their antihypertensive properties and their potential to intervene on disease pathogenesis (see online supplementary table SII). In this section, we focus on current clinical trials that seek to translate preclinical findings into clinical therapies.

Traditional medical therapy for ascending aortic aneurysms have focused on a generalised reduction of cardiovascular risk factors. A detailed account of previously completed clinical trials for ascending aortic aneurysms has been reviewed previously. More recently, experimental studies have targeted the underlying pathophysiology of ascending aortic aneurysms, thus modifying the disease process. In human clinical studies, a retrospective analysis of Marfan patients with ascending aneurysms demonstrated that administration of an AT1 receptor antagonist reduced aortic dilation. Currently, multiple large-scale clinical trials are ongoing, including the Study of the Efficacy of Losartan on Aortic Dilatation in Patients with Marfan Syndrome (MARFANSARTAN; NCT00763893) and the Oxford Marfan Trial (NCT01949233), which seek to evaluate whether AT1 receptor antagonism attenuates aortic expansion in Marfan patients. Clinical trials are also investigating the effects of AT1 receptor antagonism on preventing ascending aortic dilation and inflammatory markers in non-Marfan patients (NCT01202721; table 3).

**POTENTIAL FUTURE THERAPEUTIC STRATEGIES FOR TAAS**

Advances in understanding the pathogenesis of ascending aortic aneurysms at the genetic and molecular level have provided novel preclinical opportunities for approaching therapeutic treatment (table 4). TGF-β signalling has emerged as an important factor in aneurysm development in Marfan and non-Marfan
patients. Direct inhibition of this pathway by a polyclonal antibody that neutralises biological activity of TGF-β attenuate aortic dilation in mouse models.\(^{5,6}\) Recently, a number of early phase clinical trials have begun to investigate the safety and efficacy of TGF-β neutralising antibodies, specifically Fresolimumab, in cancer therapy (NCT01112293). If promising results occur within these trials, TAAs may be an additional avenue to investigate the use of TGF-β neutralisation. However, caution should be taken with using TGF-β neutralising antibodies, as the role of TGF-β signalling in the exacerbation or attenuation of other aortic diseases, specifically AAAs, remains unclear.\(^{11}\) Furthermore, a recent study with a TGF-β receptor conditional deletion demonstrated that a basal level of TGF-β signalling may be necessary to maintain aortic wall homeostasis and prevent aneurysmal disease.\(^{5,6}\) Additionally, little is known about the pathogenic sequence downstream of TGF-β that is involved in TAA progression. Most studies on TGF-β signalling have focused on the canonical (Smad dependent) signalling pathway with human TAA samples and mouse models demonstrating upregulation. More recently, there is emerging evidence that a non-canonical pathway specifically signalling through the ERK pathway is responsible for aortic dilation.\(^{5,5}\) Further investigation into the role of ERK signalling and potential inhibitors of this pathway may prove a valuable therapeutic target to attenuate TAA progression.

Another interesting therapeutic approach consists of antagonising the fibrillin-1 Gly-x-x-Pro-Gly derived fragments (GxxPG). Elastin and fibrillin-1 are dominant proteins in the tunica media of the aortic wall, and each contains multiple copies of GxxPG sequence. Fragments of these proteins containing the GxxPG motif induce a number of effects including macrophage chemotaxis and increased MMP activity. Antagonism of GxxPG fragments through injection of a motif-specific antibody ameliorate disease in the Marfan mouse model.\(^{6,5}\)

Finally, as with AAAs, microRNAs, specifically miR-29b have demonstrated promising results in the pathogenesis of early aneurysm development in the Marfan mouse model. Antagonism of miR-29b, through locked nucleic acid antisense oligonucleotides prevented aneurysm development, VSMC apoptosis, and extracellular matrix degeneration.\(^{5,6}\) As with all these approaches, they remain preliminary preclinical studies. Additional research will be required to assess the role in aortic tissue before development of clinical treatment strategies.

### Conclusion

AAAs and TAAs are an asymptomatic and potentially lethal disease. In recent years, a considerable increase in research on aneurysm pathogenesis has resulted in the discovery of novel mechanisms and implementation of clinical trials that seek to assess strategies that limit aneurysm expansion. Despite this progress, there remain many perplexities regarding exact mechanisms leading from aneurysm formation to progression and eventual rupture. This uncertainty highlights the importance of continual cooperation between preclinical and clinical researchers in validating findings from preclinical studies to human disease, in order to discover medical treatments that halt aneurysmal disease.

### Table 3

Ongoing clinical trials investigating medical management of ascending aortic aneurysms

<table>
<thead>
<tr>
<th>Trial registration</th>
<th>Study phase</th>
<th>Study size</th>
<th>Intervention</th>
<th>Primary outcome</th>
<th>Secondary outcome</th>
<th>Estimated completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01202721</td>
<td>Phase 3</td>
<td>416</td>
<td>Atenolol (25/50/100 mg) vs placebo; Telmisartan (40–80 mg) vs placebo; Atenolol plus Telmisartan vs double placebo</td>
<td>Ascending aorta by MRI</td>
<td>Rate of change measured by TTE</td>
<td>2016</td>
</tr>
<tr>
<td>NCT00763893</td>
<td>Phase 3</td>
<td>303</td>
<td>Losartan (50 mg daily), &lt;50 kg: 100 mg daily, &gt;50 kg vs placebo</td>
<td>Aortic sinus diameter</td>
<td>Cardiac surgery, mortality</td>
<td>2014</td>
</tr>
<tr>
<td>NCT01949233</td>
<td>Phase 2</td>
<td>56</td>
<td>Irbesartan (150–300 mg daily) vs doxycycline (100–200 mg daily) vs placebo</td>
<td>Aortic distensibility by CMR</td>
<td>Aortic dimensions, wall shear stress, serum TGF-β levels</td>
<td>2015</td>
</tr>
<tr>
<td>NCT00429364</td>
<td>Phase 3</td>
<td>604</td>
<td>Losartan (0.3–1.4 mg/kg) vs atenolol (0.5–4 mg/kg)</td>
<td>Rate of change in aortic root by echocardiography</td>
<td>AV regurgitation, mortality, adverse drug reactions</td>
<td>2014</td>
</tr>
<tr>
<td>NCT00683124</td>
<td>Phase 3</td>
<td>291</td>
<td>Losartan (100 mg daily) vs nebivolol (10 mg daily) vs losartan and nebivolol</td>
<td>Age-adjusted aortic root diameter by echocardiography</td>
<td>Serum TGF-β levels, LV EF, AV regurgitation</td>
<td>2014</td>
</tr>
<tr>
<td>NCT00782237</td>
<td>Phase 3</td>
<td>174</td>
<td>Losartan (50 mg daily, &lt;50 kg: 100 mg daily, &gt;50 kg) vs placebo</td>
<td>Aortic root growth by echocardiography</td>
<td>Aortic stiffness, LV function, surgery, mortality</td>
<td>2014</td>
</tr>
<tr>
<td>ISRCTN90011794</td>
<td>Phase 3</td>
<td>490</td>
<td>Irbesartan (75/150/300 mg) vs placebo</td>
<td>Aortic root growth by echocardiography</td>
<td>Mortality, LV function, surgery</td>
<td>2017</td>
</tr>
</tbody>
</table>

Trials listed above are in compliance with time frame for updating registration records as specified by the FDA.

AV, aortic valve; BAV, bicuspid aortic valve; CMR, Cardiac MRI; TGF-β, Transforming Growth Factor-β; TTE, transthoracic echocardiography.

### Table 4

Potential future pharmacological strategies for TAAs in preclinical investigation

<table>
<thead>
<tr>
<th>Cellular or molecular pathway</th>
<th>Target</th>
<th>Intervention: mechanism</th>
<th>Response on ascending aortic aneurysm in mouse model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular signalling</td>
<td>TGF-β</td>
<td>Polyclonal anti-TGF-β: neutralising antibody(^{6,5})</td>
<td>↓</td>
</tr>
<tr>
<td>Extracellular matrix</td>
<td>GxxPG fragments</td>
<td>Monoclonal antibody BA4: GxxPG specific antibody(^{6,5})</td>
<td>↓</td>
</tr>
<tr>
<td>MicroRNAs</td>
<td>miR-29b</td>
<td>anti-miR-29b: miR29b antagonist(^{5,6})</td>
<td>↓</td>
</tr>
</tbody>
</table>

GxxPG, Gly-x-x-Pro-Gly; miR, microRNA; TGF-β, transforming growth factor-β; TAA, thoracic aortic aneurysms.
Review

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Mechanisms of aortic aneurysm formation: translating preclinical studies into clinical therapies

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