Signalling, inflammation and arthritis

MAPKs and their relevance to arthritis and inflammation

T. Thalhamer, M. A. McGrath and M. M. Harnett

Rheumatoid arthritis (RA) is a chronic autoimmune disease in which imbalances in pro- and anti-inflammatory cytokines promote the induction of autoimmunity, inflammation and joint destruction. The importance of inflammatory cytokines in the pathogenesis of RA has been underscored by the success of biologics that act to block the effects of cytokines, such as tumour necrosis factor-α, interleukin (IL)-1 or IL-6, in treating disease. Mitogen-activated protein kinases (MAPKs) have been implicated as playing key regulatory roles in the production of these pro-inflammatory cytokines and downstream signalling events leading to joint inflammation and destruction. This article reviews the evidence that MAPKs play important roles in the pathogenesis of RA and discusses their therapeutic potential as drug targets.

KEY WORDS: Mitogen-activated protein kinases, Extracellular signal-regulated kinases, C-jun N-terminal kinase, p38 Mitogen-activated protein kinase, Rheumatoid arthritis, Cytokines.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease in which imbalances in pro- and anti-inflammatory cytokines promote the induction of autoimmunity, inflammation and joint destruction [1, 2]. The initial stages of autoimmunity, which can precede articular inflammation by several years, are the result of breakdowns in B- and T-cell tolerance that can occur at a number of central or peripheral checkpoints and are evidenced by the production of rheumatoid factors [antibodies specific for immunoglobulin G (IgG)] or anti-citrullinated peptide antibodies. Poorly defined physiochemical, genetic and environmental factors then drive the development of articular disease that recruits a wide range of activated immune effector cells [including macrophages, mast cells, neutrophils, B-, T- and natural killer (NK) cells] into the affected joint(s). Cytokines [including the master regulators interleukin (IL)-1, IL-17 and tumour necrosis factor-α (TNFα)] and downstream inflammatory mediators [such as prostaglandins and the matrix metalloproteinases (MMPs)] produced by such cellular infiltrates promote the autoimmunity and chronic inflammation of the synovium that is required for the ultimate destruction of the cartilage and the underlying bone driven by synovial fibroblasts, chondrocytes and osteoclasts [1, 2]. Synovial inflammation and bone destruction are intimately linked as IL-1 and TNF-α drive production of MMPs by synovial fibroblasts to further promote inflammation and facilitate invasion of articular cartilage, whilst IL-1 and IL-17 convert chondrocytes to a phenotype that degrades, rather than synthesizes, matrix, resulting in cartilage destruction [1, 3, 4]. Similarly, TNF-α and IL-17 induce the production of M-CSF that is required for the early differentiation of osteoclasts, and along with IL-1, IL-6 and prostaglandin E2 (PGE2) up-regulates receptor activator of nuclear factor kappa B ligand (RANKL) expression on synovial fibroblasts to mediate differentiation of the bone-resorption capabilities of osteoclasts. Moreover, the potential for compensatory new bone formation is essentially ablated in RA as TNF-α also acts to suppress the maturation and function of bone-producing osteoblasts [1, 5–8].

The importance of inflammatory cytokines in the pathogenesis of RA has been underscored by the success of biologics in treating disease by blocking the effects of cytokines such as TNF-α, IL-1 or IL-6. However, there remains a significant unmet clinical need for new therapies as existing regimens are effective in only a proportion of patients and crucially leave the host at significantly higher risk of overwhelming infection, e.g. tuberculosis, entailing careful screening and limitations of use in some populations. Moreover, the high cost, short half-life and i.v. application of these drugs have made it necessary to consider alternative treatments such as orally active small molecule inhibitors of signalling cascades relating to these important inflammatory mediators. Much interest has focused on inhibitors of the mitogen-activated protein kinases (MAPKs) primarily because they have been implicated as key regulators of pro-inflammatory cytokine (e.g. IL-1, IL-6, IL-12, IL-23 and TNF) production as well as playing crucial roles in signalling via the B cell antigen receptor (BCR), TCR/CD28 and toll-like receptor (TLR), IL-1, IL-17 and TNF-α receptors [9–14]. Indeed, p38 MAPK was initially discovered as the target of pyridinyl imidazoles [15, 16], a class of compounds that inhibit lipopolysaccharide (LPS)-stimulated production of TNF-α and IL-1 by human monocytes and p38 MAPK inhibitors are currently undergoing phase II trials for RA (www.sciosinc.com/scios_pr_1046376591; www.vpharm.com/Pressreleases2006/pr030806.html). This article reviews the evidence that MAPKs play important roles in the pathogenesis of RA and discusses their therapeutic potential as drug targets.

MAPK

MAPKs comprise a family of highly conserved serine/threonine protein kinases that have been implicated in the regulation of key cellular processes including gene induction, cell survival/apoptosis, proliferation and differentiation as well as cellular stress and inflammatory responses. There are three major classes of MAPKs in mammals, the extracellular signal-regulated kinases (ERKs) and the two stress-activated protein kinase (SAPKs) families, c-jun N-terminal kinase (JNK) and p38. MAPKs are activated via a signalling cascade that is conserved from yeast to mammals [17, 18]. Thus, stimulation of MAPKs requires the upstream activation of a MAPK kinase (termed MAPKK, MEK or MKK) and an MAPK kinase kinase (termed MAPKKK, MEKK or MKKK). MKKKs are serine/threonine protein kinases that phosphorylate and activate MKKs, whilst MKKs are dual-specificity protein kinases that phosphorylate the threonine and tyrosine residues of a conserved T–X–Y motif of the activation loop of MAPKs (Fig. 1). The X residue is different in each class of MAPKs: ERK has a threonine–glutamic acid–tyrosine (T–E–Y) motif, JNK has a threonine–proline–tyrosine (T–P–Y) motif

Division of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, UK.

Submitted 2 July 2007; revised version accepted 4 October 2007.

Correspondence to: M. M Harnett, Division of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK. E-mail: M.Harnett@bio.gla.ac.uk
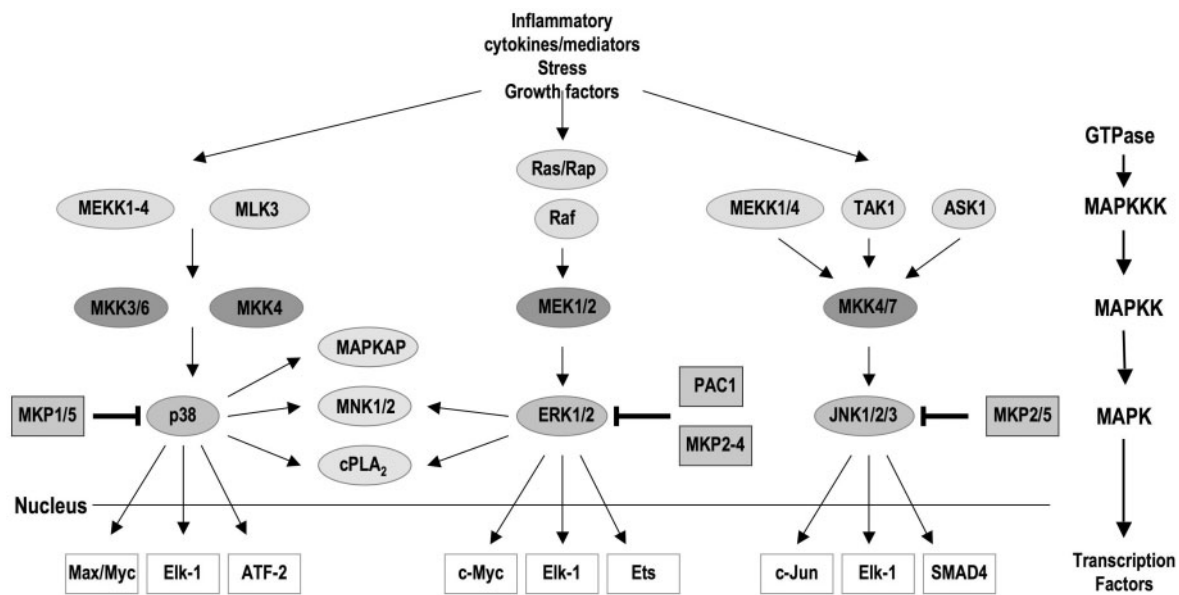T. Thalhamer and M. A. McGrath equally contributed to this work.

Fig. 1. The ERK, JNK and p38 MAPK cascades. There are three major classes of MAPKs in mammals, the ERKs and the two SAPKs families: JNK and p38. MAPKs are activated via a signalling cascade that is conserved from yeast to mammals in which stimulation of MAPKs requires the upstream activation of a MAPK kinase (termed MAPKK, MEK or MKK) and a MAPK kinase kinase (termed MAPKKK, MEKK or MKKK). Activation of the MAPKs results in phosphorylation of a specific repertoire of cytoplasmic and nuclear target proteins including various transcription factors. Negative feedback mechanisms including MAPK phosphatases (MKPs) exist to ensure MAPK enzymes are not activated constitutively. ASK, apoptosis signal-regulating kinase; ATF, activating transcription factor; ERK, extra-cellular signal-regulated kinase MAPK; JNK, c-Jun NH2-terminal kinase MAPK; MEK, MAPK kinase; MEKK, MEK kinase; MLK, mixed lineage kinase; MNK, MAPK interacting serine/threonine kinase; MAPKAP, MAPK activated protein kinase; cPLA2, cytosolic phospholipase A2; MKK, c-Jun NH2-terminal kinase MAPK kinase; Elk, Ets-like transcription factor; Ets, E26-AMV virus oncogene cellular homologue; TAK, TGFβ-activated kinase; PAC, phosphatase of activated cells; MKP, MAPK phosphatase.

and p38 has a threonine–glycine–tyrosine (T–G–Y) motif. Phosphorylation of the T–X–Y motif activates the MAPKs, allowing phosphorylation of a specific repertoire of cytoplasmic and nuclear proteins including various transcription factors. The substrate specificity of MAPKs is influenced by the X residue of the T–X–Y motif and by the amino acids surrounding this motif since this region can affect the availability or conformation of the substrate-binding groove [17, 18]. Each MAPK enzyme can be activated by a number of different upstream MKKs and MKKKs, each with unique properties regarding its regulation, substrate specificity and kinetics of activation providing specificity and allowing activation by a broad range of immunological stimuli acting via the antigen and co-stimulatory receptors, FcRs, TLRs and cytokine receptors [10, 19–23].

Negative feedback mechanisms exist to ensure that MAPK enzymes are not activated constitutively. For example, MAPKs can induce at least three different types of protein phosphatases that dephosphorylate and inhibit MAPK: dual-specificity phosphatases (DUSPs), threonine phosphatases and tyrosine phosphatases [24–27]. For example, the MAPK phosphatases for ERK2 include the dual-specificity phosphatase MKP3, the threonine phosphatase PP2A and the tyrosine phosphatase HePTP [28]. The DUSPs form a family of proteins that can dephosphorylate both the threonine and tyrosine residues of the T–X–Y motif [27]. The activity of DUSPs is largely regulated at the transcriptional level and the expression of specific protein phosphatases allows the selective inhibition of particular MAPK proteins since each phosphatase has a precise substrate specificity [29]. For example, procaspase-activating compound 1 (PAC-1) dephosphorylates ERK and p38, whilst MKP-1 acts on all three families of MAPKs. In contrast, MKP-2 preferentially dephosphorylates ERK and JNK and MKP-M dephosphorylates JNK. Indeed, each member of the DUSP family has a unique set of properties including tissue distribution, subcellular localization, regulation and substrate specificity [27, 29]. For example, PAC-1 is a DUSP predominantly located in the nucleus of haematopoietic cells that

preferentially dephosphorylates ERK MAPK, acting on the tyrosine residue of the T–E–Y motif prior to dephosphorylating the threonine residue. The expression of PAC-1 in B- and T-cells is increased by various mitogenic stimuli since the transcription of the *pac-1* gene can be activated by transcription factors that are regulated by ERK MAPK. DUSPs often have a short half-life to prevent excessive inhibition of MAPK. However, the degradation of DUSPs can also be regulated by MAPKs thus creating the potential for prolonged activation of ERK [24–27, 29].

Whilst all three classes of MAPKs have been shown to be expressed in the synovial tissue of RA and osteoarthritis patients, phosphorylation and therefore activation of the enzymes could be detected only in tissue from RA patients, implicating a role(s) for these MAPKs in the pathogenesis of this disease [30]. Interestingly, the activated form of the three classes of MAPKs displayed distinct localization patterns in synovial tissue from RA patients. Thus, phospho-p38 MAPK was expressed in both the endothelium of the synovial microvessels and in the cells of the synovial lining layer. In contrast, activation of ERK MAPK was detected in mononuclear infiltrates and cells within the synovial lining area around synovial microvessels, whilst JNK MAPK was shown to be active predominantly in mononuclear cell infiltrates in the sublining areas [30]. TNF-α, IL-1 and IL-6 have been proposed to play important roles in promoting RA by inducing production of IL-8, MMPs and adhesion molecules by many of the cells present in the synovium, thereby further enhancing cell infiltration, inflammation and cartilage destruction. To address the role(s) of MAPKs in these processes, the ability of such pro-inflammatory cytokines to induce MAPK activation in synovial fibroblasts and chondrocytes was tested *in vitro*. TNF-α, IL-1 and IL-6 were found to activate all three classes of MAPKs in synovial fibroblasts [30] and TNF-α and IL-1 were similarly found to activate ERK, JNK and p38 MAPK [31–33], with consequent induction of MMP-1 and MMP-13 in chondrocytes [33–35]. Moreover, TNF-α and IL-1 were shown to play important *in vivo* roles in the activation of p38 and ERK MAPKs in inflamed

synovial tissue as TNF-α or IL-1 blockade reduced the activation of these MAPKs in the synovium of TNF-α-expressing transgenic mice [36]. Furthermore, MAPK phosphatase-1 (MKP-1) deficiency exacerbates murine collagen-induced arthritis (CIA), underlining the importance of negative feedback mechanisms in keeping MAPK signalling and consequent inflammation in check [37]. However, PAC-1 (DUSP2) knockout mice exhibit reduced inflammatory responses and less-severe histological changes in the joints. These rather paradoxical results in PAC-1-deficient mice appear to reflect unforeseen reductions in ERK and p38 MAPK activity due to increased JNK MAPK signalling [25], highlighting the unpredictable cross-talk that can occur amongst MAPK cascades in the presence of inhibitors [14, 19] and that dictates that side effects are likely to arise even with highly isoform-specific inhibitors. Nevertheless, collectively, these findings indicate that in inflamed synovial tissue, all three MAPKs are (aberrantly) activated and the differences in their activation patterns suggest they may each play specific roles in the disease process.

ERK MAPK

Specific inhibitors (PD98059 and PD198306: Pfizer Inc., CT, USA and U0126: DuPont Inc., DE, USA) that target ERK MAPK by inhibiting the activation of the upstream regulators, MEK1/2 [38, 39] have been available for over 10 yrs but there is only limited information available concerning their therapeutic potential in chronic inflammatory conditions such as RA. For example, there are no reports that these compounds reduce inflammation or joint destruction in animal models of arthritis although PD098059-reduced nociceptive responses in an adjuvant-induced monoarthritis in rats, suggesting that ERK plays an important role in transducing chronic inflammatory articular pain [40]. However, ERK MAPK inhibitors have been found to be successful in reducing inflammation in an ear oedema model in mice and in an experimental osteoarthritis model in rabbits [41, 42]. Nevertheless, ERK MAPK has been shown to be activated in synovial fibroblasts following stimulation with IL-1, TNF-α and fibroblast growth factor (FGF) and is indeed found to be activated in mononuclear cell infiltrates and synovial fibroblasts in synovial tissue from RA patients [30]. As ERK is known to be involved in the regulation of IL-6, IL-12, IL-23 and TNF-α synthesis [13, 14], these *in vitro* findings suggest a possible involvement of ERK in joint damage associated with pro-inflammatory cytokine production by macrophages. Moreover, ERK MAPK signalling could also play a role in the maintenance phase of disease, by promoting pannus formation, due to its role in phospholipase A_2 induction, prostaglandin synthesis and resultant chemotaxis of cells into the joint [30].

Indeed, paw extracts from transgenic mice over-expressing TNF-α exhibited higher levels of phosphorylated, activated ERK MAPK relative to those obtained from wild-type mice and such ERK MAPK activity was found predominantly in macrophages and fibroblasts within the synovium [36]. At present, the potential role of ERK MAPK in such joint inflammation and destruction is not clear. However, IL-1 induced MMP-1 gene expression by rabbit synovial fibroblasts requires both NF-κB and ERK MAPK activation [43, 44]. Similarly, IL-1-induced AP-1 activation and subsequent MMP-1 induction in synovial fibroblasts from RA patients was slightly decreased by inhibition of ERK MAPK, presumably due to suppression of its downstream effector, c-Fos [45]. Moreover, the MEK1/2 inhibitor PD198306 decreased MMP-1 production by chondrocytes in a rabbit model of osteoarthritis [42] and the ERK inhibitors, U0126, and to a lesser extent PD98059, ablated TNF-α-induced MMP-13 (mRNA and protein) production by chondrocytes from patients with osteoarthritis [33]. Interestingly, studies with human osteoblasts indicated that culture with the ERK inhibitor PD98059 resulted in stimulation of MMP-13 production from these cells [46]. Thus, the induction of MMPs, which remove matrix components such as cartilage in order for osteoclasts to attach to bone resulting in bone resorption [46], may be a target for therapeutic intervention by ERK inhibitors.

JNK MAPK

Specific JNK MAPK inhibitors have only recently been developed and so there is also relatively little evidence regarding the therapeutic potential of JNK MAPK in RA. However, recent work employing a specific inhibitor (SP600125) and cells from JNK1 or JNK2 knockout mice has suggested that JNK MAPK also appears to play a major role in the regulation of collagenase production by synovial fibroblasts [45, 47]. Thus, for example, SP600125 suppressed IL-1-induced and JNK MAPK-mediated c-Jun phosphorylation and AP-1 up-regulation, resulting in consequent down-regulation of MMP-1 expression [45]. Similarly, synovial fibroblasts from JNK1 or JNK2-deficient mice were found to express reduced levels of MMP-3 and MMP-13. In addition, JNK1 appears to play a role in driving osteoclast differentiation as JNK1-, but not JNK2-, deficient osteoclast progenitors were unable to mature into bone-resorbing osteoclasts following exposure to RANKL stimulation [48]. Consistent with these findings, whilst prophylactic treatment with SP600125 in an adjuvant-induced arthritis model only modestly improved inflammation, it significantly reduced joint destruction [45]. The relative contributions of JNK1 and JNK2 to bone destruction *in vivo* are not clear, however, as mice defective in either JNK1 or JNK2 exhibited only marginally reduced joint inflammation and destruction [49, 50], suggesting that both isoforms may have to be targeted for effective therapy.

To identify potentially more inflammation-specific upstream targets in the JNK MAPK pathways, synovial fibroblasts were examined for the expression and activation of MAPKKs and MAPKKKs that regulate JNK MAPK signalling. This revealed that MKKK2 was the major kinase activated by IL-1 and the most potent inducer of the JNK MAPK cascade [51]. Moreover, although both MKK-4 and -7 were found to be activated downstream of MKKK2 by IL-1 [52], only MKK-7 was essential for IL-1-stimulated JNK MAPK activation and consequent c-Jun phosphorylation and AP-1 activation [53], suggesting that targeting of MKK-7 could allow specific suppression of harmful inflammation whilst leaving MKK-4-mediated activation of stress-related pathways intact.

p38 MAPK

p38 MAPK is generally considered to be the most promising MAPK therapeutic target for RA as p38 MAPK isoforms have been implicated in the regulation of many of the processes, such as migration and accumulation of leucocytes, production of cytokines and pro-inflammatory mediators and angiogenesis, that promote disease pathogenesis (reviewed in references [30, 54]). For example, inhibitors of p38 have been shown to block TNF-α, IL-1, IL-8 and cyclo-oxygenase-2 (COX-2) production by monocytes [16, 55], with p38 MAPK playing crucial roles in the regulation of IL-1 and TNF-α synthesis at both the transcriptional and translational levels [16, 56, 57]. Similarly, p38 MAPK regulates IL-1-stimulated production of IL-6 and IL-8 and induction of COX-2 and MMP-1 and -3 in fibroblasts [58–60] and IL-1-stimulated nitric oxide production by chondrocytes [61]. Moreover, p38 MAPK also appears to play a key role in regulating osteoclast differentiation induced by M-CSF/RANKL or TNF-α by down-regulating tartarate-resistant acid phosphatase (TRAP) mRNA levels [62, 63], although it does not appear to be required for the survival or function of mature osteoclasts [63]. In addition, inhibition of p38 MAPK activity suppresses the TNF-α/IL-1-mediated induction of IL-6 by osteoblasts [64] and chondrocytes [65] that plays a role in osteoclast formation and bone resorption [64, 66]. Finally, and consistent with reports of the crucial role of p38 MAPK in IL-17

TABLE 1. MAPK inhibitors in clinical trial

Target	Compound	Company	Disease	Clinical Trial
p38	BIRB 796	Boehringer Ingelheim GmbH	RA, Psoriasis, Crohn's disease	III (RA)[79, 96] http://www.invitrogen.com/content.cfm?pageid=10561
p38	RWJ67657	Johnson & Johnson		[80, 81]
p38	SCIO-496	Scios Inc.	RA, multiple myeloma	III (RA)[97] http://www.arthritissupport.com/library/showarticle.cfm/ID/644/t/Arthritis http://www.invitrogen.com/content.cfm?pageid=10561
p38	VX-745	Vertex Pharmaceuticals	RA	II [98] http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/01-04-2001/0001396593&EDATE
p38	VX-702	Vertex Pharmaceuticals	Acute coronary syndrome (ACS), RA	II (ACS)[99] http://www.vpharm.com/Pressreleases2004/pr101804.html http://www.invitrogen.com/content.cfm?pageid=10561
p38	SB681323	GlaxoSmith Kline	COPD, RA	I/II http://www.druglib.com/trial/33/NCT00380133.html
JNK + p38	CNI-1493	Cytokine Pharma Sciences Inc.	Crohn's disease	I [100]
JNK	CEP-1347	Cephalon	Parkinson's disease	I (neuro-degeneration) II/III (Parkinsons, discontinued) [101] http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/05-11-2005/0003595559&EDATE http://www.invitrogen.com/content.cfm?pageid=10561
MEK	AZD6244	AstraZeneca	Cancer	I/II http://www.medicalnewstoday.com/articles/44838.php http://www.invitrogen.com/content.cfm?pageid=10561
MEK	CI-1040	Pfizer Ltd	Cancer	I/II [102–104]

signalling [11, 12], IL-17-mediated enhancement of IL-6 production by osteoblasts following TNF-α stimulation is abrogated by inhibition of p38 MAPK [67].

The importance of these p38 MAPK targets *in vivo* has been demonstrated in a range of different animal models of RA. Thus, p38 MAPK inhibitors have been proven to be effective in prophylactically reducing clinical severity, paw swelling, inflammation, cartilage breakdown and bone erosion in the rat streptococcal cell wall arthritis model [68, 69], the CIA model in mice [70] and the adjuvant and CIA models in rats [71, 72]. Importantly, these disease-suppressing effects could also be seen in animals treated therapeutically [68–72], and as the inhibitors were able to inhibit further bone erosion in such regimens [70], these studies suggest that p38 MAPK inhibitors indeed have therapeutic potential for patients with well-established disease. The ability to inhibit further bone erosion is likely to be mainly due to the inhibition of osteoclast differentiation [63] as osteoclast numbers were shown to be reduced in the affected joints in animals treated with p38 MAPK inhibitors [70, 72, 73]. Moreover, the suppression of TNF-α and IL-1 production by inhibition of p38 MAPK may also contribute to reduced osteoclast numbers as both of these cytokines are known to induce osteoclast differentiation independently of RANKL [74–76]. Additional validation of p38 MAPK as a target as well as the identification of potential novel targets was provided by mice deficient in a downstream effector of p38 MAPK, MAPKAP kinase-2 (MK-2) as such mice showed lower severity scores and incidence in a CIA model [77]. These effects appeared to be due to MK-2 playing a crucial role in the induction of TNF-α as deletion of the MK-2 gene in mice led to a 90% reduction of serum TNF-α levels after LPS challenge [78].

Such validation of the therapeutic potential of p38 MAPK inhibitors in inflammatory disease has led to the development of a large number of inhibitory compounds, such as RWJ67657 and BIRB-796 have been tested in clinical studies in humans (Table 1) and found to be safe [79–83]. Indeed, a single dose application has proved sufficient at reducing p38 MAPK activation in leucocytes, cytokine production (IL-6, IL-8 and TNF-α), C-reactive protein release in serum as well as clinical symptoms (fever) upon endotoxin challenge in healthy volunteers [82, 83]. Hence, studies to evaluate the safety and efficacy of these and other compounds such as VX-745, VX-702 and SCIO-469 in patients with RA are currently underway with several companies [84]. To date, efficacy of these compounds in RA appears limited with respect to placebo controls and there are significant adverse reactions and hepatotoxicity problems (reviewed in reference [21]). Moreover, because p38 MAPK plays key roles in cellular processes unrelated to

inflammation, it is likely that such compounds could result in serious side-effects. Thus, the search for more inflammation-specific p38 MAPK inhibitors is underway, focusing on the therapeutic potential of different isoforms of p38 MAPK (p38α and p38γ appear to be the dominant isoforms expressed and activated in synovial tissue from RA patients) and regulatory elements upstream of p38 activation [54, 85–87]. Thus, for example, both MKK-3 and MKK-6 have been found to be activated in synovial fibroblasts and the synovium of RA patients [88] and the use of dominant negative mutants revealed that they are both able to activate p38 MAPK in synovial fibroblasts [89]. The relative importance of MKK-3 *vs* MKK-6 in p38 MAPK signalling in inflammation, however, was dissected by exploiting MKK-3 knockout mice to show that LPS-stimulation of IL-1 and IL-6 production is not affected by MKK-3 deficiency *in vivo*. In contrast, MKK-3 deficiency not only leads to ablation of IL-1 and IL-6 production in response to TNF-α but also to reduced severity of disease in a passive model (K/BxN) of arthritis [90]. Taken together, these *in vivo* data suggest that MKK-3 is a more selective candidate target for inhibition of the p38 MAPK signalling pathway in inflammation.

Conclusions and future perspectives

Clearly, MAPKs play important roles in transducing inflammation and joint destruction and therefore are key molecular targets for therapeutic intervention in chronic inflammatory diseases such as RA. However, there are multiple isoforms of these kinases (ERK-1–8, JNK1–3 and p38-α, -β, -γ and -δ) that have been implicated in the regulation of a plethora of essential cellular responses [91–94], dictating that inhibitors that simply ablate ERK, JNK or p38 MAPK activities are likely to have serious side effects. Moreover, most of the current inhibitor compounds under trial are competitors for the adenosine triphosphate (ATP)-binding site of p38 MAPK and hence are likely to induce side effects due to cross inhibition of other classes of kinases *in vivo* [19]. Interestingly, the ERK/MEK inhibitors are not ATP competitive but rather act to prevent the MAPKKK, Raf1 from activating MEK and thus their observed relative lack of side effects probably reflects that they do not non-specifically inhibit other kinases *in vivo* [19]. Similarly, peptide-based approaches to disrupting JNK signalling by targeting scaffold protein interactions show promise but as yet have not been tested in *in vivo* models of inflammation [21]. The future therefore lies in the development of inhibitors that target inflammation-restricted MAPK signalling and, in particular, the aberrant inflammation

related to chronic disease whilst leaving intact the 'healthy' inflammation that is essential for fighting infection. The recent explosion in identifying isoforms of the major components (MAPKs, MAPKKs, MAPKKKs and DUSPs) of MAPK cascades [21, 24, 93–95] and the delineation of their specific roles in coupling individual receptors to particular ERK, JNK and p38 MAPK signals and their functional outcomes will ultimately lead to the unravelling of disease pathogenesis and the development of specific inhibitors that will provide novel, safe small-molecule therapeutics for arthritis.

Rheumatology key messages

- MAPKs play key roles in the molecular and cellular events underpinning the pathogenesis of RA.
- MAPKs are rational targets of drug design for novel therapies in RA.

Acknowledgements

References

1 McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol 2007;7:429–42.

2 Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–61.

3 Cai L, Yin JP, Starovasnik MA et al. Pathways by which interleukin 17 induces articular cartilage breakdown in vitro and in vivo. Cytokine 2001;16:10–21.

4 Muller-Ladner U, Pap T, Gay RE, Neidhart M, Gay S. Mechanisms of disease: the molecular and cellular basis of joint destruction in rheumatoid arthritis. Nat Clin Pract Rheumatol 2005;1:102–10.

5 Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM. Mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest 2003;111:821–31.

6 Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. IL-1 mediates TNF-induced osteoclastogenesis. J Clin Invest 2005;115:282–90.

7 Wong PK, Quinn JM, Sims NA, van Nieuwenhuijze A, Campbell IK, Wicks IP. Interleukin-6 modulates production of T lymphocyte-derived cytokines in antigen-induced arthritis and drives inflammation-induced osteoclastogenesis. Arthritis Rheum 2006;54:158–68.

8 Sato K, Suematsu A, Okamoto K et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 2006;203:2673–82.

9 Liu JO. The yins of T cell activation. Sci STKE 2005;2005:re1.

10 Harnett MM, Katz E, Ford CA. Differential signalling during B-cell maturation. Immunol Lett 2005;98:33–44.

11 Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity 2004;21:467–76.

12 Lubberts E, Koenders MI, van den Berg WB. The role of T-cell interleukin-17 in conducting destructive arthritis: lessons from animal models. Arthritis Res Ther 2005;7:29–37.

13 Goodridge HS, Harnett W, Liew FY, Harnett MM. Differential regulation of interleukin-12 p40 and p35 induction via Erk mitogen-activated protein kinase-dependent and -independent mechanisms and the implications for bioactive IL-12 and IL-23 responses. Immunology 2003;109:415–25.

14 Feng GJ, Goodridge HS, Harnett MM et al. Extracellular signal-related kinase (ERK) and p38 mitogen-activated protein (MAP) kinases differentially regulate the lipopolysaccharide-mediated induction of inducible nitric oxide synthase and IL-12 in macrophages: Leishmania phosphoglycans subvert macrophage IL-12 production by targeting ERK MAP kinase. J Immunol 1999;163:6403–12.

15 Han J, Lee JD, Bibbs L, Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science 1994;265:808–11.

16 Lee JC, Laydon JT, McDonnell PC et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 1994;372:739–46.

17 Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. Annu Rev Immunol 2002;20:55–72.

18 Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 2002;298:1911–2.

19 Ropert C. Translating MAPK inhibitors to anti-inflammatory compounds. Curr Enz Inhib 2005;1:75–84.

20 Sweeney SE, Firestein GS. Signal transduction in rheumatoid arthritis. Curr Opin Rheumatol 2004;16:231–7.

21 Sweeney SE, Firestein GS. Mitogen activated protein kinase inhibitors: where are we now and where are we going? Ann Rheum Dis 2006;65(Suppl 3):iii83–8.

22 Vincenti MP, Brinckerhoff CE. The potential of signal transduction inhibitors for the treatment of arthritis: Is it all just JNK? J Clin Invest 2001;108:181–3.

23 Kumar S, Blake SM, Emery JG. Intracellular signaling pathways as a target for the treatment of rheumatoid arthritis. Curr Opin Pharmacol 2001;1:307–13.

24 Jeffrey KL, Camps M, Rommel C, Mackay CR. Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discov 2007;6:391–403.

25 Jeffrey KL, Brummer T, Rolph MS et al. Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. Nat Immunol 2006;7:274–83.

26 Owens DM, Keyse SM. Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. Oncogene 2007;26:3203–13.

27 Camps M, Nichols A, Arkinstall S. Dual specificity phosphatases: a gene family for control of MAP kinase function. FASEB J 2000;14:6–16.

28 Zhou B, Wang ZX, Zhao Y, Brautigan DL, Zhang ZY. The specificity of extracellular signal-regulated kinase 2 dephosphorylation by protein phosphatases. J Biol Chem 2002;277:31818–25.

29 Saxena M, Mustelin T. Extracellular signals and scores of phosphatases: all roads lead to MAP kinase. Semin Immunol 2000;12:387–96.

30 Schett G, Tohidast-Akrad M, Smolen JS et al. Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-JUN N-terminal kinase, and p38 mitogen-activated protein kinase, in synovial tissue and cells in rheumatoid arthritis. Arthritis Rheum 2000;43:2501–12.

31 Geng Y, Valbracht J, Lotz M. Selective activation of the mitogen-activated protein kinase subgroups c-Jun NH2 terminal kinase and p38 by IL-1 and TNF in human articular chondrocytes. J Clin Invest 1996;98:2425–30.

32 Masuko-Hongo K, Berenbaum F, Humbert L, Salvat C, Goldring MB, Thirion S. Up-regulation of microsomal prostaglandin E synthase 1 in osteoarthritic human cartilage: critical roles of the ERK-1/2 and p38 signaling pathways. Arthritis Rheum 2004;50:2829–38.

33 Liacini A, Sylvester J, Li WQ et al. Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. Exp Cell Res 2003;288:208–17.

34 Mengshol JA, Vincenti MP, Coon CI, Barchowsky A, Brinckerhoff CE. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappaB: differential regulation of collagenase 1 and collagenase 3. Arthritis Rheum 2000;43:801–11.

35 Miller C, Zhang M, He Y et al. Transcriptional induction of cyclooxygenase-2 gene by okadaic acid inhibition of phosphatase activity in human chondrocytes: co-stimulation of AP-1 and CRE nuclear binding proteins. J Cell Biochem 1998;69:392–413.

36 Gortz B, Hayer S, Tuerck B, Zwerina J, Smolen JS, Schett G. Tumour necrosis factor activates the mitogen-activated protein kinases p38alpha and ERK in the synovial membrane in vivo. Arthritis Res Ther 2005;7:R1140–7.

37 Salojin KV, Owusu IB, Millerchip KA, Potter M, Platt KA, Oravecz T. Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. J Immunol 2006;176:1899–907.

38 Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR. A synthetic inhibitor of the mitogen-activated protein kinase cascade. Proc Natl Acad Sci USA 1995;92:7686–9.

39 Favata MF, Horiuchi KY, Manos EJ et al. Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J Biol Chem 1998;273:18623–32.

40 Cruz CD, Neto FL, Castro-Lopes J, McMahon SB, Cruz F. Inhibition of ERK phosphorylation decreases nociceptive behaviour in monoarthritic rats. Pain 2005;116:411–9.

41 Jaffee BD, Manos EJ, Collins RJ et al. Inhibition of MAP kinase kinase (MEK) results in an anti-inflammatory response in vivo. Biochem Biophys Res Commun 2000;268:647–51.

42 Pelletier JP, Fernandes JC, Brunet J et al. In vivo selective inhibition of mitogen-activated protein kinase kinase 1/2 in rabbit experimental osteoarthritis is associated with a reduction in the development of structural changes. Arthritis Rheum 2003;48:1582–93.

43 Barchowsky A, Frleta D, Vincenti MP. Integration of the NF-kappaB and mitogen-activated protein kinase/AP-1 pathways at the collagenase-1 promoter: divergence of IL-1 and TNF-dependent signal transduction in rabbit primary synovial fibroblasts. Cytokine 2000;12:1469–79.

44 Pillinger MH, Rosenthal PB, Tolani SN et al. Cyclooxygenase-2-derived E prostaglandins down-regulate matrix metalloproteinase-1 expression in fibroblast-like synoviocytes via inhibition of extracellular signal-regulated kinase activation. J Immunol 2003;171:6080–9.

45 Han Z, Boyle DL, Chang L et al. c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. J Clin Invest 2001;108:73–81.

46 Rifas L, Arackal S. T cells regulate the expression of matrix metalloproteinase in human osteoblasts via a dual mitogen-activated protein kinase mechanism. Arthritis Rheum 2003;48:993–1001.

47 Han Z, Boyle DL, Aupperle KR, Bennett B, Manning AM, Firestein GS. Jun N-terminal kinase in rheumatoid arthritis. J Pharmacol Exp Ther 1999;291:124–30.

48 David JP, Sabapathy K, Hoffmann O, Idarraga MH, Wagner EF. JNK1 modulates osteoclastogenesis through both c-Jun phosphorylation-dependent and -independent mechanisms. J Cell Sci 2002;115:4317–25.

49 Han Z, Chang L, Yamanishi Y, Karin M, Firestein GS. Joint damage and inflammation in c-Jun N-terminal kinase 2 knockout mice with passive murine collagen-induced arthritis. Arthritis Rheum 2002;46:818–23.

50 Koller M, Hayer S, Redlich K *et al.* JNK1 is not essential for TNF-mediated joint disease. Arthritis Res Ther 2005;7:R166–73.

51 Hammaker DR, Boyle DL, Chabaud-Riou M, Firestein GS. Regulation of c-Jun N-terminal kinase by MEKK-2 and mitogen-activated protein kinase kinase kinases in rheumatoid arthritis. J Immunol 2004;172:1612–8.

52 Sundarrajan M, Boyle DL, Chabaud-Riou M, Hammaker D, Firestein GS. Expression of the MAPK kinases MKK-4 and MKK-7 in rheumatoid arthritis and their role as key regulators of JNK. Arthritis Rheum 2003;48:2450–60.

53 Inoue T, Hammaker D, Boyle DL, Firestein GS. Regulation of JNK by MKK-7 in fibroblast-like synoviocytes. Arthritis Rheum 2006;54:2127–35.

54 Korb A, Tohidast-Akrad M, Cetin E, Axmann R, Smolen J, Schett G. Differential tissue expression and activation of p38 MAPK alpha, beta, gamma, and delta isoforms in rheumatoid arthritis. Arthritis Rheum 2006;54:2745–56.

55 Westra J, Doornbos-van der Meer B, de Boer P, van Leeuwen MA, van Rijswijk MH, Limburg PC. Strong inhibition of TNF-alpha production and inhibition of IL-8 and COX-2 mRNA expression in monocyte-derived macrophages by RWJ 67657, a p38 mitogen-activated protein kinase (MAPK) inhibitor. Arthritis Res Ther 2004;6:R384–92.

56 Campbell J, Ciesielski CJ, Hunt AE *et al.* A novel mechanism for TNF-alpha regulation by p38 MAPK: involvement of NF-kappa B with implications for therapy in rheumatoid arthritis. J Immunol 2004;173:6928–37.

57 Baldassare JJ, Bi Y, Bellone CJ. The role of p38 mitogen-activated protein kinase in IL-1 beta transcription. J Immunol 1999;162:5367–73.

58 Suzuki M, Tetsuka T, Yoshida S *et al.* The role of p38 mitogen-activated protein kinase in IL-6 and IL-8 production from the TNF-alpha- or IL-1beta-stimulated rheumatoid synovial fibroblasts. FEBS Lett 2000;465:23–7.

59 Westra J, Limburg PC, de Boer P, van Rijswijk MH. Effects of RWJ 67657, a p38 mitogen activated protein kinase (MAPK) inhibitor, on the production of inflammatory mediators by rheumatoid synovial fibroblasts. Ann Rheum Dis 2004;63:1453–9.

60 Ridley SH, Sarsfield SJ, Lee JC *et al.* Actions of IL-1 are selectively controlled by p38 mitogen-activated protein kinase: regulation of prostaglandin H synthase-2, metalloproteinases, and IL-6 at different levels. J Immunol 1997;158:3165–73.

61 Badger AM, Cook MN, Lark MW *et al.* SB 203580 inhibits p38 mitogen-activated protein kinase, nitric oxide production, and inducible nitric oxide synthase in bovine cartilage-derived chondrocytes. J Immunol 1998;161:467–73.

62 Matsumoto M, Sudo T, Saito T, Osada H, Tsujimoto M. Involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-kappa B ligand (RANKL). J Biol Chem 2000;275:31155–61.

63 Li X, Udagawa N, Itoh K *et al.* p38 MAPK-mediated signals are required for inducing osteoclast differentiation but not for osteoclast function. Endocrinology 2002;143:3105–13.

64 Ishimi Y, Miyaura C, Jin CH *et al.* IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 1990;145:3297–303.

65 Kumar S, Votta BJ, Rieman DJ, Badger AM, Gowen M, Lee JC. IL-1- and TNF-induced bone resorption is mediated by p38 mitogen activated protein kinase. J Cell Physiol 2001;187:294–303.

66 Roodman GD. Interleukin-6: an osteotropic factor? J Bone Miner Res 1992;7:475–8.

67 Tokuda H, Kanno Y, Ishisaki A, Takenaka M, Harada A, Kozawa O. Interleukin (IL)-17 enhances tumor necrosis factor-alpha-stimulated IL-6 synthesis via p38 mitogen-activated protein kinase in osteoblasts. J Cell Biochem 2004;91:1053–61.

68 McLay LM, Halley F, Souness JE *et al.* The discovery of RPR 200765A, a p38 MAP kinase inhibitor displaying a good oral anti-arthritic efficacy. Bioorg Med Chem 2001;9:537–54.

69 Mbalaviele G, Anderson G, Jones A *et al.* Inhibition of p38 mitogen-activated protein kinase prevents inflammatory bone destruction. J Pharmacol Exp Ther 2006;317:1044–53.

70 Medicherla S, Ma JY, Mangadu R *et al.* A selective p38 alpha mitogen-activated protein kinase inhibitor reverses cartilage and bone destruction in mice with collagen-induced arthritis. J Pharmacol Exp Ther 2006;318:132–41.

71 Badger AM, Griswold DE, Kapadia R *et al.* Disease-modifying activity of SB 242235, a selective inhibitor of p38 mitogen-activated protein kinase, in rat adjuvant-induced arthritis. Arthritis Rheum 2000;43:175–83.

72 Nishikawa M, Myoui A, Tomita T, Takahi K, Nampei A, Yoshikawa H. Prevention of the onset and progression of collagen-induced arthritis in rats by the potent p38 mitogen-activated protein kinase inhibitor FR167653. Arthritis Rheum 2003;48:2670–81.

73 Zwerina J, Hayer S, Redlich K *et al.* Activation of p38 MAPK is a key step in tumor necrosis factor-mediated inflammatory bone destruction. Arthritis Rheum 2006;54:463–72.

74 Jimi E, Nakamura I, Duong LT *et al.* Interleukin 1 induces multinucleation and bone-resorbing activity of osteoclasts in the absence of osteoblasts/stromal cells. Exp Cell Res 1999;247:84–93.

75 Kobayashi K, Takahashi N, Jimi E *et al.* Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000;191:275–86.

76 Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A. Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. J Biol Chem 2000;275:4858–64.

77 Hegen M, Gaestel M, Nickerson-Nutter CL, Lin LL, Telliez JB. MAPKAP kinase 2-deficient mice are resistant to collagen-induced arthritis. J Immunol 2006;177:1913–7.

78 Kotlyarov A, Neininger A, Schubert C *et al.* MAPKAP kinase 2 is essential for LPS-induced TNF-alpha biosynthesis. Nat Cell Biol 1999;1:94–7.

79 Schreiber S, Feagan B, D'Haens G *et al.* Oral p38 mitogen-activated protein kinase inhibition with BIRB 796 for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol 2006;4:325–34.

80 Parasrampuria DA, de Boer P, Desai-Krieger D, Chow AT, Jones CR. Single-dose pharmacokinetics and pharmacodynamics of RWJ 67657, a specific p38 mitogen-activated protein kinase inhibitor: a first-in-human study. J Clin Pharmacol 2003;43:406–13.

81 Fijen JW, Tulleken JE, Kobold AC *et al.* Inhibition of p38 mitogen-activated protein kinase: dose-dependent suppression of leukocyte and endothelial response after endotoxin challenge in humans. Crit Care Med 2002;30:841–5.

82 Fijen JW, Zijlstra JG, De Boer P *et al.* Suppression of the clinical and cytokine response to endotoxin by RWJ-67657, a p38 mitogen-activated protein-kinase inhibitor, in healthy human volunteers. Clin Exp Immunol 2001;124:16–20.

83 Branger J, van den Blink B, Weijer S *et al.* Anti-inflammatory effects of a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. J Immunol 2002;168:4070–7.

84 Smith RJ. Therapies for rheumatoid arthritis: hope springs eternal. Drug Discov Today 2005;10:1598–606.

85 Lee MR, Dominguez C. MAP kinase p38 inhibitors: clinical results and an intimate look at their interactions with p38alpha protein. Curr Med Chem 2005;12:2979–94.

86 Schieven GL. The biology of p38 kinase: a central role in inflammation. Curr Top Med Chem 2005;5:921–8.

87 Popa-Burke I, Birkos S, Blackwell L *et al.* Discovery of highly selective inhibitors of p38alpha. Curr Top Med Chem 2005;5:941–51.

88 Chabaud-Riou M, Firestein GS. Expression and activation of mitogen-activated protein kinase kinases-3 and -6 in rheumatoid arthritis. Am J Pathol 2004;164:177–84.

89 Inoue T, Hammaker D, Boyle DL, Firestein GS. Regulation of p38 MAPK by MAPK kinases 3 and 6 in fibroblast-like synoviocytes. J Immunol 2005;174:4301–6.

90 Inoue T, Boyle DL, Corr M *et al.* Mitogen-activated protein kinase kinase 3 is a pivotal pathway regulating p38 activation in inflammatory arthritis. Proc Natl Acad Sci USA 2006;103:5484–9.

91 Aouadi M, Binetruy B, Caron L, Le Marchand-Brustel Y, Bost F. Role of MAPKs in development and differentiation: lessons from knockout mice. Biochimie 2006;88:1091–8.

92 Meloche S, Pouyssegur J. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. Oncogene 2007;26:3227–39.

93 Cuevas BD, Abell AN, Johnson GL. Role of mitogen-activated protein kinase kinases in signal integration. Oncogene 2007;26:3159–71.

94 Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. Oncogene 2007;26:3100–12.

95 Bogoyevitch MA. The isoform-specific functions of the c-Jun N-terminal Kinases (JNKs): differences revealed by gene targeting. Bioessays 2006;28:923–34.

96 Branger J, van den Blink B, Weijer S *et al.* Inhibition of coagulation, fibrinolysis, and endothelial cell activation by a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. Blood 2003;101:4446–8.

97 Nikas SN, Drosos AA. SCIO-469 Scios Inc. Curr Opin Investig Drugs 2004;5:1205–12.

98 Ding C. Drug evaluation: VX-702, a MAP kinase inhibitor for rheumatoid arthritis and acute coronary syndrome. Curr Opin Investig Drugs 2006;7:1020–5.

99 Haddad JJ. VX-745. Vertex Pharmaceuticals. Curr Opin Investig Drugs 2001;2:1070–6.

100 Hommes D, van den Blink B, Plasse T *et al.* Inhibition of stress-activated MAP kinases induces clinical improvement in moderate to severe Crohn's disease. Gastroenterology 2002;122:7–14.

101 Group PS. The safety and tolerability of a mixed lineage kinase inhibitor (CEP-1347) in PD. Neurology 2004;62:330–2.

102 LoRusso PM, Adjei AA, Meyer MB *et al.* A phase 1 clinical and pharmacokinetic evaluation of the oral MEK inhibitor, CI-1040, administered for 21 consecutive days, repeated every 4 weeks in patients with advanced cancer. 2002 ASCO Annual Meeting; 2002; 2002.

103 Lorusso PM, Adjei AA, Varterasian M *et al.* Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. J Clin Oncol 2005;23:5281–93.

104 Rinehart J, Adjei AA, Lorusso PM *et al.* Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. J Clin Oncol 2004;22:4456–62.