Multiple roles of macrophage migration inhibitory factor in pulmonary hypertension
Gael Jalce, Christophe Guignabert

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The Multiple Roles of Macrophage Migration Inhibitory Factor in Pulmonary Hypertension

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Short title: MIF in PH/PAH

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Abstract

Pulmonary hypertension (PH) is a life-threatening condition arising from the loss and obstructive remodeling of the pulmonary arteries, leading to the sustained elevation of pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR) and subsequently right ventricular (RV) failure and death. PH encompasses a group of multifactorial diseases, such as pulmonary arterial hypertension (PAH) and chronic thromboembolic PH (CTEPH), for which there is no treatment that can stop or reverse the progression of remodeling of the pulmonary vasculature. The identification of new molecular targets for the development of more effective drugs is thus urgently needed. In this context, macrophage migration inhibitory factor (MIF), a pleiotropic upstream pro-inflammatory mediator, is emerging as a promising molecular target, as it contributes to perivascular inflammation and pulmonary arterial remodeling, two key hallmarks of PAH that are not specifically targeted by currently approved therapies. The objective of this review is to summarize the scientific evidence on the pathogenic roles of MIF and its potential as a biomarker and therapeutic target in PH/PAH.

**Keywords:** macrophage migration inhibitory factor, vascular remodeling, inflammation, endothelial dysfunction, target, therapeutics
1. Introduction

Pulmonary hypertension (PH) is a hemodynamic and pathophysiological condition defined as a mean pulmonary arterial pressure (mPAP) of greater than 20 mmHg at rest, measured during right heart catheterization (RHC), which frequently culminates in right ventricular failure and death (17, 53). PH can result from pre-capillary (arterial) or post-capillary (venous) pathomechanisms. The new classification of PH proposed by the 6th World Symposium (2019) gathers the various PH entities into five groups based on their similar clinical presentation, pathological findings, hemodynamic characteristics, and treatment strategy: Group 1, pulmonary arterial hypertension (PAH); Group 2, PH due to left heart disease; Group 3, PH due to lung diseases and/or hypoxia; Group 4, PH due to pulmonary artery obstructions; Group 5, PH with unclear and/or multifactorial mechanisms (53).

PAH, defined as an increased mPAP > 20 mmHg and pulmonary vascular resistance (PVR ≥ 3 Wood units) with normal left heart filling pressures (pulmonary artery wedge pressure ≤ 15 mmHg) measured during resting RHC, includes the most serious and lethal forms of PH but is relatively rare (17, 53). In Europe, the prevalence and incidence of PAH are in the range of 15 to 60 subjects per million of the population and 5 to 10 cases per million per year, respectively. Although mortality rates have improved in the last few decades with the current available therapeutic options, most patients still die from PAH or fail to adequately respond to medical therapy, with a five-year survival rate of 59% (7). Pulmonary vascular remodeling in PAH, which is the cause of increased mPAP and PVR in PAH patients, is a complex and multifactorial process that involves multiple mechanisms, including, among others: dysfunction of the pulmonary endothelium, sustained vasoconstriction, chronic lung inflammation and dysimmunity, altered communication between the various types of vascular cells within the pulmonary arterial wall (pulmonary endothelial (ECs) and vascular smooth muscle cells (PA-SMCs), fibroblasts, myofibroblasts, and pericytes) and between the pulmonary vascular cells and the various types of immune cells, as well as excessive activation of signaling pathways and transcription factors by various key growth factors, hormones, chemokines, and cytokines (21, 22, 29, 32). In addition, several genetic and environmental factors are known to play a predisposing or facilitating role in the development of PAH (46) (Figure 1).
Currently approved therapies mainly focus on the imbalance between vasoconstriction and vasodilation. Although such therapies have improved patient outcomes over the last 20 years, they all consist of symptomatic vasodilators, with little impact on the progression of aberrant pulmonary arterial remodeling. Median survival is thus still unsatisfactory (7) and new drugs that could halt or even reverse the progression of the disease by targeting other causal mechanisms that drive pulmonary vascular remodeling are urgently needed.

This review provides insights concerning the anti-inflammatory strategy in PH/PAH that aims to target key immune system mediators, with a focus on the pro-inflammatory mediator macrophage migration inhibitory factor (MIF).

2. Targeting the vicious cycle between the dysfunctional pulmonary endothelium and the immune/inflammatory component

The structural and functional alterations of the endothelium in pulmonary arteries are now well established to be central in the pathogenesis of PAH (22, 29, 31). Briefly, the main characteristics of
pulmonary EC alterations or dysfunction in PAH include, among others, a reduction in the secretion of vasodilator molecules, such as prostacyclin (PGI$_2$) and nitric oxide (NO), and an increase in the potent vasoconstrictor endothelin (ET)-1. However, the dysfunctional pulmonary endothelial cells in PAH also exhibit a decrease in tube formation capacity, metabolic alterations, and a pro-inflammatory phenotype (32). Indeed, the dysfunctional pulmonary endothelium in PAH overexpresses E-selectin, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1 and secretes locally excessive amounts of numerous inflammatory mediators, such as interleukin (IL)-1$\alpha$, IL-6, IL-8, IL-12, CCL2 (MCP-1), and MIF (39). As previously reviewed (48), this pro-inflammatory EC phenotype, along with other abnormalities affecting the immune/inflammatory component of PAH, facilitates the infiltration of inflammatory cells in the perivascular area. The pro-inflammatory environment within the pulmonary arterial walls plays a central role in pulmonary vascular remodeling through the promotion of cell survival and migration (39, 58, 61).

In accordance with these features, a link between circulating levels of various pro-inflammatory mediators and clinical outcomes and/or survival has been described in PH/PAH, suggesting a possible relationship between chronic lung inflammation and disease progression (33, 54, 58). Recently, four PAH clusters with distinct proteomic immune profiles have been found in cohorts of PAH patients by unsupervised analysis of blood proteomic profiles, highlighting the potential existence of distinct immune phenotypes (57). The authors found tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), C-C motif chemokine ligand (CCL)-5, CCL7, CCL4, TNF-β, and MIF to be among the panel of up-regulated circulating pro-inflammatory mediators central to immune network-cluster 1, which was associated with the most unfavorable five-year transplant-free survival rates (57). These authors also identify two other different panels of circulating mediators (composed of specific inflammatory molecules and growth factors) that were able to distinguish between groups of patients with low- (cluster 3) and intermediate-risk (cluster 4). Using this specific multiplex approach, they also found a subgroup of patients with intermediate-risk (cluster 2) who had low levels of these circulating mediators similar to healthy controls (56). These observations highlight the need to confirm these findings in large, well-characterized populations with different technologies and arise
the question on how these events detected in the circulation can be used to guide the management of these patients, and how they reflect what is occurring in the PAH lung.

The interplay between alterations of the pulmonary endothelium and the inflammatory component is also illustrated by the presence of circulating autoantibodies directed against antinuclear antigens, ECs, and fibroblasts in several forms of PH/PAH. Maladaptation of the immune response is also underscored by the presence of lymphoid neogenesis, T helper 17 (Th17) cell immune polarization, and dendritic-cell recruitment in the lungs of PH/PAH patients. As a result, the clinical evaluation of anti-inflammatory drugs has become a recent focus in PAH clinical trials. The anti-IL6 and anti-CD20 monoclonal antibodies tocilizumab and rituximab are examples of anti-inflammatory agents under clinical investigation in PAH (ClinicalTrials.gov identifiers NCT02676947 and NCT01086540). Patients with rheumatoid arthritis (RA) that responded to tocilizumab treatment were those that showed significantly lower MIF serum levels, indicating a potential interplay between these two cytokines in RA (34).

Despite its considerable therapeutic potential, targeting inflammation or the vicious cycle between the dysfunctional pulmonary endothelium and the inflammatory component is still highly challenging and highlights the importance of understanding the mechanistic diversity of inflammatory pathways that contribute to PH/PAH (30). Because the excessive activity of the MIF/CD74 axis in pulmonary ECs has been shown to be partly involved in the acquisition and maintenance of the pro-inflammatory endothelial phenotype in PAH (39), MIF and its signaling are emerging as promising therapeutic targets.

3. Macrophage migration inhibitory factor (MIF) and its signaling in PH/PAH

MIF is a pro-inflammatory mediator with multiple biological functions and pleiotropic effects that is expressed by a variety of immune cells, including macrophages and lymphocytes, as well as endothelial and epithelial cells under certain conditions. MIF occupies an upstream position in the inflammatory cascade and promotes the production of various other pro-inflammatory mediators, such
as TNF-alpha, IL-6, IL-1 beta, etc. Because MIF is associated with acute and chronic inflammatory disorders, as well as glucocorticoid resistance, it represents a promising therapeutic target in several diseases, such as rheumatoid arthritis (RA), irritable bowel disease (IBD), systemic lupus erythematosus (SLE), multiple sclerosis (MS), type 1 diabetes mellitus, and PH/PAH (25).

3.1. **Circulating MIF levels: a new emerging PAH marker?**

Even if concentration levels vary greatly in the existing literature, circulating MIF levels have been found to be elevated in several forms of PH/PAH (Table 1). Importantly, these results support the notion that circulating MIF levels could serve, alone or in conjunction with other inflammatory mediators, as a potential biomarker of PAH severity (14, 20, 35, 44, 57). Of note, no significant difference in blood levels of the MIF homolog d-dopachrome tautomerase (DDT or MIF-2) between patients with PAH and control subjects without established cardiovascular disease (7.4 ± 2.2 versus 4.6 ± 1.0 ng/mL) (39).

**Table 1. Circulating levels of MIF in patients with PAH:**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Sample type &amp; Technology</th>
<th>PAH subgroups</th>
<th>Number of patients</th>
<th>Circulating MIF concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>[38]</td>
<td>Serum &amp; ELISA</td>
<td>Idiopathic and heritable PAH</td>
<td>40</td>
<td>147 ± 14 ng/mL.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control subjects</td>
<td>20</td>
<td>66 ± 7 ng/mL.</td>
</tr>
<tr>
<td>[34]</td>
<td>Serum &amp; ELISA</td>
<td>PAH (both systemic sclerosis-associated and idiopathic)</td>
<td>101</td>
<td>1170 pg/mL.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Healthy controls</td>
<td>35</td>
<td>175 pg/mL.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAH subgroup</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Idiopathic PAH</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Idiopathic PAH</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drugs and toxin-induced PAH</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAH associated with congenital heart disease</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAH associated with connective tissue disease</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAH discovery cohort (n=281):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[56]</td>
<td>Plasma &amp; Bio-Plex®</td>
<td>PAH discovery cohort (n=281):</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 1 (n=58): high-risk</td>
<td>715 pg/mL (598-917)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 2 (n=109): intermediate-risk</td>
<td>534 pg/mL (451-644)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 3 (n=77): low-risk</td>
<td>565 pg/mL (517-687)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 4 (n=37): intermediate-risk</td>
<td>599 pg/mL (501-714)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>PAH external validation cohort (n=104):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Idiopathic PAH</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAH associated with congenital heart disease</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAH associated with congenital heart disease</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Portopulmonary hypertension</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 1 (n=26): high-risk</td>
<td>640 pg/mL (531-816)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 2 (n=33): intermediate-risk</td>
<td>467 pg/mL (354-622)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 3 (n=36): low-risk</td>
<td>732 pg/mL (575-832)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cluster 4 (n=9): intermediate-risk</td>
<td>733 pg/mL (620-931)</td>
<td></td>
</tr>
<tr>
<td>[54]</td>
<td>Serum &amp; ELISA</td>
<td>Idiopathic PAH</td>
<td>13</td>
<td>270 ± 636.2 pg/mL.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Systemic sclerosis-associated PAH</td>
<td>15</td>
<td>333 ± 520 pg/mL.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Systemic sclerosis without PAH</td>
<td>14</td>
<td>175 ± 68.68 pg/mL.</td>
</tr>
<tr>
<td>[14]</td>
<td>Plasma &amp; ELISA</td>
<td>Portopulmonary hypertension</td>
<td>21</td>
<td>46.68 ng/mL.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls with liver disease without both pulmonary hypertension and hepatopulmonary syndrome</td>
<td>31</td>
<td>31.19 ng/mL.</td>
</tr>
</tbody>
</table>

The variability around the reported results, probably due to the type of samples, technology or to the small numbers of patients studied, emphasizes the need to conduct a replicative study with a
sufficiently large sample size and a reproducible technology to measure MIF levels. Indeed, a positive correlation between MIF serum levels and pulmonary vascular resistance (PVR), which is used as a primary endpoint in phase II PAH clinical trials, has been found in both idiopathic and systemic sclerosis-associated PAH (SSc-PAH patients) (35). Consistent with this notion, MIF levels in patients with porto-pulmonary hypertension (PoPH) were found to positively correlate with PVR and inversely with cardiac output (CO), two hemodynamic parameters of disease severity in PAH patients (14). Stefanantoni et al. (55) also reported increased MIF levels in idiopathic PAH and SSc-PAH patients with higher NYHA functional classes. More recently, Marshall et al. (44) reported significant positive correlations between MIF levels and those of seven angiogenic factors (angiopoietin-1, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-BB, fibroblast growth factor (FGF)-2, epidermal growth factor (EGF), placent al growth factor (PLGF), and Dickkopf (DKK)-1) in patients with idiopathic PAH and those with connective tissue disease-associated PAH (CTD-PAH) in a cohort of patients enrolled in the FREEDOM C-2 trial. However, the authors did not find any correlation between MIF levels and prognostic markers, such as six-minute walk distance (6MWD) or the composite clinical outcome, in this cohort (44). The potential of MIF as a PAH severity biomarker is also supported by the recent work of Sweat et al. (57), who used unsupervised machine learning to categorize PAH patients into four proteomic immune clusters with distinct circulating cytokine signatures that were independent of PAH etiology and associated with various clinical-risk parameters and the long-term prognosis (outcome). Cluster 1, which contains a set of selective upregulated cytokines, including MIF, was associated with high risk. Cluster 1 was comprised of a high proportion of PAH patients with functional class IV symptoms (22.4%), NT-proBNP > 1500 pg/mL (39.7%), high-risk composite REVEAL, elevated mPAP (52 mmHg [45-60]) and right atrial pressure (11 mmHg [5-14]), impaired right ventricular function, and low five-year survival rates (47.6%, CI 35.4-64.1%) (57). This study provides support for the use of immune phenotyping to potentially help in selecting PAH patients who are the most likely to respond to cytokine and growth-factor antagonists, such as MIF antagonists. Interestingly, T-cell lymphocytes were shown to be a source of MIF overabundance in patients with PAH, with no significant difference between those with idiopathic and heritable forms (39).
3.2. Elevated levels of MIF in other types of PH

a) PH associated with heart failure (HF) with preserved ejection fraction (PH-HFpEF)

Heart failure (HF) with preserved ejection fraction (HFpEF) is currently the dominant form of HF and a common disease among older adults. Up to 80% of these patients develop PH, which is associated with worse symptoms and higher mortality. The prevalence of PH in HFpEF, based on echocardiographic diagnosis, is 83% and five-year overall mortality is more than 50% from the time of diagnosis (27, 37). In HFpEF patients, plasma MIF levels above the median value (51.58 ng/mL) were found to be associated with higher pulmonary artery systolic pressure (PASP), a surrogate parameter of pulmonary artery pressure (PAP). In addition, MIF levels were also found to correlate with markers of heart failure, the level of natriuretic peptides BNP and NT-proBNP, and a combined endpoint of death or hospitalization after 180 days. These results suggest that MIF could be a useful biomarker, as it is associated with clinical outcomes and could be involved in the pathophysiology of patients with HFpEF and PH (41, 42).

b) PH associated with congenital cardiac shunts

PH is a relatively common complication of congenital heart disease (CHD), with an adult prevalence that ranges from 5 to 10% (15, 19, 60). Serum MIF levels are higher in pediatric patients with PH associated with congenital cardiac shunt, a type of CHD, than those of controls. In these patients, high circulating MIF levels were found to be associated with medial hypertrophy of the small pulmonary arteries and elevated PVR (43) and inversely associated with pulmonary blood flow, estimated noninvasively by transthoracic echocardiography (VTIPV) (67). A common single nucleotide polymorphism (SNP) in the Carbamyl-Phosphate Synthetase I (CPSI) gene is known to cause the substitution of asparagine (Asn) for threonine (Thr) at position 1405 (T1405N) in the critical cofactor-binding domain of the enzyme (56). The CPSI T1405N functional polymorphism has been shown to be associated with the development of PH in the pediatric population (9). Remarkably, patients with a high PVR and the AC-CPSI T1405N genotype profile were those with the highest MIF levels.
c) PH associated with congenital diaphragmatic hernia (CDH)

Congenital diaphragmatic hernia (CDH) is a rare congenital anomaly and life-threatening disease characterized by a diaphragmatic defect. PH is a common complication in newborn infants with CDH, in addition to lung hypoplasia. The vascular defects associated with PH, which are characterized by increased muscularization of the arterioles and capillaries, start to develop early in gestation (67). MIF expression has been shown to be 10-fold higher in newborns than in children and adults (50). In the rat neonate model of CHD induced by nitrofen, which mimics the pulmonary abnormalities described for human CDH, including lung hypoplasia and pulmonary vascular remodeling, treatment with the MIF inhibitor ISO-92 led to decreased pulmonary cellularity, vascular wall thickness, and right ventricular systolic pressure (RVSP). However, in contrast to CD74 expression in fetuses of CHD rats, ISO-92 did not affect MIF expression or secretion relative to the nitrofen group (47).

d) PH caused by chronic lung diseases

PH is a common and severe complication of interstitial lung diseases (ILDs), such as idiopathic pulmonary fibrosis (IPF), and can adversely affect symptoms, functional capacity, and survival. PH affects nearly 8 to 15% of patients with IPF at diagnosis and 30 to 50% at the time of evaluation for lung transplantation. Drugs approved for the treatment of PAH are ineffective in IPF-PH and there are currently no approved treatments for PH in IPF (IPF-PH) (11).

Zhang et al. (66) reported that median plasma MIF levels were higher in patients with interstitial lung disease-associated PH (ILD-PH) (median 1,424 pg/mL; range 519-4,396; p = 0.007) than in those with ILD without PAH (median 803 pg/mL; p = 0.076) or a randomly recruited population-based control group (median 365 pg/mL; range 142-4,707).

MIF has also been found to be particularly abundant in the bronchoalveolar lavage (BAL) of patients with IPF and expressed in actively fibrosing areas, such as fibroblast foci and lung remodeling zones (2). Interestingly, higher numbers of cells positive for MIF or its two main receptors, CD74 and CXCR4, have been found in the perivascular area and vascular wall in the lungs of IPF-PH patients (23). In contrast, fibroblast foci of IPF-PH patients have been found to not express CD74, but low
levels of MIF and CXCR4 (23). MIF levels have also been shown to be higher in the sputum and BAL of patients with chronic obstructive pulmonary disease (COPD) than those of non-smokers and healthy smokers (51).

### 3.3. **Dysregulation of the MIF signaling pathway and its role in PAH**

Engagement of MIF with CD74, CXC chemokine receptors (CXCR)-2, CXCR4 and CXCR7 is partly responsible for the multiple biological activities associated with MIF (1, 24, 49). Interestingly, several of these receptors exhibit a more intense immunoreactivity in lungs from patients with PAH and from rodents with established PH (5, 10, 16, 18, 23, 40, 45, 52, 65).

Consistent with these observations, emerging evidence support the notion that MIF and its signaling could contribute to the progression of the pulmonary vascular remodeling associated with PAH. Activation of the MIF/CXCR2 and MIF/CXCR4 axes is known to facilitate leukocyte recruitment (3), induce PASMCs proliferation (64, 66), and promote fibroblast migration in scratch-wounded monolayers in vitro (13). In addition, up-regulation of the MIF/CD74 axis in human pulmonary ECs induces the expression of specific adhesion molecules (*i.e.* ICAM-1, VCAM-1, and E-selectin), facilitating the adhesion and recruitment of inflammatory cells to the pulmonary vascular wall (Figure 2). Although CXCR2 and CXCR4 mRNA levels in pulmonary ECs derived from patients with idiopathic PAH are unaltered (3), it cannot be excluded that MIF could act through these endothelial receptors to interfere with particular endothelial functions. Interestingly, Burton *et al.* have reported that the loss of signaling through the bone morphogenetic protein receptor type II (BMPR-II) increased CXCR2 protein levels in human pulmonary artery endothelial cells (8). Furthermore, the excessive secretion of MIF by dysfunctional pulmonary ECs has been shown to increase FoxM1 expression and activity in PA-SMCs, mediating SMC proliferation and pulmonary vascular remodeling (12).

Despite this knowledge, there are still many challenges and open questions. It is necessary to define more precisely the role of MIF on the maintenance of cardiac homeostasis under stress and during the right ventricular hypertrophy that occurs in PAH (59). Although several studies support that cardiac-derived MIF may exert cardioprotection in some ischemia models, recent findings also suggest a
detrimental effect of MIF in myocardial ischemia under certain circumstances. Plasma MIF levels also have been reported to be associated with infarct size and the extent of post-infarct cardiac remodeling (62). Inhibition of MIF in mice with myocardial ischemia reduced inflammatory cell infiltration, expression of CCL2 and decreased the incidence of post-myocardial ischemia cardiac rupture (62). Thus, a better understanding of the temporal MIF expression during cardiac ischemia and repair is needed. Given that MIF has both intracellular and extracellular activity, further studies are also required to precise their functions and to address the role of MIF polymorphisms in PAH. Bossini-Castillo et al. (6) found an association of the MIF rs755622*C allele with a higher risk of developing PAH in patients with the diffuse cutaneous form of SSc (dcSSc).

![Figure 2: The MIF/CD74 axis contributes to the endothelial pro-inflammatory phenotype and leukocyte recruitment in pulmonary arterial hypertension (PAH).](image)

4. Therapeutic potential of MIF inhibition in preclinical models

Although animal models do not recapitulate the full spectrum of the human disease (4), several preclinical studies have demonstrated that the inhibition of MIF may provide a novel efficient anti-inflammatory approach in the treatment of PH/PAH. Phenotypically, MIF-deficient (KO Mif /-) mice
appear to live longer and develop normally on multiple genetic backgrounds (26, 28), and are protected against chronic hypoxia-induced PH (36, 66).

In addition, chronic treatment with the prototypical MIF inhibitor ISO-1 or its analogue ISO-92 (Figure 3) have been reported to reduce pulmonary vascular remodeling, cardiac hypertrophy, and right ventricular systolic pressure in the mouse model of PH induced by chronic hypoxia (63, 64, 66).

It has been shown that MIF inhibition with ISO-92 enhances pulmonary angiogenesis and lung development in a model of congenital diaphragmatic hernia by increasing the activity of p-eNOS and VEGF and reducing that of arginase 1, arginase 2, and Sflt-1 (47). These observations are consistent with the findings obtained by Le Hiress et al., who demonstrated that curative treatment with the MIF antagonists ISO-1 or N-(3-hydroxy-4-fluorobenzyl)-5 trifluoromethylbenzoazol-2-thione 31 or anti-CD74 antibodies reversed established PH in monocrotaline-injected rats, reducing total PVR and lung remodeling (Figure 3) (38). MIF inhibition was shown to also attenuate the increase in IL-6 and CCL2 (MCP-1) serum levels in monocrotaline-injected rats, two key pro-inflammatory cytokines known to contribute to the pathogenesis of PAH. However, treatment of monocrotaline-injected rats with ISO-1 has been shown to promote cardiac fibrosis relative to chronic treatment with anti-CD74 antibodies or compound 31 (38, 39). In accordance with these results, Günther et al. reported that chronic treatment of bleomycin-injected mice with ISO-1 or compound 31 attenuated bleomycin-induced inflammation and lung fibrosis and prevented the development of PH in mice (23).

![Figure 3](image-url)  
*Figure 3.* Chemical structure of MIF inhibitors tested against PH in animal models
5. Conclusions

Although the exact pathophysiology of PH/PAH is still unknown, there is increasing evidence that inflammation plays an important role in remodeling of the pulmonary arteries, leading to the development and progression of PH/PAH (Figure 4). MIF is an upstream pleiotropic proinflammatory mediator associated with acute and chronic inflammatory disorders and glucocorticoid resistance. Elevated MIF levels in the blood of patients with PH/PAH have been shown to correlate with disease severity, suggesting that serum MIF levels may be a potential biomarker. Pharmacological inhibition of MIF with the small molecules ISO-1, ISO-92, or compound 31, as well neutralizing CD-74 antibodies, significantly improves PH in various animal models of the disease. These pharmacological efficacy studies suggest that targeting MIF may be an effective approach to treat pulmonary vascular remodeling, particularly in PH/PAH patients with high circulating levels of MIF. Efforts are currently underway to design small molecule MIF inhibitors with optimized drug properties for clinical trials in immune, inflammatory, and cardiologic diseases and cancer. To date, the most advanced anti-MIF product is Imalumab, a monoclonal anti-oxMIF antibody that is currently under clinical investigation for cancer treatment (NCT01765790). The importance of understanding the pathogenic role of inflammation and the vicious cycle between the dysfunctional pulmonary endothelium and the inflammatory component that contributes to PH/PAH is essential for the design of new therapies for targeting relevant inflammatory pathways in PH/PAH.
Figure 4: Schematic diagram summarizing the contribution of MIF to the 3 main components of the pulmonary vascular remodeling associated to pulmonary arterial hypertension (PAH).
Acknowledgments:
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6. References


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