Macrophage migration inhibitory factor (MIF) as a therapeutic target for rheumatoid arthritis and systemic lupus erythematosus

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Abstract

Introduction.—Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory cytokine with upstream regulatory roles in innate and adaptive immunity and is implicated in the pathogenesis of autoimmune diseases including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Several classes of MIF inhibitors such as small molecule inhibitors and peptide inhibitors are in clinical development.

Areas Covered.—The role of MIF in the pathogenesis of RA and SLE is examined; the authors review the structure, physiology and signaling characteristics of MIF and the related cytokine D-DT/MIF-2. The preclinical and clinical trial data for MIF inhibitors are also reviewed; information was retrieved from PubMed and ClinicalTrials.gov using the keywords MIF, D-DT/MIF-2, CD74, CD44, CXCR2, CXCR4, Jab-1, rheumatoid arthritis, systemic lupus erythematosus, MIF inhibitor, small molecule, anti-MIF, anti-CD74, and peptide inhibitor.

Expert Opinion.—Studies in mice and genetic and clinical studies in humans demonstrate the therapeutic potential of MIF inhibition for RA and SLE. MIF- directed approaches could be particularly efficacious in patients with high expression MIF genetic polymorphisms. In patients with RA and SLE and high expression MIF alleles, targeted pharmacologic MIF inhibition could be part of a precision medicine approach. Anti-MIF pharmacotherapies could ultimately also be steroid-sparing in patients with chronic glucocorticoid dependence and/or refractory autoimmune disease.

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Keywords

CD44; CD74; CXCR2; CXCR4; CXCR7; macrophage migration inhibitory factor (MIF); D-DT/MIF-2; precision medicine

1. Introduction.

Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory cytokine important in the upstream regulation of both innate and adaptive immunological responses and has been implicated in the pathogenesis of autoimmune diseases including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). MIF was the first "cytokine" function to be described in a report published in the *Bulletin of the Johns Hopkins Hospital* in 1932, which highlighted the ability of *Mycobacteria*-sensitized lymphocytes to arrest tissue macrophage migration *in vitro*.[1] While MIF has direct chemotactic effects similar to macrophage inflammatory protein-1α/β (MIP-1α/β) and monocyte chemoattractant protein 1 (MCP-1), its eponymic function ultimately was found to arise from its ability to desensitize the chemokine receptors CXCR2 and CXCR4.[2] The unambiguous molecular cloning of the human *MIF* gene was achieved and reported in 1993 following identification of its unique role in regulating the effects of endogenous cortisol.[3]

1.1 MIF Structure.

The MIF structure comprises a homo-trimer with a primary sequence that is well-conserved across evolutionary phylogeny ranging from mammals to invertebrates, plants, and protozoan species.[4] The human MIF monomer subunit is composed of 114 amino acids and has a mass of 12.5 kilo-Daltons.[5, 6] At the interface between monomer subunits, there is a tautomerase enzymatic activity. This enzymatic activity may be of vestigial function, as its physiologic substrate remains unknown.[7] The N-terminal proline residue within the tautomerase active site appears to interface with the region of the protein that binds to the MIF cognate receptor CD74.[8] (Figure 1) Mutation of this proline resulted in decreased MIF-CD74 binding *in vitro* and reduced MIF function in a genetic mouse model.[9]

1.2 MIF Physiology.

Systemic MIF levels in humans follow a diurnal rhythm, peaking in the early morning hours in coordination with the levels of endogenous cortisol.[10] The normal MIF serum levels are in the 2–6 ng/mL range, but in the setting of acute stressors and/or inflammatory stimuli such as bacterial lipopolysaccharide (LPS), tumor necrosis factor (TNF), or interferon-γ (IFN- γ),[11] MIF levels increase several-fold subsequent to release from many cellular and tissue subtypes,[12] including monocytes/macrophages, B and T lymphocytes, granulocytes, platelets, dendritic cells, endothelial cells, and mesenchymal cells.[10, 13] Unlike most inflammatory cytokines, MIF is constitutively synthesized and stored in pre-formed intracellular pools. Following an acute stressor or inflammatory stimulus, MIF is then released from the cells through a process involving the Golgi-associated chaperone protein p115,[14] though in some cases export also may involve membrane blebbing and exosomes. [15]

Following its release, MIF acts in an autocrine and/or paracrine fashion to upregulate the synthesis of secondary inflammatory cytokines.[16] MIF also has an upstream role in regulating its own synthesis.[17] In a murine sepsis model, genetic *Mif* deletion resulted in decreased plasma levels of TNF, without an effect on IL-6 and IL-12 levels.[18] However, *Mif* deletion did result in decreased renal IL-6 levels in a mouse glomerulonephritis model, [19] and decreased serum IL-12 levels in response to systemic infection.[20] Overall studies have observed *Mif* knockout mice to have decreased macrophage synthesis of TNF, IL-1β, and prostaglandin E_2,[21] and decreased lymphocyte production of IL-1β, IL-6, IL-17, and IL-23 in particular.[22] MIF-activated lymphocytes secrete IL-2, IL-17, and IFN-γ.[22, 23] MIF was shown to have a role in promoting neutrophil IL-1β release in a murine model of acute gout.[24] Though the mechanism for this remains loosely defined, MIF was found to co-localize with the NLRP3 and ASC proteins and regulated their expression and activation. [25] In human patients with SLE, immune complexes containing anti-U1-snRNP antibodies were shown to upregulate MIF expression in monocytes, leading to the increased activation of the NLRP3 inflammasome and resulting increased production of IL-1β.[26, 27]

In addition to these roles, MIF signaling is important for the appropriate surface expression of pattern recognition receptors in the innate immune response against infections. MIF stimulates increased activity of the PU.1 transcription factor, leading to upregulation of Toll-like receptor 4 (TLR4). MIF-deficient macrophages are less responsive to bacterial LPS due to downregulated surface expression of TLR4[28] and are similarly hypo-responsive to mycobacterial β-glucans due to downregulation of surface dectin-1.[29]

MIF signaling leads to enhanced cell survival and proliferation through several related pathways which differ between cell types. MIF signaling promotes cell survival through direct activation of the Akt pathway[30] and prevents apoptosis through inhibition of the tumor suppressor protein p53, a mechanism which is common between many cell types.[21] Given these roles in cell proliferation and survival, MIF also contributes to the inflammatory pathogenesis of different cancers.[31] High MIF expression has been reported in various tumors including glioblastoma multiforme, melanoma, and prostate, gastric, pancreatic, and lung cancers.[32–34]

In endothelial cells, MIF affects the expression of adhesion molecules necessary for leukocyte and lymphocyte migration and trafficking into inflamed tissues. Endogenous MIF signaling leads to endothelial cell expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin, as well as synthesis of the chemokines IL-8 and MCP-1. These effects facilitate leukocyte recruitment into inflamed tissues. Conversely, exogenous MIF signaling in the setting of concurrent TNF leads to endothelial cell expression of P-selectin, facilitating leukocyte rolling.[35]

1.3   D-DT/MIF-2.

A more recently characterized member of the MIF family that also binds to CD74 with high affinity is D-dopachrome tautomerase (D-DT) (also called MIF-2).[36] MIF-2 has many overlapping signaling functions as compared with MIF, as well as a described role in tissue protection from injury following ischemia-reperfusion.[37] (Figure 2) Like MIF, D-DT/ MIF-2 is produced by many tissue and immune cell types, and its circulating levels in blood

are similar to MIF.[38] D-DT/MIF-2 may also share a pro-oncogenic role with MIF, with signaling by both cytokines exerting a cooperative role in tumorigenesis.[39] D-DT/MIF-2 expression levels have been found to be increased in glioblastoma multiforme [40], pancreatic adenocarcinoma,[41] and renal cell carcinoma in particular.[39]

Despite having many overlapping functions, some studies report distinct roles for D-DT/MIF-2 in adipogenesis in particular.[42] D-DT/MIF-2 also lacks the *pseudo*-(E)LR motif present in MIF that is required for CXCR2 binding and activation.[43] As such, its role in inflammation and migration may be narrower than MIF. Another difference is that common genetic polymorphisms have not been described in the human D-DT/MIF-2 gene.

2. MIF Genetics.

During an investigation of RA patients, sequencing of the *MIF* locus revealed a variant four nucleotide microsatellite within the promoter region. This microsatellite was comprised of a C-A-T-T repeat present in 5–8 copies (*e.g.*, −794 $CATT_{5-8}$) (*rs5844572*); higher numbers of CATT repeats were found to be associated with higher baseline and stimulated *MIF* transcription.[44] These variant *MIF* alleles are common throughout different human populations. High expression *MIF* alleles in particular have been implicated in the pathogenesis of various autoimmune diseases, and the frequency of these alleles in human populations can exceed 20%.[45] The transcription factor ICBP90 was recently found to be the major protein regulating *MIF* mRNA transcription from this $CATT_{5-8}$ promoter locus. [46] (Figure 3)

A second *MIF* promoter single nucleotide polymorphism (SNP) at −173*G/C (*rs755622*) has been identified, with the *C allele in linkage disequilibrium with the −794 $CATT_7$ microsatellite.[47] Multiple studies have reported the −173*C allele and $CATT_7$ in association with increased systemic MIF and an increased propensity for and/or severity of inflammatory rheumatological disease.[47–54]

3. MIF Receptors and Ligands.

3.1 CD74 and CD44.

The cognate MIF receptor comprises a signaling complex of ligand-binding CD74 coupled to signal-transducing CD44.[55, 56] CD74 is the cell surface expressed form of the class II invariant chain, its intracellular function to facilitate the loading of peptide fragments into MHC class II for antigen presentation. In the context of its function as the cognate MIF receptor, CD74 is expressed on the cell surface independent of MHC class II expression and can be found on virtually all nucleated cell types, either constitutively or after stimulatory induction.[57, 58]

CD44 is a broadly expressed cell adhesion molecule that mediates the activation of Src family kinases.[59] The gene for CD44 comprises 20 exons, 10 of which can undergo alternative splicing to generate various isoforms which differ in the length and structure of the protein ectodomain.[60] MIF signaling increases synthesis of the Tra2α splicing factor, which in turn leads to the transcription of CD44v3-v6 isoforms. These larger isoforms of

CD44 are limited to expression in epithelium, proliferating cells, and in certain malignancies. The larger ectodomains facilitate extracellular matrix migration and provide binding sites for matrix metalloproteinases (MMPs) and growth factors such as fibroblast growth factor (FGF).[60]

Upon ligand binding with MIF, CD74 recruits CD44, whereupon both proteins become phosphorylated at their intracellular motifs and initiate downstream signal transduction.[61] (Figure 4) In monocytes and stromal cells such as fibroblasts, initial activation of CD44-associated Src tyrosine kinase and MEK leads to phosphorylation of ERK1/2 MAP kinases, activation of cytosolic phospholipase A_2 (cPLA$_2$), and the inhibition of p53 (blocking activation-induced apoptosis and contributing to MIF's inflammatory and pro-survival effects).[62]

In B cells, CD44-associated Syk tyrosine kinase leads to Akt phosphorylation and downstream NFκB activation.[63] At least among B cells, MIF binding to CD74 also results in the intra-membrane cleavage of CD74 via the SPPL2a protease to produce a 42 amino acid intracellular domain which has a role in the positive regulation of B cell maturation.[64] This fragment translocates to the nucleus to activate p65-NFκB, upregulate TAp63, and stimulate Bcl-2 leading to enhanced cell survival.[65] Another signaling pathway in B cells involves the engagement of the tyrosine kinase receptor c-Met with CD74/CD44, which permits sensitization of c-Met to autocrine hepatocyte growth factor/scatter factor (HGF). Signaling through the latter supplements the proliferation and survival mechanisms in B cells.[66]

A soluble form of the CD74 ectodomain (sCD74) formed by apparent membrane proteolysis also has been described, initially in the context of patients with autoimmune hepatitis and primary biliary cholangitis.[67] This sCD74 receptor binds to extra-cellular MIF, neutralizes its activity, and may function in regulating systemic MIF signaling.[68] There is evidence that SPPL2a protease cleavage of CD74 may account for the formation of sCD74.[64]

3.2 CXCR2, CXCR4, and CXCR7.

The CXCR2, CXCR4, and CXCR7 chemokine receptors associate with CD74, and MIF is a non-cognate ligand for these receptors.[69] MIF signaling through the CD74-CXCR2 complex elicits monocyte/macrophage chemotaxis, and migration arrest results from CXCR2 desensitization.[2] The binding of MIF to CXCR2 requires the presence of the *pseudo*-(E)LR motif (Asp-44-X-Arg-11) formed by the folding of non-adjacent aspartate and arginine residues in the MIF protein.[43] The interaction between MIF and CXCR4 requires an arginine-leucine-arginine (RLR) structural motif that binds to the N-terminus of CXCR4.[70] MIF signaling through the CD74-CXCR4-CXCR7 complex has been demonstrated to have a role in lymphocyte chemotaxis particularly in B cells.[71] These chemokine receptors are variably expressed on different stromal and leukocyte populations, conferring tissue specificity to MIF action in different physiologic and pathologic contexts. [2, 72]

3.3 Jab-1.

In the cytosol, endogenous MIF can bind to Jun-c activation domain-binding protein 1 (Jab-1) and inhibits its function, resulting in the downregulation of Jab-1 signaling pathways. The MIF and Jab-1 interaction leads to the reduced transcription of AP-1 specific pathways, while the NFκB pathway remains unaffected. In addition, MIF interaction with Jab-1 leads to the inhibition of JNK signaling pathways, which are otherwise activated by unbound Jab-1. Another activity of Jab-1 involves the degradation of the cyclin dependent kinase inhibitor p27Kip1, which promotes cell proliferation. The MIF-Jab-1 interaction thus opposes this latter effect, causes persistence of p27Kip1 rather than degradation, and results in cell cycle growth arrest.[73] Overall, the MIF-Jab-1 interaction appears to counter-regulate the inflammatory effects of MIF signaling through the cognate CD74 receptor.

4. MIF and Steroids.

MIF antagonizes and regulates the immunosuppressive effects of endogenous cortisol. MIF suppresses the cortisol-induced expression of the NFκB inhibitor IκB, resulting in increased NFκB-associated transcription of inflammatory genes.[74] MIF also suppresses the glucocorticoid-induced expression of MAP kinase phosphatase-1, which normally dephosphorylates and inactivates the inflammatory ERK1/2, JNK, and p38 MAP kinase signaling pathways.[75, 76] MIF activation of ERK1/2 MAP kinase reverses the corticosteroid-mediated suppression of phospholipase A_2 activity, resulting in the enhanced synthesis of arachidonic acid, which promotes the activation of JNK-mediated inflammatory signaling pathways.[62]

Wang and colleagues studied human patients with SLE and reported higher MIF levels in serum and in peripheral blood mononuclear cells from patients with "steroid-resistant" disease, as well as increased NFκB and decreased IκB levels in this group.[77] Among SLE patients, systemic MIF levels have been considered as a potential biomarker for steroid-resistant disease.[78] Griga and colleagues studied patients with Crohn's Disease and determined that those with the −173*C *MIF* promoter SNP, which is in linkage disequilibrium with the high expression, functional $CATT_7$ microsatellite, had greater overall glucocorticoid requirements for disease management compared to those without this variant.[79]

5. MIF and Rheumatoid Arthritis (RA).

Collagen-induced arthritis (CIA) is a mouse model for RA that follows immunization with a mixture of complete Freund's adjuvant and type II collagen. Affected mice develop inflammatory arthritis in the context of anti-type II collagen auto-antibodies and a primarily T cell-driven response. Genetic *Mif* deficiency reduces arthritis severity in this model and in the related adjuvant-induced arthritis (AIA) animal model, revealing MIF to be a critical mediator of inflammation and joint destruction in these diseases.[80–82]

Patients with RA who carry the $CATT_5$ allele have been reported to have milder, non-erosive disease, while patients with $CATT_{6–8}$ alleles have more severe and erosive phenotypes.[44] These results were subsequently confirmed by Radstake and colleagues, in which the high

expression $CATT_7$ allele was correlated with increased circulating MIF levels and more severe erosive radiologic joint damage.[49]

Patients with RA have elevated serum and synovial tissue MIF levels compared to healthy controls,[83, 84] and patients with high expression *MIF* alleles have increased serum levels compared to those with low expression alleles.[83] MIF is present at increased concentrations within synovial macrophages and fibroblast-like synoviocytes (FLS) of patients with RA, as well as within the synovial fluid of inflamed joints.[85]

MIF has numerous roles in the maintenance of inflammation within the synovial microenvironment of the RA joint. MIF induces expression of phospholipase A_2 and cyclooxygenase-2 (COX-2) in FLS cells, leading to increased expression of IL-1, IL-6, IL-8, prostaglandin E_2, and MMPs, the latter directly contributing to cartilage destruction and bone erosions. MIF signaling leads to FLS cell proliferation and inhibits apoptosis, resulting in synovial hyperplasia and pannus formation.[86]

MIF signaling also leads to upregulated expression of MMP-9 and MMP-13 from murine osteoblasts,[87] a mechanism that may be involved in the development of bone erosions in human patients with RA. Another mechanism may involve T cell-derived MIF upregulating RANKL expression, which leads to osteoclastogenesis.[88]

Treatment of cultured FLS from patients with RA with anti-MIF results in decreased IL-1β-mediated transcription of phospholipase A_2 and COX-2.[89] The role of MIF signaling in FLS contributes to synovial hyperplasia and the pannus formation that is one of the hallmarks of RA. Rheumatoid pannus demonstrates features of a locally-invasive tumor.[90] MIF signaling resulting in cell proliferation, angiogenesis,[91] and inhibition of apoptosis contributes to this invasive pannus formation, and these pathophysiological mechanisms may be similar to MIF implicated malignant transformation.

6. MIF and Systemic Lupus Erythematosus (SLE).

In two different mouse models of SLE (MRL/*lpr* and NZB/NZW F1), treatment with either an anti-MIF antibody or a small molecule MIF antagonist resulted in a similar degree of decreased leukocyte recruitment to the kidneys, improved renal function and histological glomerulonephritis, and reduced systemic inflammatory cytokine and chemokine production, in particular TNF and the monocyte chemoattractant CCL2. However, MIF inhibition did not affect T and B cell activation, anti-dsDNA antibody levels, or glomerular IgG deposition.[92] Genetic *Mif* deficiency in MRL/*lpr* mice resulted in a similar amelioration of inflammation as compared with pharmacologic MIF inhibition.[19, 92]

A role for MIF in the pathophysiology of human SLE was supported by the finding of a correlation between increased systemic MIF levels and Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) disease activity index scores.[93] In a genetic study by Sreih and colleagues, a cohort of 1,369 patients with SLE were assessed for *MIF* genotype and circulating MIF levels. Among patients with established disease, high expression *MIF* alleles ($CATT_{6-8}$) and increased circulating MIF levels correlated with serositis, nephritis, and central nervous system involvement.[94]

Elevated MIF levels in the serum,[95, 96] kidneys,[97] and urine[98] have been correlated with active systemic disease as well.

However, among patients with high expression *MIF* alleles (CATT$_7$, −173*C haplotype), there was a lower rate of SLE and antinuclear antibody (ANA) positivity compared to healthy controls.[94] Dysregulated clearance of apoptotic debris has been implicated in the pathogenesis of the loss of self-tolerance and autoantibody formation against nuclear components in SLE.[99] It has been hypothesized that among patients without SLE, high systemic MIF expression facilitates the clearance of apoptotic debris and immune complexes leading to a reduced risk of developing pathologic autoantibodies and loss of immune tolerance.[94] Conversely, low systemic MIF expression may lead to the persistence of apoptotic and immune complex debris, resulting in B cell expansion and the development of antinuclear antibodies.

A particular role for MIF in mediating the inflammation of glomerulonephritis has been suggested by studies correlating immune-mediated renal damage with local tissue MIF levels. In human patients with various forms of proliferative glomerulonephritis, Lan and colleagues associated up-regulated tissue MIF expression with renal insufficiency, cellular damage, and leukocyte infiltrates.[97] These mechanisms may similarly account for the development of glomerulonephritis in the context of SLE.

7. MIF Directed Therapeutics.

MIF as a therapeutic target could be particularly advantageous for patients with higher background and inducible MIF expression, such as among those with CATT$_{6-8}$ alleles or the −173*C/CATT$_7$ haplotype. Furthermore, MIF inhibition might be particularly efficacious for patients with autoimmune inflammatory conditions and concurrent steroid refractoriness or long-standing glucocorticoid dependence.[100] (Table 1)

7.1 Small Molecule Inhibitors.

Small molecule MIF inhibitors have been identified through various screening strategies, [101–103] and there has been a particular focus upon exploiting the MIF tautomerase site, which is involved in the interaction with CD74, in the design of such inhibitors.[104]

The first small molecule inhibitor of MIF that was identified was the acetaminophen metabolite N-acetyl-p-benzoquinone (NAPQI), which was reported to irreversibly bind to the proline-1 residue in the MIF tautomerase active site and decrease its cell-binding activity. [105] This finding opened the way for further investigations of small molecule inhibitors involving the tautomerase active site.

Subsequently, the first synthetically designed small molecule MIF inhibitor to be described was the isoxazoline compound ISO-1. ISO-1 binds in the MIF tautomerase active site and inhibits downstream signaling effects.[106] In the experimental autoimmune encephalomyelitis (EAE) animal model of multiple sclerosis (MS), ISO-1 treatment reduced disease severity and duration. Further, ISO-1 decreased synthesis of TNF, IFN-γ, IL-4, and

IL-17 from murine splenocytes of mice with EAE.[107] ISO-1 treatment also has been shown to reduce proliferation and mitogenic signaling in glioblastoma cells.[32]

The small molecule antagonist 4-iodo-6-phenylpyrimidine (4-IPP) covalently binds to the N-terminal proline within both the MIF and D-DT/MIF-2 tautomerase sites and arrests their signaling functions.[108] 4-IPP was found to have 5–10 times greater specificity for MIF than for D-DT/MIF-2 when compared to ISO-1, and resulted in decreased migration and growth of human lung adenocarcinoma cells.[109] Treatment of human melanoma cells *in vitro* with 4-IPP suppresses their endogenous expression of programmed death ligand 1 (PDL-1)[110], and this effect could account for the decreased tumor growth and angiogenesis in response to 4-IPP treatment in a mouse model of human melanoma.[111] Combined inhibition of both MIF and D-DT/MIF-2 has the potential for greater therapeutic efficacy in conditions driven by the overlapping signaling functions of these cytokines.[112]

SCD-19 is a small molecule inhibitor that binds in the MIF tautomerase active site and blocks its extracellular signaling functions. SCD-19 treatment resulted in decreased tumor volumes in a mouse model of lung cancer,[113] and ameliorated the inflammatory response in a model of chronic *Pseudomonas aeruginosa* lung disease.[114]

The small molecule inhibitor ibudilast is furthest in clinical evaluation. Ibudilast is a non-selective but principally phosphodiesterase 4 inhibitor being investigated for the treatment of MS,[115] and is a non-competitive inhibitor of the MIF tautomerase enzymatic activity, binding not at the N-terminal proline but adjacent to the active site.[116] Ibudilast binds MIF with micromolar affinity, and decreases mononuclear cell chemotaxis and downstream synthesis of MIF-dependent cytokines including IL-1β, IL-6, and TNF.[112]

Ebselen is a synthetic organo-selenium compound being investigated for use in various inflammatory disorders. Ebselen has a unique mechanism of causing MIF trimer disassociation into monomer subunits. Ouertatani-Sakouhi and colleagues determined that ebselen-mediated MIF disassociation disrupts MIF-CD74 signaling interactions, and results in hyperresponsiveness of signaling through the non-cognate receptors CXCR2 and CXCR4.[117]

The inhibitor p425 is an azo compound sulfonated organic acid that has a unique mechanism of action. It binds allosterically to the MIF trimer surface, not within the tautomerase site, through hydrophobic bonds and blocks the interaction between MIF and its cognate receptor CD74.[118]

7.2 Monoclonal antibodies.

Initial studies involving treatment of mice with CIA with an anti-MIF neutralizing antibody led to delayed onset and reduced frequency of disease.[119] Similar findings were observed in a related anti-type II collagen/LPS (anti-CII Ab/LPS) inflammatory arthritis mouse model, with decreased inflammatory cytokine levels and synovial inflammation in those animals treated with anti-MIF antibodies.[82] Lan and colleagues pursued experiments with a neutralizing murine anti-MIF monoclonal antibody for the treatment of experimentally-induced anti-glomerular basement membrane disease (anti-GBM) in a rat model. Anti-MIF

treatment resulted in decreased proteinuria, maintained renal function, and decreased histological inflammatory changes.[120]

The fully human anti-MIF monoclonal antibody imalumab is the only candidate that has advanced in clinical testing, initially in patients with lupus nephritis (, discontinued with limited enrollment). Imalumab has been more extensively studied for the treatment of cancer (for malignant solid tumors, for ovarian cancer, and for metastatic colorectal cancer). Outcomes from these early phase trials in heavily-pretreated patients with metastatic cancer reported that imalumab was well-tolerated by the patients.[121]

The anti-CD74 monoclonal antibody milatuzumab is a humanized form of the mouse anti-CD74 LL1.[122] Milatuzumab was evaluated in a 24-week phase 1b clinical trial for the treatment of SLE (), with patients demonstrating improvement per the British Isles Lupus Assessment Group (BILAG) index and SLE Disease Activity Index (SLEDAI) scoring systems, and only mild-moderate adverse effects.[123] Further safety and efficacy trials have been pursued in patients with graft-versus-host disease (), multiple myeloma (and), chronic lymphocytic leukemia (and), and B-cell non-Hodgkin lymphoma (). With these early phase clinical trials having been completed and milatuzumab reportedly having been well-tolerated by patients, further development has nonetheless not been continued.[124] Early outcomes of the combination therapy of milatuzumab with the anti-CD20 monoclonal antibody rituximab were also promising for the treatment of mantle cell lymphoma.[125] Milatuzumab has been granted orphan designation by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) for multiple myeloma and chronic lymphocytic leukemia.[126–128]

Nanobodies, which are single domain antigen-binding fragments, are emerging as a new class of biologic therapeutics. Advantages of nanobodies include their high solubility, stability, and their greater tissue penetration compared to conventional antibodies.[129] Anti-MIF nanobodies with nanomolar affinity for both murine and human MIF have been developed and may prove beneficial for the treatment of inflammatory conditions.[130]

7.3 Peptide inhibitors.

A newer class of emerging therapeutics are small peptides that have shown potential in blocking MIF and/or its receptors.

The novel peptide inhibitor DRα1-MOG-35–55 is a chimeric molecule comprising the HLA-DRα1 domain and the myelin oligodendrocyte glycoprotein 35–55 (MOG) peptide. This peptide binds to CD74 and downregulates signaling on CD74+ monocytes, showing efficacy in reducing axonal damage and histological inflammation in a murine model of MS (EAE).[131]

The related inhibitor RTL1000 combines the DRβ1 domain (DRα1β1-MOG-35–55) with the DRα1 domain and MOG moiety and blocks MIF and MIF-2 binding to CD74.[132] A phase 1 clinical trial of RTL1000 in human patients with MS was well-tolerated without significant safety signals being observed.[133] Notably, the expression of MIF and D-DT/

MIF-2 are increased in male patients with secondary forms of MS, suggesting the possibility of a precision medicine approach to the treatment of these patients.[134]

Figueiredo and colleagues reported on the development of C36L1, a 17 amino acid peptide that binds to CD74 on monocytes and dendritic cells. Peptide binding leads to decreased MIF-CD74 signaling and results in the decreased synthesis of immunosuppressive factors including TGF-β, IL-10, and PDL-1 with resulting increased antitumoral immunity from CD8+ cytotoxic T cells in a melanoma model.[135]

Inhibition of MIF binding to its non-cognate receptors with peptide therapeutics also has been explored. The synthetic peptides MIF-(40–49) and MIF-(47–56) resemble the N-like loop of parent MIF responsible for secondary site binding to CXCR2 and compete for this binding site. *In vitro* experiments with MIF-CXCR2 peptide inhibition resulted in decreased monocyte arrest upon aortic endothelial cells.[136]

8. Conclusions.

MIF is a pleiotropic inflammatory cytokine with upstream immunoregulatory effects that is induced as part of the innate and adaptive immune responses. MIF is constitutively expressed among a broad distribution of cell types, and in the setting of an appropriate stimulus is released from intracellular pools as well as synthesized *de novo*. Its effects include stimulation of synthesis of downstream inflammatory cytokines (including IL-1β, IL-6, TNF, and IFN-γ), promotion of cell proliferation, and arrest of apoptotic pathways. MIF signaling through non-cognate cell receptors mediates leukocyte trafficking and arrest at sites of inflammation.

MIF genetic polymorphisms are common across global populations and microsatellite numbers may have expanded to increase MIF expression in response to lethal infections such as invasive pneumococcus.[137, 138] High expression *MIF* alleles have been correlated with severity and joint erosions in RA, and with end-organ manifestations in established SLE disease.

The development of MIF inhibitors is being pursued for the treatment of various malignancies and autoimmune conditions. Small molecule inhibitors binding within the MIF tautomerase site negatively affect protein binding with CD74. Small molecules with unique mechanisms of action include ebselen, which causes MIF trimer disassociation, and p425, which binds the MIF trimer surface to cause disrupted CD74 signaling. Monoclonal antibodies including imalumab (anti-MIF) and milatuzumab (anti-CD74) have been evaluated in early phase clinical trials, with reports of efficacy in the treatment of SLE and cancer. The development of peptide inhibitors as a third therapeutic class may expand the potential armamentarium of anti-MIF clinical therapeutics even further.

9. Expert Opinion.

Significant experimental, genetic, and clinical research has implicated MIF in the pathogenesis of inflammation and autoimmunity, in particular of RA and SLE. Due to the upstream regulatory role of MIF in the pathogenesis of these inflammatory diseases, it has

been considered to be a good candidate cytokine for targeted inhibition, and MIF inhibitors may have a broader spectrum of clinical effects compared to blocking more downstream inflammatory cytokines such as TNF or IL-6.

Precision medicine is an approach to patient care that allows physicians to choose treatments based on a genetic understanding of an individual patient's disease, and tailoring therapeutics to the personal level. A precision medicine approach could be advantageous in patients with RA or SLE who have high expression *MIF* alleles. Such patients might be optimally treated with MIF inhibitors with the goal to reduce systemic MIF levels to those typical of low genotypic *MIF* expressers (*e.g.*, those patients with $CATT_5$ alleles). This approach, rather than complete MIF blockade, would likely be better tolerated by patients with fewer adverse effects, as other important physiologic functions of MIF could otherwise be preserved.

Despite research advances and the recent development of biologic and small molecule tyrosine kinase inhibitor treatments for autoimmune diseases, glucocorticoids continue to be commonly used by clinicians for the treatment of autoimmunity and inflammation. Advantages of glucocorticoids include their broad immunosuppressive effect and rapid onset of action. However, many patients can become dependent on high doses of glucocorticoids to maintain disease control and/or remission status. MIF has a unique role among cytokines through its counter regulation of glucocorticoid action. As such, the pharmacologic approach of MIF inhibition could increase the potency of endogenous or exogenous steroids. This approach could reduce steroid dependence and ultimately be steroid-sparing for patients across the spectrum of autoimmune diseases. This could also have the consequence of decreasing long-term glucocorticoid morbidities in such patients, such as secondary diabetes mellitus, weight gain and obesity, and metabolic bone disease.

The development of pharmacological MIF inhibitors for indications including autoimmunity and cancer will continue. Clinical trials thus far have been limited to phase 1 or II studies, and the various therapeutics tested have in general been well tolerated with a mild-to-moderate adverse effect profile. Despite the development of small molecule and peptide inhibitors, trials involving the monoclonal antibodies imalumab and milatuzumab comprise much of the human safety and efficacy data thus far. As such, the prospect for human patient treatment with the small molecule and peptide inhibitors remains more uncertain. Ibudilast, while inhibiting MIF, also has other relevant anti-inflammatory actions related to its inhibition of phosphodiesterases.

While most of the clinical trial research with pharmacologic MIF inhibitors has been directed at cancer or MS, there is significant potential that antagonism of MIF or D-DT/ MIF-2 will be effective for the treatment of RA and SLE. Genetic *Mif* deficiency in animal models of these diseases results in phenotypic effects comparable with pharmacologic MIF inhibition. In human patients, systemic and focal MIF upregulation at sites of inflammation such as the synovium in RA and the kidneys in SLE has direct correlations with disease pathogenesis. Ibudilast and milatuzumab are furthest along in clinical development, with milatuzumab showing evidence of efficacy in a phase 1 tolerability study in human SLE.

Further exploration of treatment with small molecule, antibody, and peptide MIF inhibitors for RA and SLE are warranted.

A precision medicine approach could also streamline the clinical development of MIF inhibitors. Clinical trials would become more cost effective with recruitment limited to high genotypic *MIF* expressers. The utilization of patient-specific *MIF* genotyping may offer the possibility of directing more effective and less toxic clinical care for autoimmune diseases such as RA and SLE. Among patients with high expression *MIF* alleles and more severe disease phenotypes, and/or chronic glucocorticoid dependence or steroid resistance, the use of MIF-directed pharmacological treatment might be especially warranted.

Acknowledgments

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Article Highlights

- MIF is a pleotropic inflammatory cytokine with upstream immunoregulatory roles and its activity counter-regulates the effects of endogenous corticosteroids.

- Commonly occurring high expression *MIF* alleles, defined by the presence of greater than 5 CATT repeats at the −794 *MIF* promoter site or the −173*G/C single nucleotide polymorphism in linkage disequilibrium with $CATT_7$, are associated with greater severity and erosive rheumatoid arthritis, and greater severity of systemic lupus erythematosus in patients with established disease.

- MIF signals through the CD74/CD44 receptor complex to activate intracellular kinases and NFκB transcriptional pathways. MIF signaling through the non-cognate ligands CXCR2, CXCR4, and CXCR7 has been implicated in mediating leukocyte trafficking and migration arrest at sites of inflammation.

- Experimental animal studies of *Mif* gene deletion and anti-MIF treatment in models of rheumatoid arthritis and systemic lupus erythematosus ameliorate disease activity.

- MIF directed pharmacologic therapeutics in development for treatment of human patients include small molecule inhibitors, monoclonal antibodies and nanobodies, and peptide inhibitors.
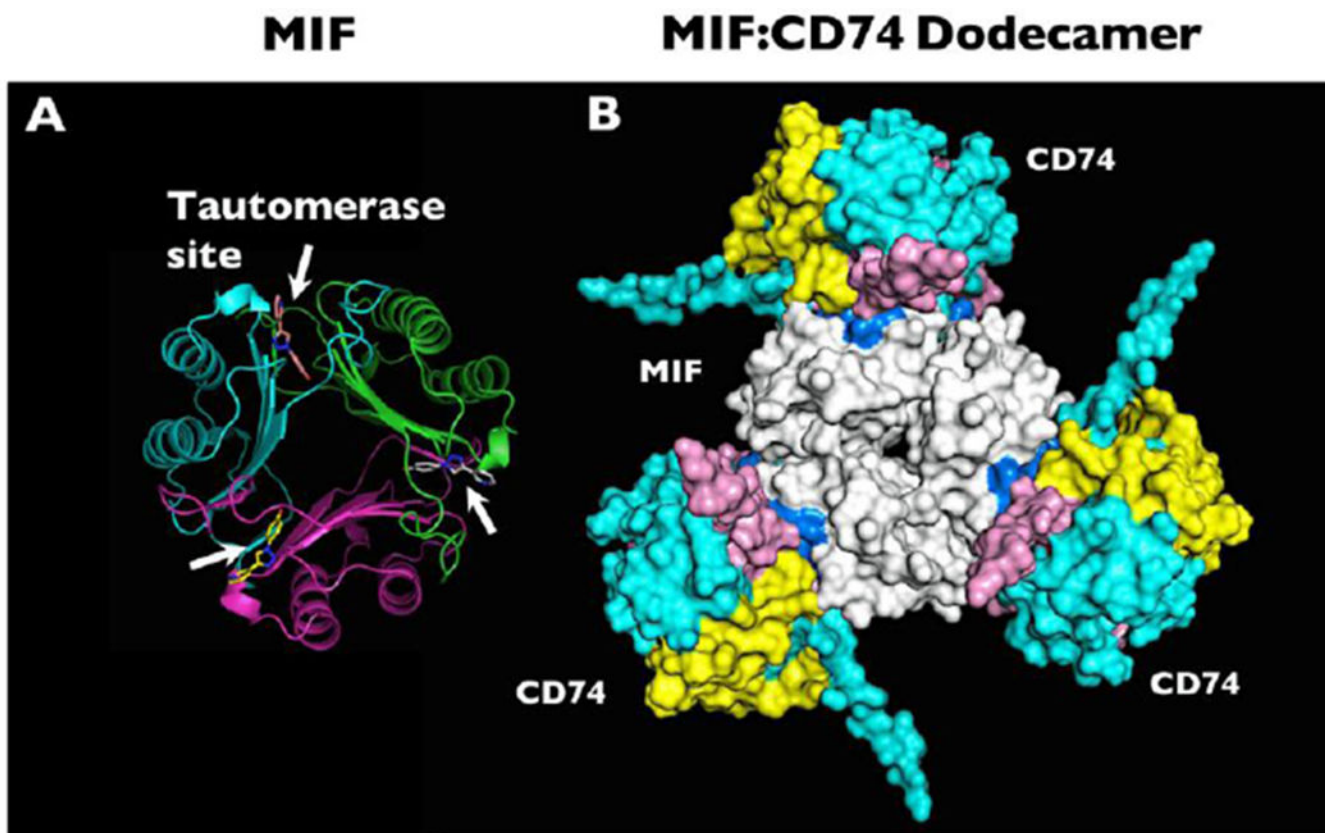
Figure 1.
(a) Molecular structure of MIF based on x-ray crystallography, with white arrows indicating the locations of the tautomerase sites between adjacent monomers. The tautomerase sites are shown occupied by the small molecule MIF20. (b) Computational model representation of the MIF trimer (white, center) engaging with CD74 trimers (blue, yellow, and pink, outer). Many small molecule MIF inhibitors can occupy the MIF tautomerase sites that appear in close apposition to the CD74 receptor. Reprinted by permission from Springer: Metabolic brain disease. Predicted structure of MIF/CD74 and RTL1000/CD74 complexes, Meza Romero R., et al, COPYRIGHT 2016.
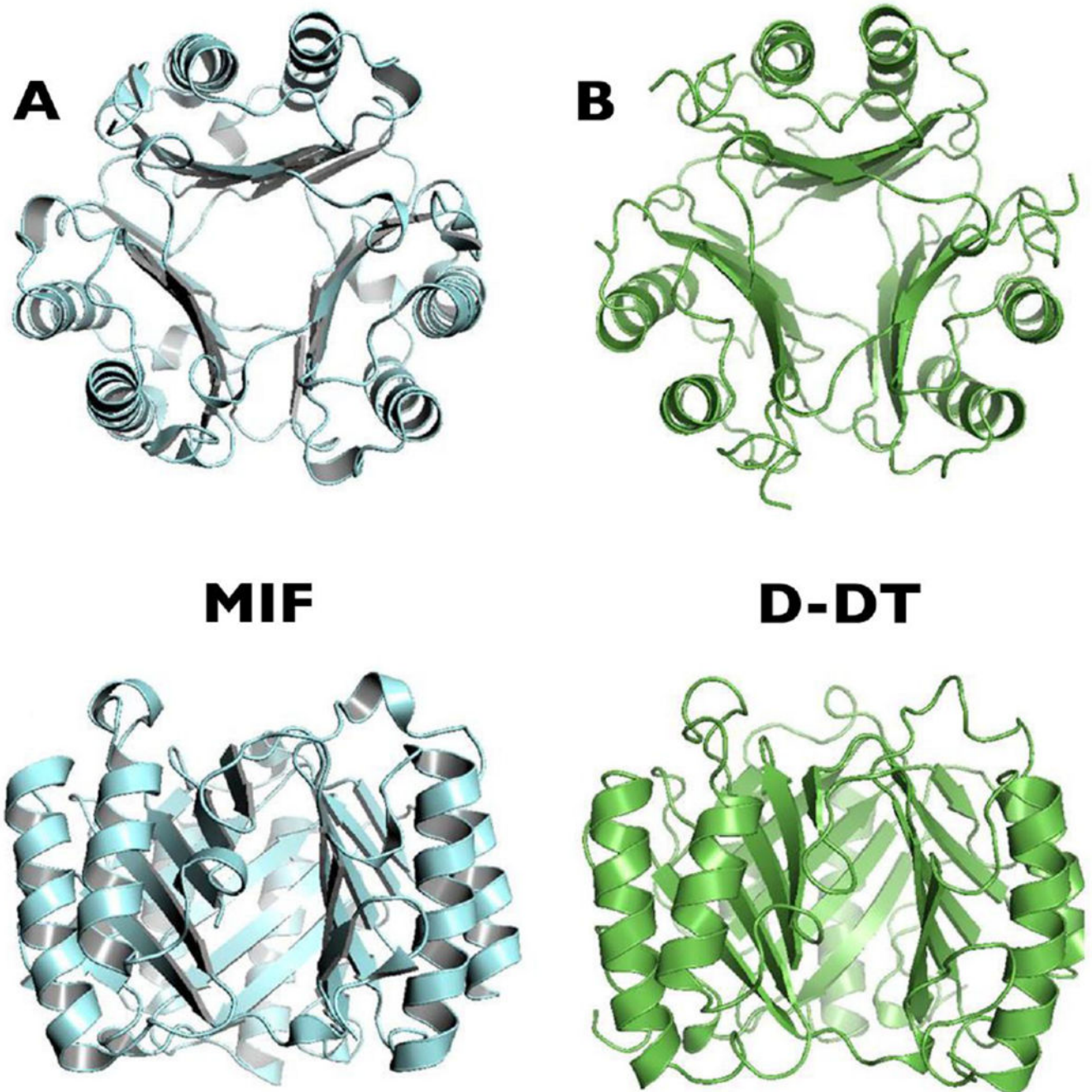
Figure 2.
MIF family protein crystal structures. (A) Human MIF trimer. (B) Human D-DT/MIF-2 trimer. Reprinted with adaptations from Cytokine, 88, Meza-Romero R., et al, Modeling of both shared and distinct interactions between MIF and its homologue D-DT with their common receptor CD74, 62–70, Copyright (2016), with permission from Elsevier.
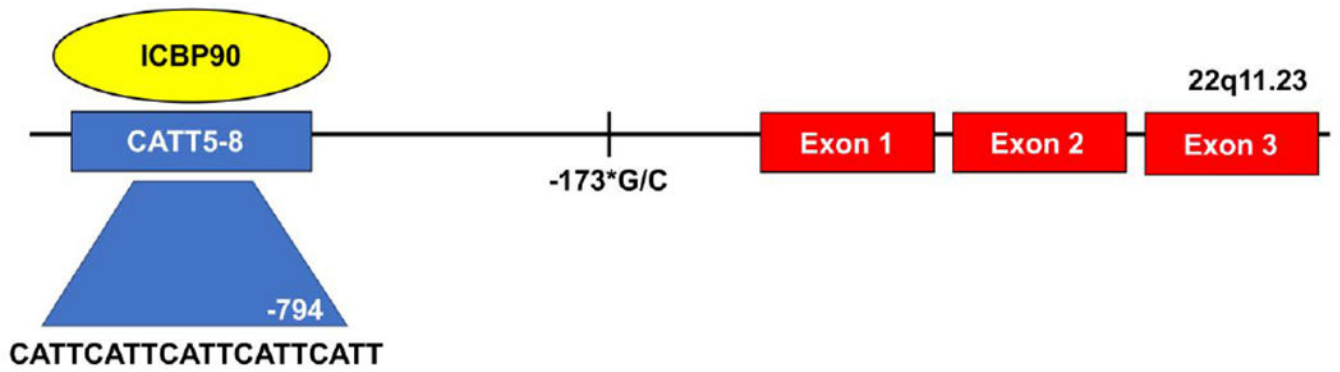
Figure 3.
Diagram of the human MIF gene with 3 downstream exons, and the upstream microsatellite −794 CATT$_{5-8}$ to which binds the transcription factor ICBP90. The number of CATT repeats correlates with basal and stimulated *MIF* expression. The single nucleotide polymorphism −173*G/C is in linkage disequilibrium with the CATT$_7$ microsatellite repeat.
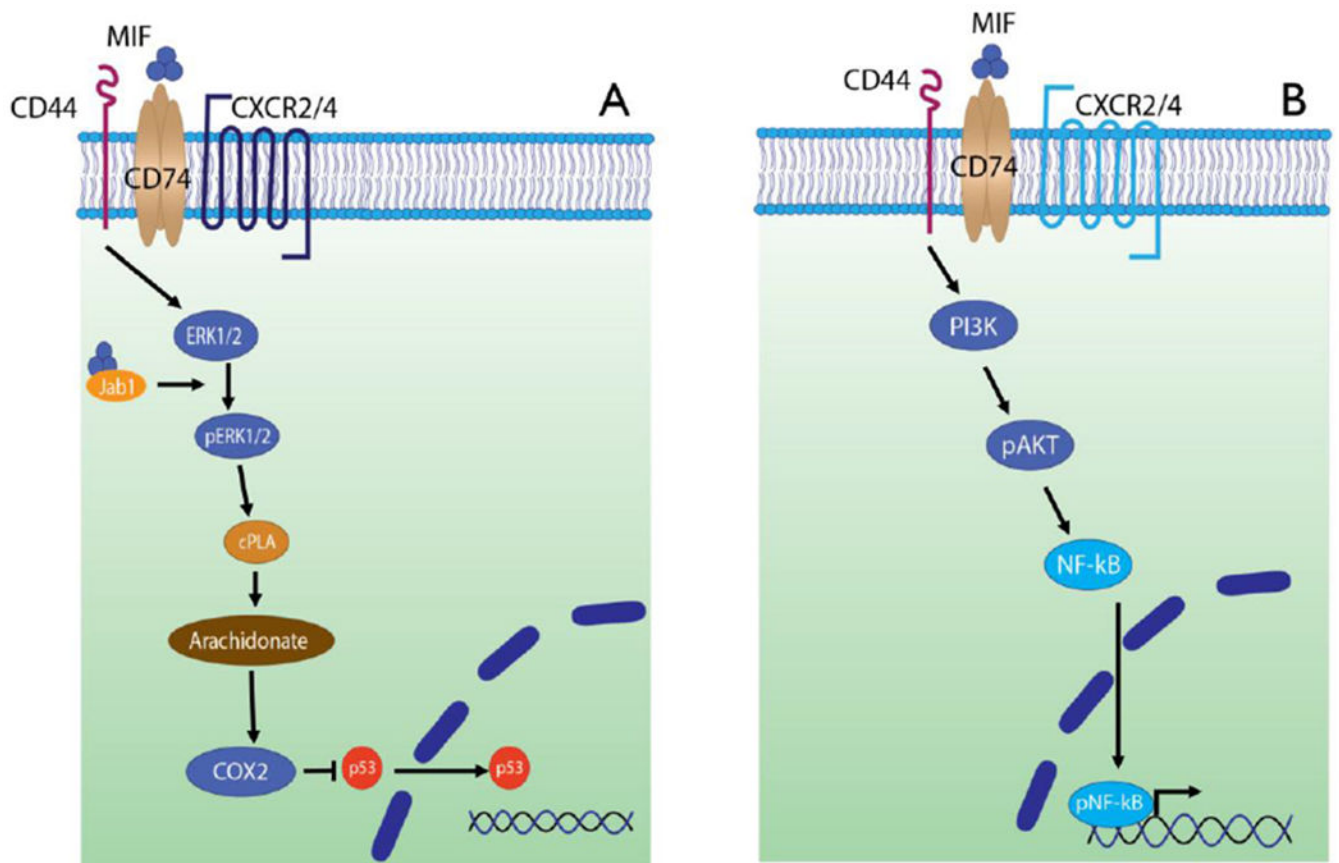
Figure 4.
MIF signaling through the cognate surface receptor CD74. (A) CD74 and CD44 co-localize to mediate intracellular phosphorylation of ERK1/2, which in turn activates phospholipase A2 (cPLA$_2$). This signaling cascade ultimately inhibits nuclear translocation of p53, promoting cell survival and proliferation. MIF interaction with intracellular Jab-1 may function to self-regulate these signaling pathways. CXCR2 is a non-cognate MIF ligand which can interact with CD74. (B) MIF signaling through CD74 leads to activation of phosphoinositide-3 kinase (PI3K), which then leads to phosphorylation and activation of Akt and then NFkB. The latter localizes to the nucleus and causes the transcription of inflammatory gene products. CXCR4 is a non-cognate MIF ligand which can interact with CD74.

Table 1.

MIF Directed Therapeutics

Small molecule inhibitors.			
Name:	**Description:**	**Target:**	**Reference:**
N-Acetyl-P-benzoquinone (NAPQI)	Metabolite of acetaminophen/paracetamol	MIF tautomerase site	105
ISO-1	4,5-dihydro-3-(4-hydroxyphenyl)-5-isoxazoleacetic acid methyl ester	MIF tautomerase site	32, 106, 107
4-IPP	4-Iodo-6-Phenylpyrimidine	MIF tautomerase site	108–112
SCD-19	Isocoumarin	MIF tautomerase site	113, 114
Ibudilast	3-isobutyryl-2-isopropylpyrazolo-(1,5-a) pyridine	MIF, proximal to the tautomerase site	112, 115, 116
Ebselen	Synthetic organo-selenium	MIF trimer	117
p425	Azo sulfonated organic acid	MIF trimer	118
Monoclonal antibodies.			
Name:	**Description:**	**Target:**	**Reference:**
imalumab	Human monoclonal	MIF	121
milatuzumab	Humanized monoclonal	CD74	122–128
Peptide inhibitors.			
Name:	**Description:**	**Target:**	**Reference:**
DRα1-MOG-35–55	HLA-DRα1 domain and myelin oligodendrocyte glycoprotein 35–55 peptide	CD74	131
RTL1000	HLA-DRα1β1 and myelin oligodendrocyte glycoprotein 35–55 peptide	CD74	132, 133
C36L1 peptide	Immunoglobulin complementarity-determining region (CDR) peptide	CD74	135
MIF-(40-49), MIF-(47-56)	Resemble the N-like loop of MIF that binds to CXCR2	CXCR2	136

MIF: macrophage migration inhibitory factor protein;

HLA: human leukocyte antigen;

MOG: myelin oligodendrocyte glycoprotein