New Therapeutic Targets in Cardiology

p38 Alpha Mitogen-Activated Protein Kinase for Ischemic Heart Disease

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The p38 mitogen-activated protein kinases (p38s) are members of a key signaling pathway that responds to varied stresses, including those that contribute to heart failure. This review will focus on the ways in which p38 can be manipulated based on its mechanisms of activation and structure and how this knowledge has led to current cardiovascular clinical trials.

Phosphorylation is the process through which extracellular signals are communicated to the interior of the cell and it is catalyzed by kinases. The protein kinases transfer the terminal phosphate group from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue. This posttranslational modification can transform the function of the substrate protein by changing its binding partners, subcellular location, stability, and/or activity. In turn, these transformations have an impact on diverse fundamental cellular processes to alter transcription, translation, metabolism, contractility, growth, death, and/or differentiation. The 518 known protein kinases occupy a relatively high proportion (1.7%) of the human proteome of which an even higher proportion (~30%) is modified by phosphorylation.

The dysregulation of kinases is a common feature of many cancers, because growth signals can be inappropriately amplified by mutations that render kinases constitutively active. Consequently, drugs to inhibit kinases are the most successful and rapidly growing of the recent advances in cancer therapy. This success has occurred despite initial concerns of the constraints imposed by the high degree of homology between the ATP binding sites of different protein kinases and of the need to compete with millimolar concentrations of ATP. Initial success was achieved with imatinib (Gleevec) that targets an abnormal fusion protein kinase formed in the translocation event that creates the Philadelphia Chromosome in chronic myeloid leukemia. Another early success was the targeting of the human epidermal growth factor receptor 2 tyrosine kinase that is abnormally active in at least 20% of breast cancers. Trastuzumab (Herceptin), the antibody-based human epidermal growth factor receptor 2 tyrosine kinase ligand, prevents downstream signaling. The major adverse event profile in early trials of imatinib and trastuzumab have illustrated the importance of kinase signaling to cardiovascular health. Based on this observed toxicity, the key question is whether the corollary is also true, namely, that the inhibition of some kinases brings cardiovascular benefits? If this is the case, the impact of kinase inhibitors on cardiovascular disease could be similarly transformational.

The p38 Mitogen-Activated Protein Kinase Family

Protein kinases are divided into 7 groups. One group phosphorylates tyrosine residues alone, whereas the other 6 groups are predominantly composed of serine/threonine kinases and include the mitogen-activated protein kinase (MAPK) family. The MAPKs comprise p38s, together with p42/44 (also known as extracellular signal-regulated kinases [ERKs] 1/2), c-jun N-terminal kinases, ERK5, and various atypical members (ERK3/4, ERK7, and Nemo-like kinase). The responses to extracellular stresses are predominantly mediated by p38s and c-jun N-terminal kinases, whereas the responses to growth factors/mitogens are predominantly mediated by ERKs.

The p38 family comprises 4 isoforms: p38α, p38β, p38γ, and p38δ. The ubiquitously expressed p38α and β isoforms are 74% identical. In contrast, the p38γ isoform, which is expressed in skeletal and cardiac muscle, and the p38δ isoform, which is expressed in lungs, kidney, testis, spleen, pancreas, and small intestine, are 60% identical to α. In common with other MAPK family members, p38s are activated by the phosphorylation of 2 residues in their activation loop by upstream MAPK kinases (MKKs). These upstream kinases are activated, in turn, by M KK kinases to form a canonical activation pathway/relay. At the end of this relay, the phosphoacceptors within the MAPK activation loop are a threonine (T) and a tyrosine (Y) residue separated by a single amino acid, which is glycine (G) in the case of p38α. In an inactive kinase, the activation loop occupies the substrate binding channel. The dual phosphorylation of the TGY motif within the activation loop results in local and distant conformational changes that coordinate kinase activation. Of the 4 isoforms, the cardiovascular importance of p38α is best recognized. Furthermore, this isoform is of greatest relevance to development because homozygous p38α knockout embryos die, whereas knockouts of the other isoforms, even in combination, result in a near-normal, adult phenotype.
For these reasons, the remainder of this review will concentrate on p38α.

**Mechanisms of p38α Activation**

As mentioned above, the classic mechanism of p38α activation is by a canonical pathway of 3 tiers of kinases with MKKs 3, 6, and 4 all capable of phosphorylating the TGY motif, their relative prominence perhaps varying by circumstance. For example, the tumor necrosis factor-α (TNF-α)–induced activation of p38α is mediated by MKK3. More recently, alternative activation pathways have been identified in which the catalytic activity of p38α is used to phosphorylate its own TGY motif (see Figure 1). These autophosphorylation mechanisms have 2 unusual features. The first is that p38α is a serine/threonine kinase, yet the TGY motif requires tyrosine phosphorylation. A similar situation arises with the autophosphorylations of glycogen synthase kinase 3β, dual-tyrosine–regulated kinases, and polo-like kinase 4 and is an enigma requiring further investigation. The second is that p38α is a serine/threonine kinase, yet the TGY motif requires tyrosine phosphorylation. A similar situation arises with the autophosphorylations of glycogen synthase kinase 3β, dual-tyrosine–regulated kinases, and polo-like kinase 4 and is an enigma requiring further investigation. Nonetheless, the autophosphorylation of p38α can be induced by the binding of a scaffold protein, transforming growth factor-β–activated protein kinase 1 (TAK1)–binding protein 1 (TAB1). This mechanism is of particular relevance to myocardial ischemia and heart failure. However, the TAB1-mediated autophosphorylation pathway is a component of a complex and intricate network of feedback loops, because TAB1 is also a substrate of p38, and, when phosphorylated, it no longer activates transforming growth factor-β–activated protein kinase 1, an MKK kinase upstream of MKKs3/6/4 (see Figure 1). In addition to the archetypal canonical activation by MKKs and the alternate TAB1-mediated autoactivation reviewed above, there are a variety of other pathways that modulate p38 activity. These include phosphatases that dephosphorylate the TGY motif, G-protein–coupled receptor kinase 2 that disrupts the docking of MKKs by phosphorylating a threonine residue on p38, a heat shock protein 90-cdc37 chaperone that stabilizes p38 preventing activation by TAB1, and the acetylation of a lysine that increases p38's affinity for ATP. The presence of numerous mechanisms modulating p38 activity is a likely testament of the need to exquisitely control this crucial kinase. In particular, circumstance-specific mechanisms of activation provide the opportunity for selective interference.

**The Substrates of p38**

The MAPKs are proline-directed kinases, because the phospho-accepting serine or threonine is generally positioned immediately N-terminal of a proline residue (see Figure 2). There are minimal additional consensus sequence requirements for the phosphorylation motif itself, complicating the identification of MAPK substrates based on their primary sequence. Furthermore, the MAPK–substrate interaction relies on various docking domains within the substrate that are remote from the site(s) of phosphorylation. The known substrates include transcription factors and other protein
kinases, termed MAPK-activated protein kinases (see Figure 2). Usually, in quiescent cells, p38α is present in both the nucleus and in the cytoplasm, and, on activation, it is translocated to the nucleus by use of the microtubule and dynein cytoskeletal architecture.41,42 There are also mechanisms for counter transport. For example, MAP kinase–activated protein kinase-2 is a known substrate of p38 that once phosphorylated forms a stable complex responsible for p38 export from the nucleus.43 An illustration of the coordinated action of p38 on nuclear and cytosolic substrates is in the orchestration of transcription and translation to drive inflammation. Many cytokines possess an AU-rich element in the 3′-untranslated region of their mRNA. Binding of proteins to the AU-rich element affects the stability of the cytokine mRNA and thus the abundance of its encoded protein. Through this mechanism, p38 has been shown to increase the transcript stability of the proinflammatory cytokines cyclooxygenase-2, interleukin-1β, and TNF-α, an effect that acts synergistically with its orchestration of transcription (see Figure 2).

**Relationship Between p38α Structure and Activity**

The tertiary arrangement of the catalytic core of p38 is typical of most kinases comprising an N-terminal lobe, predominantly of β-pleated sheets, and a larger C-terminal lobe, predominantly of α-helices (see Figure 3). These 2 lobes are linked by a flexible hinge. The ATP binding site is a cleft formed by the interface between the 2 lobes and has the hinge region as a border. All 3 of these regions contribute residues crucial for ATP binding. When p38 is activated, the N- and C-terminal lobes reorientate around the hinge to bring these
crucial residues into the alignment needed for ATP binding (see Figure 3, right). The overt consequence is a marked increase in binding affinity toward ATP. A second effect of activation is rearrangement within, rather than between, lobes. Within the C-terminal lobe, the activation loop swings away from the entrance of the catalytic site exposing the substrate binding site. This relocation is a consequence of phosphorylation of the TGY motif by upstream MKKs. The activation loop movements that enable autophosphorylation are unknown and lie at the heart of the conundrum outlined above.

Among the residues bordering the ATP binding site, threonine 106 is of particular importance to inhibitor design and is termed the gatekeeper residue, because it guards the entrance to a hydrophobic pocket. This pocket does not directly participate in binding ATP and thus is a point of divergence between kinases that can enable selection. Type I, ATP-competitive inhibitors are designed to probe this pocket to both increase their binding affinity and achieve kinase selectivity. Substitution of threonine 106 for a residue with a bulkier side chain impedes the binding of type 1 inhibitors but not of ATP. However, unlike ATP, the type I inhibitors can bind to both active and inactive p38. Type II inhibitors make use of another feature at the lower edge of the ATP-binding pocket in the C-terminal lobe. Here, the phenyl ring of phenylalanine 169 can exist in an “in” (aspartate 168, phenylanaline 169, glycine 170-in, ring pointing toward the core of the molecule), or an out (aspartate 168, phenylanaline 169, glycine 170-out) conformation. In the inactive kinase, this region is in conformational exchange creating a transient pocket in the aspartate 168, phenylanaline 169, glycine 170-out conformation that varies between kinases. This pocket is used by the type II inhibitors to achieve kinase selectivity by stabilizing an inactive conformer that then cannot be activated. Type II inhibitors, such as BIRB796, sit at the lower edge of the ATP-binding pocket, are insensitive to mutations of the gatekeeper residue, and, because they bind to a rare conformation, have a slow onset of action. Unlike the /H9251 and /H9252 isoforms of p38, the /H9253 and /H9254 isoforms have a bulkier methionine gatekeeper residue that prevents their inhibition by type I inhibitors, but they remain sensitive to type II inhibitors.

The numerous 3-dimensional structures available of p38 in complex with ATP competitive inhibitors have aided understanding of the molecular determinants of p38 activation and inhibition. As stated previously, other domains have been identified that influence the binding of p38 to its partners that include activators, substrates, and phosphatases. These domains are distinct from the phosphorylation and catalytic sites and are thought to be responsible for...
Role of p38 in Cardiovascular Biology

p38 was first recognized as a protein phosphorylated in monocytes exposed to lipopolysaccharide. Concurrent screening for inhibitors of TNF production in an identical model identified compounds that bound p38. Thus, p38 was one of the first kinases for which relatively selective inhibitors were available and their mode of binding understood at the atomic level. As a consequence, p38 is one of the most investigated kinases with a wealth of literature in models relevant to cardiovascular disease. For the purposes of this review, we focus on those biological processes that underpin myocardial infarction and subsequent remodeling, because it is in this area that current clinical trial activity is focused.

Atherosclerosis

Following the migration of monocytes and smooth muscle cells into the intimal layer of blood vessels, foam cells are formed. Oxidized low-density lipoproteins (LDLs) are detected by scavenger receptors on the macrophages, internalized, and contribute to the accumulation of lipid. Further inflammatory cues lead to the formation of fatty streaks and ultimately complex atherosclerotic plaques (for review, see ). p38 activity has been demonstrated to be important at various points in plaque maturation. Zhao et al showed that the formation of the foam cells in response to oxidized LDL depended on p38 activation. The oxidized LDL likely activates Toll-like receptors that lead to p38 activation, the degradation of extracellular matrix, and plaque instability. Thrombin, acting through p38, is a potent vascular smooth muscle cell mitogen and chemotactant. An attenuation of these responses perhaps explains the diminished neointima formation after vascular injury with pharmacological or genetic inhibition of p38. Further evidence is provided by the reduction in atherosclerosis progression that accompanies systemic use of p38 inhibitors in animal models. Studies with type 1 pharmacological inhibitors have generally reduced measures of atherosclerosis in the ApoE null mice. However, cell lineage–restricted p38α ablation on the ApoE null background either has no effect or aggravates atherosclerosis. These findings are difficult to reconcile and perhaps suggest that p38β, and/or kinase activity in multiple cell types, needs to be inhibited to derive benefit. In summary, based on these and other preclinical studies, the prediction is that pharmacological p38 inhibition will improve vascular function, slow atherosclerosis progression, and decrease the risk of plaque complications.

Myocardial Infarction and Ischemic Preconditioning

The use of pharmacological inhibitors has advanced understanding of the role of p38 in myocardial ischemia and acted as an impetus for current clinical trial activity (see below). Two or 3 minutes of myocardial ischemia are sufficient to cause robust p38 activation that persists until ~20 minutes of ischemia with reactivation in the first few minutes of reperfusion. Pharmacological inhibition of p38, predominantly with type 1 inhibitors, has been shown to reduce infarction in innumerable studies that have been reviewed elsewhere. By and large, these studies suggest the most robust cardioprotection occurs when p38 is inhibited both during ischemia and at reperfusion. When inhibition is confined to reperfusion alone, protection is attenuated and disappears altogether if inhibition is delayed by just a few minutes. We have restricted our synopsis to the most recent studies to update recent reviews.

We have used a combination of a chemical genetic approach and different classes of p38 inhibitor to better understand the isoforms and their mechanism of activation during myocardial ischemia. These studies made use of mouse lines in which either both p38α or both p38β alleles had been targeted to substitute the gatekeeper threonine for a bulkier methionine residue (see Figure 4). This substitution is known to prevent the binding of type I inhibitors but does not affect the binding of ATP or of type II inhibitors. Thus, all the off-target effects of the type I inhibitor will be shared in knock-in (KI) and wild-type mice. The only difference between the mouse lines is that, in the presence of the type I inhibitor, the targeted isoform of p38 will remain active in the KI mice. However, this difference will disappear in the presence of a type II inhibitor (see Figure 4). Myocardial infarction and p38 TGY motif phosphorylation were reduced by a type I inhibitor in p38α wild-type, but not KI hearts, whereas a type II inhibitor reduced infarction and activation on both backgrounds. The results verified that p38α was the isoform that autophosphorylates during myocardial ischemia and that this event aggravates injury.

In addition to being useful research tools, inhibitors have the potential to play a therapeutic role. A novel drug delivery system of p38 kinase inhibitor loaded microspheres injected directly in the myocardium has been reported. Injection of microspheres in the rat model of myocardial infarction resulted in inhibition of p38 for 7 days, reducing superoxide and TNF-α production. Although the release kinetics of the microspheres could not prevent short-term damage, cardiac dysfunction after 3 weeks was reduced in comparison with free inhibitor and vehicle controls. The potential adverse effects of the delivery system, such as the activation of the inflammatory pathways by the vehicle or its metabolites, were reduced by the design of neutral degradation products. Although the main findings of this study merely verify those made previously, the novelty lies in a local delivery system that could circumvent the toxicity that accompanies...
As mentioned above, p38 is active during early ischemia and early reperfusion. In the postinfarcted heart, activity increases once again with the inflammation that accompanies scar maturation. However, as with other kinases, it can be difficult to demonstrate robust p38 activation during the intermediate and late phases of postinfarction remodeling, because it is likely that kinase activity is cyclic.76 Thus, in heart samples from patients with end-stage heart failure, p38 activity is on average higher than in healthy controls, but there is substantial variability.77 In contrast, and in common with the preclinical literature, p38 activity is more consistently increased in ischemic human myocardium.78,79

Despite the difficulties in demonstrating consistent and chronic p38 activation in the postmyocardial infarction setting, the effect of chronic exposure to p38 inhibitors consistently improves structural remodeling, and contractility, as well (see59 for review), benefits that can persist beyond the period of p38 inhibition.80,81 The question is, which of the biological processes implicated in progressive adverse remodeling and heart failure are influenced by p38 inhibition? The most thoroughly investigated are the processes of hypertrophy, cell death, and contraction.

Early studies examining the role of p38 in hypertrophy relied predominantly on isolated neonatal rat cardiomyocytes.82,83 Adenoviral transfection of constitutively active MKK3 and MKK6, the upstream activators of p38, resulted in increased cell size, hypertrophy-associated gene expression, and sarcomeric reorganization. Overexpression of p38β enhanced the hypertrophic response, whereas p38α overexpression increased apoptosis, suggesting distinct roles.83 However, later findings in animal models have been less consistent, reflecting the difficulty of extrapolating from isolated myocytes to the in vivo heart.84 For example, although ventricular-specific overexpression of constitutively active MKK3 caused fibrosis, wall thinning, and left ventricular dysfunction, myocyte hypertrophy was not evident.85 Similarly, myocyte hypertrophy in response to pressure overload did not differ between wild-type and p38α cardiac-restricted knockout mice.86 In dominant negative p38α transgenic mice, the level of hypertrophy observed was similar to wild-type controls despite the reduced levels of p38α kinase activity.87 In fact, even in the absence of a stimulus, the dominant negative p38α transgenic mice exhibited basal hypertrophy suggesting an anti- rather than prohypertrophic role through the regulation of nuclear factor of activated T-cell transcriptional activity.88 This contrasted with p38 activation causing SERCA2 downregulation, a prolonged calcium transient and thereby the activation, rather than inhibition, of nuclear factor of activated T-cell signaling.89 Most recently, Koivisto and colleagues90 used direct injection of adenoviruses into rat left ventricular myocardium to separately examine contributions of p38α and p38β. On this basis, p38β drove B-type natriuretic peptide transcription, whereas p38α increased the expression of genes associated with fibrosis. Thus, although it seems that p38β, and perhaps p38α, can drive cardiac hypertrophy in cultured isolated myocytes, their role in vivo is far less clear.

**Postmyocardial Infarction Remodeling and Hypertrophy**

systemic p38 inhibition in many clinical trials (see below). A similar strategy has been described by Gray et al74 and involves the design of a carbohydrate moiety that is N-acetylglucosamine linked to a p38 inhibitor to target cardiomyocytes. Although the majority of studies indicate the prolonged p38 activation that accompanies lethal ischemia is detrimental, the same is not true for the brief activation that accompanies the bursts of ischemia/reperfusion that result in preconditioning. Rather, under this circumstance, p38 activation leads to adaptation and the protection of ischemic preconditioning.75 One explanation for these dichotomous effects was that p38α was the isoform leading to injury, whereas p38β led to protection.59 Although it is likely that the activation of these isoforms has different consequences, it does not explain p38’s involvement in ischemic preconditioning. For example, we found, using the chemical genetic approach outlined above, that the activation of p38α, rather than p38β, was responsible for infarct size reduction following ischemic preconditioning and that this phenomenon persisted on the p38β null background.12 Thus, depending on circumstance, the activation of a single p38 isoform can have diametrically opposite consequences.

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Figure 4. The use of a chemical genetic approach to determine the isoforms involved in ischemic preconditioning and myocardial infarction. Wild-type p38α and p38β isoforms possess a hydrophobic pocket adjacent to the ATP binding site. Type I inhibitors make use of this pocket to achieve selective binding and inhibition. Access to the pocket is determined by the size of the gatekeeper residue, which is threonine in p38α and p38β. p38γ and p38δ are resistant to type I inhibitors as a result of a bulkier methionine gatekeeper, which sterically obstructs the binding of the inhibitor. We have used KI mouse lines in which both p38α, or both p38β, alleles have been targeted to substitute the gatekeeper threonine for methionine. In these mouse hearts the wild-type p38 kinase can bind ATP, type I inhibitor and type II inhibitor, whereas the mutated, KI kinase can bind ATP and type II, but not type I, inhibitor. The elegance of this technique is that the mouse hearts behave identically unless a type I inhibitor is present. In the presence of a type I inhibitor, the only difference between the hearts is the absence of near-instantaneous inhibition of either p38α or p38β in KI hearts. The off-target effects of the type I inhibitor will be shared across genotypes. This technique has been used to show p38α activates through autophosphorylation and is responsible for both the aggravation of myocardial infarction and the protection initiated by ischemic preconditioning (see text for details).
Apoptosis, necrosis, and autophagy are the principal processes responsible for cell loss during adverse remodeling. p38 phosphorylates the antiapoptotic protein Bcl-X<sub>L</sub> in response to TNF, reducing its expression and inducing apoptosis in endothelial cells. The phosphorylation of Bad, a proapoptotic protein, was attenuated by p38, thereby promoting its mitochondrial translocation and inducing endothelial cell apoptosis. In neonatal myocytes, overexpression of activated p38 reduces Bcl-2 protein levels. These findings are reinforced by studies of myocardial ischemia/reperfusion in isolated-perfused rabbit hearts in which p38 inhibition diminished apoptosis and in the in situ mouse heart in which expression of dominant negative p38α reduced Bcl-X<sub>L</sub> deamidation/inactivation and apoptosis.

The loss of cells through apoptosis, necrosis, and autophagy may be counterbalanced by hyperplasia or stem cell differentiation. Cardiomyocyte proliferative potential has been found to be inversely proportional to p38 activity, an effect also seen in other cardiovascular progenitors. These studies suggest p38 activation adversely affects both sides of the balance in the stressed myocardium by promoting cell death while impairing cell replacement.

In addition to the effect of p38 inhibition on cardiac structure by reducing myocardial infarction and/or postinfarction remodeling, there is also an acute effect on contractility. p38α activation has a negative inotropic effect on cardiac myocytes by reducing the myofilament response to calcium, thereby negatively regulating cell contractility. Furthermore, the inhibition of p38 following myocardial infarction decreases TNF-α production, reduces type I collagen expression, and abrogates the negative inotropic action of TNF-α. p38 has also been implicated in the maladaptive vasoconstriction accompanying heart failure, because its inhibition results in a decreased myogenic response in the mesenteric arteries and diminished systemic peripheral resistance. In combination, these effects would be expected to limit remodeling and improve contractility in heart failure.

In summary, based on preclinical models, p38 signaling influences each of the processes in the cascade that most commonly causes heart failure, namely atherosclerosis progression, myocardial infarction, and adverse ventricular remodeling. The key question is whether any of the findings from these basic science studies provide potential therapies/treatments in the clinical arena.

### Clinical Experience With p38 Inhibitors

In the past, many clinical trials involving p38 inhibitors failed to enter the public domain. Currently, there are ~60 such trials listed on http://clinicaltrials.gov of which 16 are actively recruiting. The majority of previous studies were in inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. More recent trials focus on p38 inhibition in chronic pulmonary disease, neuropathic and other forms of pain, malignancy, and cardiovascular diseases (ClinicalTrials.gov). These trials are supported by a variety of companies including GlaxoSmithKline, Bristol-Myers Squibb, Pfizer, and Hoffman-La Roche and involve at least 16 different p38 inhibitors (ClinicalTrials.gov) (see Figure 5).
The most mature experience is in the treatment of rheumatoid arthritis where those immersed in the field are not encouraged by the clinical findings to date and lament the fact that much data are not publically available.\textsuperscript{101,102} Based on published manuscripts, abstracts, and personal communications, the conclusions are that liver toxicity, skin rashes, and intercurrent infections were more frequent in patients allocated active p38 inhibitor than placebo.\textsuperscript{101,102} In addition, this side-effect profile was observed despite molecules having different structural backbones and belonging to type I and type II inhibitor classes.\textsuperscript{101,103} Furthermore, the evidence of clinical efficacy was limited, especially when the comparator was an established therapy for rheumatoid arthritis or the p38 inhibitor was administered on the background of active treatment.\textsuperscript{101–103} Furthermore, the evidence of clinical efficacy was limited, especially when the comparator was an established therapy for rheumatoid arthritis or the p38 inhibitor was administered on the background of active treatment.\textsuperscript{101–103} Therefore, it is likely active in diseased human blood vessels, but this is more difficult to demonstrate because the kinase activity is likely cyclic and a sufficient mass of tissue cannot be harvested rapidly. Thus, driven by the preclinical findings summarized above, a number of exploratory phase 1/2 clinical trials of p38 inhibition for cardiovascular indications are ongoing (see Table). An advantage of the cardiovascular programs is that they have the benefit of past experience. So far, there has been no suggestion of toxicity, although this is probably the result of careful dose titration to ensure only partial inhibition of p38 kinase activity. For example, as measured with use of a whole blood assay, p38 activity was decreased by an average of 45% at 3 hours, and 35% at 6 hours, after the last oral dose of an inhibitor with a half-life of \textasciitilde12 hours.\textsuperscript{61} Furthermore, these values were obtained after 28 days of dosing, presumably at steady state.\textsuperscript{61} In this particular trial, the inhibitor was administered twice per day suggesting p38 activity at trough, immediately before the next dose, would be \textasciitilde20%.\textsuperscript{61} Another potential advantage is the availability of more refined inhibitor scaffolds. The clinical explorations to date have followed the preclinical experience and have been designed to look for vascular and myocardial benefits.

Thus far, 3 clinical trials of p38 inhibition for atherosclerosis are in the public domain. GlaxoSmithKline have recently reported the full results of a trial with losmapimod (also known as GW85655 and GSK-AHAB, NCT00474864) in patients with hypercholesterolemia-associated endothelial dysfunction.\textsuperscript{61} This was of a double-blind, placebo-controlled, parallel group design involving 56 patients with hypercholesterolemia (fasting LDL \textasciitilde4.1 mmol/L), randomly assigned on a 1:1 basis to oral losmapimod 7.5 mg BID or placebo for 28 days.\textsuperscript{61} Vascular function was assessed before and at the end of the 28-day exposure by forearm venous occlusion plethysmography. The primary end point was the change in endothelial function in comparison with baseline as assessed by vasodilation in response to intra-arterial acetylcholine. Secondary end points were changes in endogenous nitric oxide release, as measured by vasoconstriction in response to \textasciitildeL-arginine and endothelial-independent function as measured by vasodilatation in response to sodium nitroprusside. Before drug exposure, endothelial function and basal nitric oxide release were impaired.

### Clinical Trials of p38 Inhibition for Cardiovascular Indications

As mentioned previously, a number of investigators have shown that p38 is active in diseased human myocardium.\textsuperscript{77–79} It is also likely active in diseased human blood vessels, but this is more difficult to demonstrate because the kinase activity is likely cyclic\textsuperscript{76} and a sufficient mass of tissue cannot be harvested rapidly. Thus, driven by the preclinical findings summarized above, a number of exploratory phase

### Table. Summary of Ongoing and Completed Cardiovascular Trials That Involve p38 Inhibition

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Dose/Duration</th>
<th>Study Size</th>
<th>Registration</th>
<th>Sponsor</th>
<th>Indication</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losmapimod (GW85655)</td>
<td>7.5 mg BID/28 d</td>
<td>68</td>
<td>NCT00474864</td>
<td>GlaxoSmithKline</td>
<td>Hypercholesterolemia-induced endothelial dysfunction</td>
<td>Improved endothelial-dependent and independent function without alteration in LDL\textsuperscript{61}</td>
</tr>
<tr>
<td>Losmapimod (GW85655)</td>
<td>7.5 mg once daily and 7.5 mg BID/84 d</td>
<td>99</td>
<td>NCT00633022</td>
<td>GlaxoSmithKline</td>
<td>Documented atherosclerosis</td>
<td>Less FDG uptake on PET/CT (only on post hoc analysis)\textsuperscript{106}</td>
</tr>
<tr>
<td>BMS582949</td>
<td>100 mg once daily/84 d</td>
<td>72</td>
<td>NCT00570752</td>
<td>Bristol-Myers Squibb</td>
<td>Documented atherosclerosis</td>
<td>Completed December 2010, but findings not reported</td>
</tr>
<tr>
<td>VX-702</td>
<td>5–40 mg once daily/5 d</td>
<td>45</td>
<td>Unknown</td>
<td>Vertex</td>
<td>Elective PCI</td>
<td>Suppression of systemic inflammatory response post-PCI\textsuperscript{107}</td>
</tr>
<tr>
<td>SB681323</td>
<td>5 mg morning, 2.5 mg evening/28 d</td>
<td>93</td>
<td>NCT00291902</td>
<td>GlaxoSmithKline</td>
<td>Elective PCI</td>
<td>Suppression of systemic inflammatory response post-PCI and reduced cardiac events\textsuperscript{108}</td>
</tr>
<tr>
<td>Losmapimod (GW85655)</td>
<td>7.5 mg BID/84 d</td>
<td>540</td>
<td>NCT00910962</td>
<td>GlaxoSmithKline</td>
<td>Non–ST-elevation myocardial infarction</td>
<td>Completed April 2012. Main end points are safety, systemic inflammation, and size of myocardial infarction</td>
</tr>
</tbody>
</table>

Registration refers to entry number on ClinicalTrials.gov. Only those trials in the public domain have been included. FDG indicates \textsuperscript{18}F-fluorodeoxyglucose; PCI, percutaneous coronary intervention; PET, positron emission tomography; and CT, computed tomography.
in both active and placebo groups in comparison with a control cohort of healthy volunteers. After 28 days, endothelial function, but not basal nitric oxide release, significantly improved in the losmapimod group. Interestingly, endothelial-independent function was similarly improved by losmapimod. In a post hoc analysis, vascular function after 28 days in the losmapimod group no longer differed from that in healthy controls. These benefits of losmapimod were accompanied by an \( \approx 50\% \) reduction in serum C-reactive protein but no change in LDL cholesterol. There was no excess in adverse events or signs of liver toxicity in the losmapimod group. The investigators interpreted the results as showing that p38 inhibition with losmapimod improved the endothelial dysfunction associated with hypercholesterolemia, whereas the effect on endothelial-independent function may be the result of increased sensitivity of vascular smooth cells to sodium nitroprusside–derived nitric oxide.

There are also 2 trials of p38 inhibition for established atherosclerosis, one sponsored by GlaxoSmithKline (with losmapimod, identifier NCT00633022) and the other by Bristol-Myers Squibb (with BMS582949, identifier NCT00570752). Both of these studies seem to have a similar design with a primary end point of change in inflammation within atherosclerotic plaques as assessed by 18F-fluorodeoxyglucose (FDG) uptake on positron emission tomography/computed tomography. In both studies, dosing is for 3 months with a presumed primary end point of change in FDG uptake at baseline in comparison with day 84. The GlaxoSmithKline study involving 99 patients has appeared in abstract form and examined 2 dosing regimens with losmapimod at 7.5 mg once daily or 7.5 mg BID in comparison with placebo. Eligibility was based on a tissue-to-background FDG uptake ratio of \( \geq 1.6 \) in the carotids or ascending aorta. The study failed to reduce the primary end point, which was a reduction in average tissue-to-background FDG uptake. However, both doses of losmapimod did reduce this signal if analysis was confined to the active qualifying segments, an effect not seen with placebo. In addition, there were reductions in multiple systemic markers of inflammation that were most marked at the higher losmapimod dose. There was no evidence of significant adverse events. The Bristol-Myers Squibb study has been completed with 72 patients dosed with BMS582949 at 100 mg once daily in comparison with both a traditional placebo and an active comparator of atorvastatin 80 mg once daily. The active comparator is particularly valuable given the uncertainty of the \( ^{18}\text{F}-\text{FDG} \) end point as a measure of atherosclerosis activity; however, results are still awaited.

Clinical trials addressing the potential effects of p38 inhibition on the myocardium have until recently been limited to small, detailed, pathophysiological studies. Vertex’s compound VX-702 was used in a dose-escalation study in 45 patients undergoing planned coronary artery angioplasty (percutaneous coronary intervention). The salient finding was a suppression of the elevation in CRP and other inflammatory markers (monocytes, neutrophils, and total white blood cell count) for 3 to 4 days postprocedure. Oral dosing with the inhibitor (5–40 mg, once daily) was for 5 days starting the day before the procedure and was well tolerated. In a study of a similar design, SB681323 at a dose of 7.5 mg (5 mg morning and 2.5 mg evening) was compared with placebo in 93 patients with 1:1 randomization (identifier NCT00291902). Dosing in this double-blind study was for 28 days, starting 3 days before planned percutaneous coronary intervention. In the SB681323 group, 38 patients, and, in the placebo group, 35 patients completed the study. SB681323 was associated with a reduction in CRP both immediately before, and after, percutaneous coronary intervention. The mean reduction in CRP area under the curve over the 28 days was 40%. There were also reductions in other inflammatory markers such as myeloperoxidase, interleukin-6, and interleukin-8. The study was not powered to look at clinical end points, but there was a significant reduction in episodes of angina and major adverse cardiac events, albeit on a post hoc analysis. There was no significant difference in procedure-related troponin I release between groups. The results suggest that p38 inhibition can suppress the chronic systemic inflammatory state associated with atherosclerosis, and the response associated with acute injury, as well. The lack of effect on myocardial infarction is perhaps not surprising given the prerequisites for cardioprotection to manifest, including complete and timely reperfusion are absent with most periprocedural PCI-related injury.

Perhaps the most interesting and ambitious study is in the setting of non–ST-elevation myocardial infarction by GlaxoSmithKline (with losmapimod at 7.5 mg BID with or without a 15-mg loading dose for 12 weeks in comparison with placebo, NCT00910962). In this study 540 patients with non–ST-elevation myocardial infarction will be randomly assigned. The primary end points relate principally to safety in this high-risk population. However, there are measures of efficacy such as alterations in CRP and measures of infarct size and cardiac stress based on biomarkers. In addition, there is a substudy in which changes in infarction and cardiac function will be analyzed by MRI. This study was scheduled to be completed in late 2012 and will be the first in which potential effects on myocardial remodeling may be discerned.

**Concluding Remarks**

It is currently unclear if kinase inhibitors will have an impact on specialties outside oncology. In the cardiovascular system, there is a great deal of preclinical evidence that p38 activity worsens vascular and myocardial structure and function under pathological circumstances. However, there are also numerous studies that show this kinase is vital to homeostasis and adaptation to stress in the adult and the embryo. This situation is similar to that of the adrenergic and renin-angiotensin systems, suggesting inhibition can still have a therapeutic benefit. Preliminary clinical trials suggest that p38 inhibition can improve vascular function and suppress systemic and perhaps local inflammation associated with atherosclerosis. So far, these benefits have not been accompanied by an increase in adverse events. In part, this has been achieved by dosing regimens designed to achieve only partial inhibition of p38 kinase activity. Whether these early encouraging signals of success survive the rigors of phase III clinical trials will only be told by time.

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Disclosures
Dr Marber, through King’s College London, has acted as a consultant to GlaxoSmithKline and is a member of the Steering Committee of the SOLSTICE trial.

References


