



Multiple roles of macrophage migration inhibitory factor in pulmonary hypertension

Gael Jalce, Christophe Guignabert

► To cite this version:

Gael Jalce, Christophe Guignabert. Multiple roles of macrophage migration inhibitory factor in pulmonary hypertension. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 2020, 318 (1), pp.L1-L9. 10.1152/ajplung.00234.2019 . inserm-02454988

HAL Id: inserm-02454988

<https://inserm.hal.science/inserm-02454988>

Submitted on 19 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The Multiple Roles of Macrophage Migration Inhibitory Factor in Pulmonary Hypertension

Gael Jalce¹ and Christophe Guignabert^{2, 3}

Short title: MIF in PH/PAH

¹APAXEN, Gosselies, Belgium; gael.jalce@apaxen.com

²INSERM UMR_S 999, Hôpital Marie Lannelongue, Le Plessis-Robinson, France

³Faculté de Médecine, Université Paris-Sud, Université Paris-Saclay, Le Kremlin-Bicêtre, France.

Contents:

1. Introduction.....	2
2. Targeting the vicious cycle between the dysfunctional pulmonary endothelium and the inflammatory component	3
3. Macrophage migration inhibitory factor (MIF) and its signaling in PH/PAH	5
3.1. MIF circulating levels: a new emerging PAH marker?	6
3.2. Elevated levels of MIF in other types of PH.....	8
3.3. Dysregulation of the MIF signaling pathway and its role in PAH.....	10
4. Therapeutic potential of MIF inhibition in preclinical models	11
5. Conclusions	13
6. References	16

Conflict of interest: G.J. and C.G. are the inventors on patent WO2015173433 and have no conflict of interest to disclose.

Manuscript word count: 3,209

Corresponding author:

Christophe Guignabert, PhD
INSERM UMR_S 999
133, Avenue de la Résistance,
92350 Le Plessis-Robinson, France.
Tel: +33-1-40948833; Fax: +33-1-40942522
Email: christophe.guignabert@inserm.fr

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 \geq 3 Wood units) with normal left heart filling pressures (pulmonary artery wedge pressure \leq 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**).

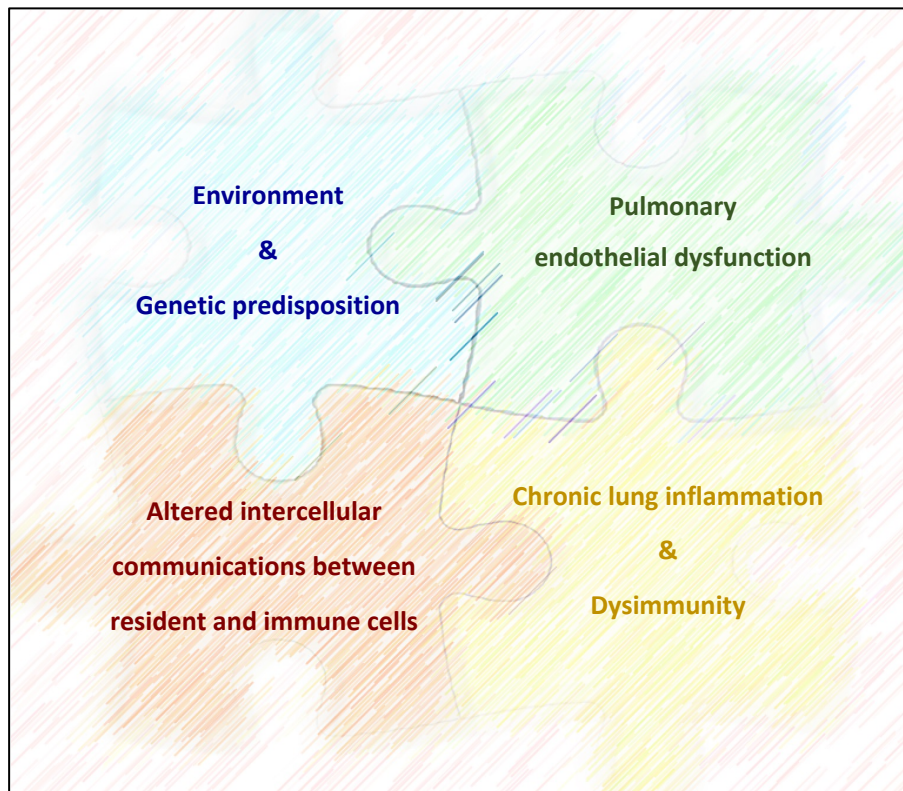


Figure 1: Components of the pulmonary vascular remodeling associated with pulmonary arterial hypertension (PAH).

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 (PGI₂) 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 α , 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:

Ref.	Sample type & Technology	PAH subgroups	Number of patients	Circulating MIF concentration	
[38]	Serum & ELISA	Idiopathic and heritable PAH	40	147 ± 14 ng/mL	
		Control subjects	20	66 ± 7 ng/mL	
[34]	Serum & ELISA	PAH (both systemic sclerosis-associated and idiopathic)	101	1170 pg/mL	
		Healthy controls	35	175 pg/mL	
[56]	Plasma & Bio-Plex® multiplex immunoassay	• PAH discovery cohort (n=281):		Cluster 1 (n=58): high-risk Cluster 2 (n=109): intermediate-risk Cluster 3 (n=77): low-risk Cluster 4 (n=37): intermediate-risk	715 pg/mL (598-917) 534 pg/mL (451-644) 565 pg/mL (517-687) 599 pg/mL (501-714)
		PAH associated with connective tissue disease	87		
		Idiopathic PAH	84		
		Drugs and toxin-induced PAH	49		
		PAH associated with congenital heart disease	38		
		Portopulmoanry hypertension	7		
		• PAH external validation cohort (n=104):		Cluster 1 (n=26): high-risk Cluster 2 (n=33): intermediate-risk Cluster 3 (n=36): low-risk Cluster 4 (n=9): intermediate-risk	640 pg/mL (531-816) 467 pg/mL (354-622) 732 pg/mL (575-832) 733 pg/mL (620-931)
		Idiopathic PAH	53		
		PAH associated with connective tissue disease	37		
		PAH associated with congenital heart disease	7		
		Portopulmoanry hypertension	7		
		• Healthy controls	88	504 pg/mL (496-555)	
[54]	Serum & ELISA	Idiopathic PAH	13	270 ± 636.2 pg/mL	
		Systemic sclerosis-associated PAH	15	333 ± 520 pg/mL	
		Systemic sclerosis without PAH	14	175 ± 68.68 pg/mL	
[14]	Plasma & ELISA	Portopulmonary hypertension	21	46.68 ng/mL	
		Controls with liver disease without both pulmonary hypertension and hepatopulmonary syndrome	31	31.19 ng/mL	

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), placental 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 (BMPRII) 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).

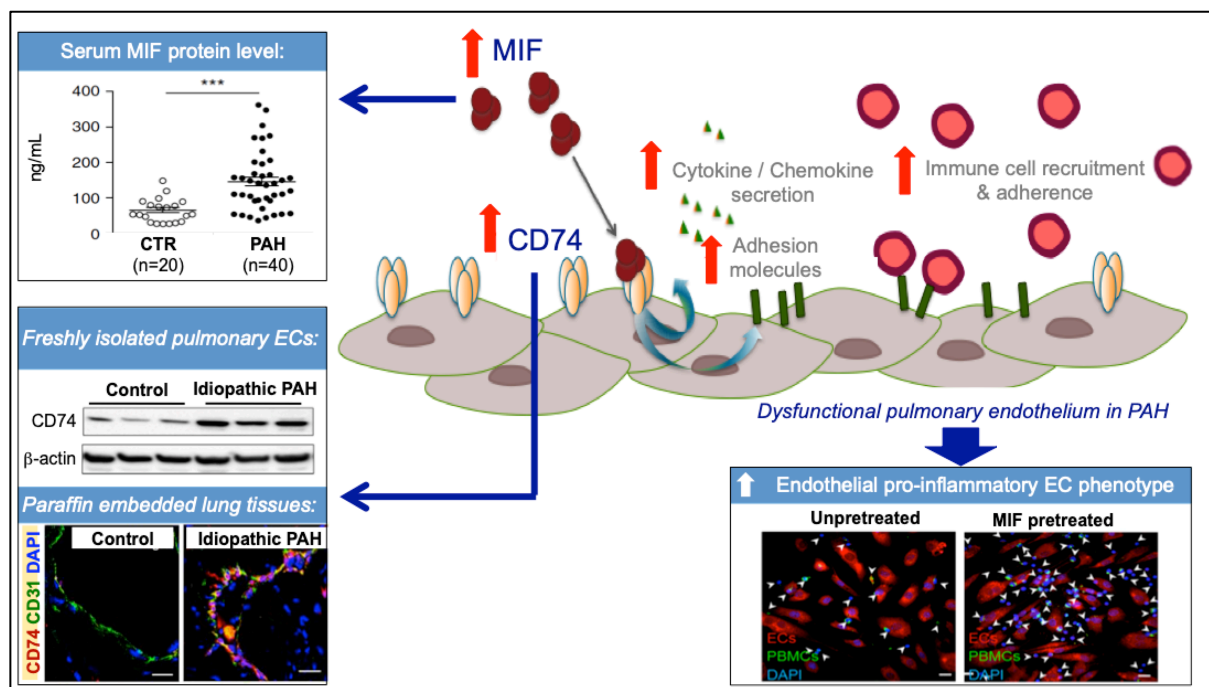


Figure 2: The MIF/CD74 axis contributes to the endothelial pro-inflammatory phenotype and leukocyte recruitment in pulmonary arterial hypertension (PAH).

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 trifluoromethylbenzoxazol-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).

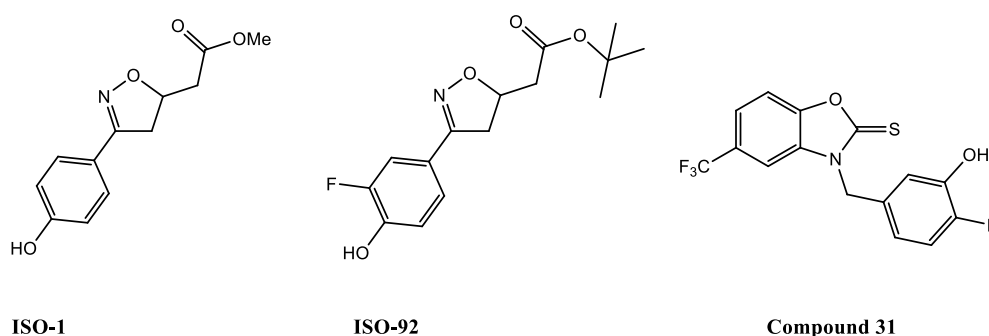


Figure 3. Chemical structure of MIF inhibitors tested against PH in animal models

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 cardiological 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.

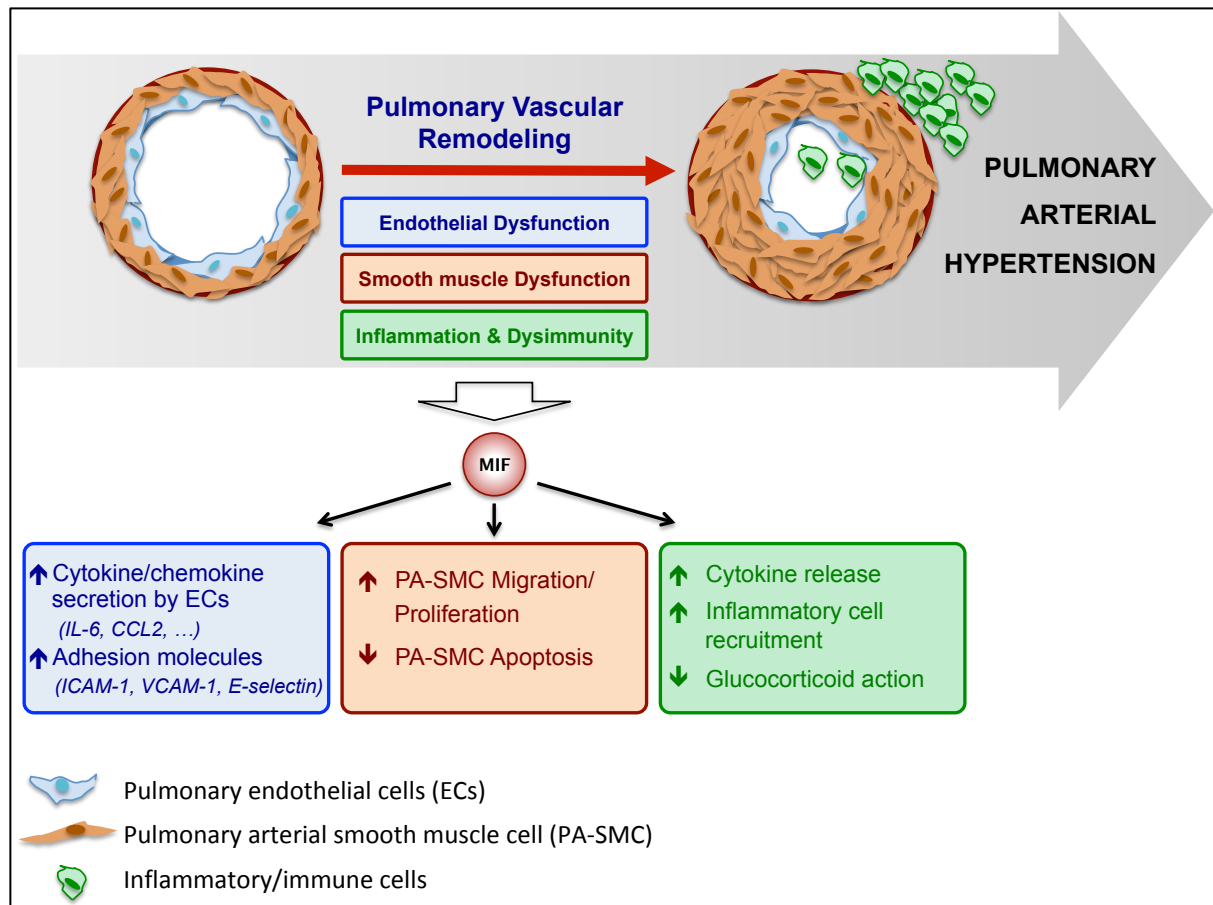


Figure 4: Schematic diagram summarizing the contribution of MIF to the 3 main components of the pulmonary vascular remodeling associated to pulmonary arterial hypertension (PAH).

Acknowledgments:

This research was supported by grants from the French National Institute for Health and Medical Research (INSERM), the University of Paris-Sud and the Université Paris-Saclay, the Marie Lannelongue Hospital, the French National Agency for Research (ANR) grant no. ANR-16-CE17-0014 (TAMIRAH), the Fondation pour la Recherche Médicale (FRM) grant no. DEQ20150331712 (Equipe FRM 2015), and in part by the Département Hospitalo-Universitaire (DHU) Thorax Innovation (TORINO), the Assistance Publique-Hôpitaux de Paris (AP-HP), Service de Pneumologie, Centre de Référence de l'Hypertension Pulmonaire Sévère, LabEx LERMIT (grant no. ANR-10-LABX-0033), the French PAH patient association (HTAP France), and the french Fonds de Dotation "Recherche en Santé Respiratoire" - (FRSR) - Fondation du Souffle (FdS).

6. References

1. **Alampour-Rajabi S, El Bounkari O, Rot A, Muller-Newen G, Bachelier F, Gawaz M, Weber C, Schober A, and Bernhagen J.** MIF interacts with CXCR7 to promote receptor internalization, ERK1/2 and ZAP-70 signaling, and lymphocyte chemotaxis. *FASEB J* 29: 4497-4511, 2015.
2. **Bargagli E, Olivieri C, Nikiforakis N, Cintorino M, Magi B, Perari MG, Vagaggini C, Spina D, Prasse A, and Rottoli P.** Analysis of macrophage migration inhibitory factor (MIF) in patients with idiopathic pulmonary fibrosis. *Respir Physiol Neurobiol* 167: 261-267, 2009.
3. **Bernhagen J, Krohn R, Lue H, Gregory JL, Zerneck A, Koenen RR, Dewor M, Georgiev I, Schober A, Leng L, Kooistra T, Fingerle-Rowson G, Ghezzi P, Kleemann R, McColl SR, Bucala R, Hickey MJ, and Weber C.** MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat Med* 13: 587-596, 2007.
4. **Bonniaud P, Fabre A, Frossard N, Guignabert C, Inman M, Kuebler WM, Maes T, Shi W, Stampfli M, Uhlig S, White E, Witzernath M, Bellaye PS, Crestani B, Eickelberg O, Fehrenbach H, Guenther A, Jenkins G, Joos G, Magnan A, Maitre B, Maus UA, Reinhold P, Vernooy JHJ, Richeldi L, and Kolb M.** Optimising experimental research in respiratory diseases: an ERS statement. *Eur Respir J* 51: 2018.
5. **Bordenave J, Thuillet R, Tu L, Phan C, Cumont A, Marsol C, Huertas A, Savale L, Hibert M, Galzi JL, Bonnet D, Humbert M, Frossard N, and Guignabert C.** Neutralization of CXCL12 attenuates established pulmonary hypertension in rats. *Cardiovasc Res* 2019.
6. **Bossini-Castillo L, Campillo-Davo D, Lopez-Isac E, Carmona FD, Simeon CP, Carreira P, Callejas-Rubio JL, Castellvi I, Fernandez-Nebro A, Rodriguez-Rodriguez L, Rubio-Rivas M, Garcia-Hernandez FJ, Madronero AB, Beretta L, Santaniello A, Lunardi C, Airo P, Hoffmann-Vold AM, Kreuter A, Riemekasten G, Witte T, Hunzelmann N, Vonk MC, Voskuyl AE, de Vries-Bouwstra J, Shiels P, Herrick A, Worthington J, Radstake T, Martin J, and Spanish Scleroderma G.** An MIF Promoter Polymorphism Is Associated with Susceptibility to Pulmonary Arterial Hypertension in Diffuse Cutaneous Systemic Sclerosis. *J Rheumatol* 44: 1453-1457, 2017.
7. **Boucly A, Weatherald J, Savale L, Jais X, Cottin V, Prevot G, Picard F, de Groote P, Jevnikar M, Bergot E, Chaouat A, Chabanne C, Bourdin A, Parent F, Montani D, Simonneau G, Humbert M, and Sitbon O.** Risk assessment, prognosis and guideline implementation in pulmonary arterial hypertension. *Eur Respir J* 50: 2017.
8. **Burton VJ, Holmes AM, Ciucan LI, Robinson A, Roger JS, Jarai G, Pearce AC, and Budd DC.** Attenuation of leukocyte recruitment via CXCR1/2 inhibition stops the progression of PAH in mice with genetic ablation of endothelial BMPR-II. *Blood* 118: 4750-4758, 2011.
9. **Canter JA, Summar ML, Smith HB, Rice GD, Hall LD, Ritchie MD, Motsinger AA, Christian KG, Drinkwater DC, Jr., Scholl FG, Dyer KL, Kavanaugh-McHugh AL, and Barr FE.** Genetic variation in the mitochondrial enzyme carbamyl-phosphate synthetase I predisposes children to increased pulmonary artery pressure following surgical repair of congenital heart defects: a validated genetic association study. *Mitochondrion* 7: 204-210, 2007.
10. **Costello CM, McCullagh B, Howell K, Sands M, Belperio JA, Keane MP, Gaine S, and McLoughlin P.** A role for the CXCL12 receptor, CXCR7, in the pathogenesis of human pulmonary vascular disease. *Eur Respir J* 39: 1415-1424, 2012.

11. **Cottin V, Price LC, and Valenzuela C.** The unmet medical need of pulmonary hypertension in idiopathic pulmonary fibrosis. *Eur Respir J* 51: 2018.
12. **Dai Z, Zhu MM, Peng Y, Jin H, Machireddy N, Qian Z, Zhang X, and Zhao YY.** Endothelial and Smooth Muscle Cell Interaction via FoxM1 Signaling Mediates Vascular Remodeling and Pulmonary Hypertension. *American journal of respiratory and critical care medicine* 198: 788-802, 2018.
13. **Dewor M, Steffens G, Krohn R, Weber C, Baron J, and Bernhagen J.** Macrophage migration inhibitory factor (MIF) promotes fibroblast migration in scratch-wounded monolayers in vitro. *FEBS Lett* 581: 4734-4742, 2007.
14. **DuBrock HM, Rodriguez-Lopez JM, LeVarge BL, Curry MP, VanderLaan PA, Zsengeller ZK, Pernicone E, Preston IR, Yu PB, Nikolic I, Xu D, Thadhani RI, Channick RN, and Ananth Karumanchi S.** Macrophage migration inhibitory factor as a novel biomarker of portopulmonary hypertension. *Pulm Circ* 6: 498-507, 2016.
15. **Engelfriet PM, Duffels MG, Moller T, Boersma E, Tijssen JG, Thaulow E, Gatzoulis MA, and Mulder BJ.** Pulmonary arterial hypertension in adults born with a heart septal defect: the Euro Heart Survey on adult congenital heart disease. *Heart* 93: 682-687, 2007.
16. **Farkas D, Kraskauskas D, Drake JI, Alhussaini AA, Kraskauskiene V, Bogaard HJ, Cool CD, Voelkel NF, and Farkas L.** CXCR4 inhibition ameliorates severe obliterative pulmonary hypertension and accumulation of C-kit(+) cells in rats. *PLoS One* 9: e89810, 2014.
17. **Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, and Hoeper M.** 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Respir J* 46: 903-975, 2015.
18. **Gambaryan N, Perros F, Montani D, Cohen-Kaminsky S, Mazmanian M, Renaud JF, Simonneau G, Lombet A, and Humbert M.** Targeting of c-kit+ haematopoietic progenitor cells prevents hypoxic pulmonary hypertension. *Eur Respir J* 37: 1392-1399, 2011.
19. **Gatzoulis MA, Beghetti M, Landzberg MJ, and Galie N.** Pulmonary arterial hypertension associated with congenital heart disease: recent advances and future directions. *Int J Cardiol* 177: 340-347, 2014.
20. **Grieb G, Kim BS, Simons D, Bernhagen J, and Pallua N.** MIF and CD74 - suitability as clinical biomarkers. *Mini Rev Med Chem* 14: 1125-1131, 2014.
21. **Guignabert C, and Dorfmüller P.** Pathology and Pathobiology of Pulmonary Hypertension. *Seminars in respiratory and critical care medicine* 38: 571-584, 2017.
22. **Guignabert C, Tu L, Girerd B, Ricard N, Huertas A, Montani D, and Humbert M.** New molecular targets of pulmonary vascular remodeling in pulmonary arterial hypertension: importance of endothelial communication. *Chest* 147: 529-537, 2015.
23. **Gunther S, Bordenave J, Hua-Huy T, Nicco C, Cumont A, Thuillet R, Tu L, Quatremerre T, Guilbert T, Jalce G, Batteux F, Humbert M, Savale L, Guignabert C, and Dinh-Xuan AT.** Macrophage Migration Inhibitory Factor (MIF) Inhibition in a Murine Model of Bleomycin-Induced Pulmonary Fibrosis. *Int J Mol Sci* 19: 2018.

24. **Gunther S, Fagone P, Jalce G, Atanasov A, Guignabert C, and Nicoletti F.** Role of MIF and D-DT in immune-inflammatory, autoimmune, and chronic respiratory diseases: from pathogenic factors to therapeutic targets. *Drug Discov Today* 2018.
25. **Gunther S, Fagone P, Jalce G, Atanasov AG, Guignabert C, and Nicoletti F.** Role of MIF and D-DT in immune-inflammatory, autoimmune, and chronic respiratory diseases: from pathogenic factors to therapeutic targets. *Drug discovery today* 24: 428-439, 2019.
26. **Harper JM, Wilkinson JE, and Miller RA.** Macrophage migration inhibitory factor-knockout mice are long lived and respond to caloric restriction. *FASEB J* 24: 2436-2442, 2010.
27. **Hoeper MM, Lam CSP, Vachiery JL, Bauersachs J, Gerges C, Lang IM, Bonderman D, Olsson KM, Gibbs JSR, Dorfmüller P, Guazzi M, Galie N, Manes A, Handoko ML, Vonk-Noordegraaf A, Lankeit M, Konstantinides S, Wachter R, Opitz C, and Rosenkranz S.** Pulmonary hypertension in heart failure with preserved ejection fraction: a plea for proper phenotyping and further research. *European heart journal* 38: 2869-2873, 2017.
28. **Honma N, Koseki H, Akasaka T, Nakayama T, Taniguchi M, Serizawa I, Akahori H, Osawa M, and Mikayama T.** Deficiency of the macrophage migration inhibitory factor gene has no significant effect on endotoxaemia. *Immunology* 100: 84-90, 2000.
29. **Huertas A, Guignabert C, Barbera JA, Bartsch P, Bhattacharya J, Bhattacharya S, Bonsignore MR, Dewachter L, Dinh-Xuan AT, Dorfmüller P, Gladwin MT, Humbert M, Kotsimbos T, Vassilakopoulos T, Sanchez O, Savale L, Testa U, and Wilkins MR.** Pulmonary vascular endothelium: the orchestra conductor in respiratory diseases: Highlights from basic research to therapy. *The European respiratory journal* 51: 2018.
30. **Huertas A, Perros F, Tu L, Cohen-Kaminsky S, Montani D, Dorfmüller P, Guignabert C, and Humbert M.** Immune dysregulation and endothelial dysfunction in pulmonary arterial hypertension: a complex interplay. *Circulation* 129: 1332-1340, 2014.
31. **Huertas A, Tu L, and Guignabert C.** New targets for pulmonary arterial hypertension: going beyond the currently targeted three pathways. *Curr Opin Pulm Med* 23: 377-385, 2017.
32. **Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, and Rabinovitch M.** Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J* 53: 2019.
33. **Humbert M, Monti G, Brenot F, Sitbon O, Portier A, Grangeot-Keros L, Duroux P, Galanaud P, Simonneau G, and Emilie D.** Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. *American journal of respiratory and critical care medicine* 151: 1628-1631, 1995.
34. **Kasama T, Isojima S, Umemura M, Tsukamoto H, Tokunaga T, Furuya H, Yanai R, Takahashi R, Nakamura M, and Inagaki K.** Serum macrophage migration inhibitory factor levels are correlated with response to tocilizumab therapy in patients with rheumatoid arthritis. *Rheumatol Int* 34: 429-433, 2014.
35. **Kolb TM, Rafaels N, Gao L, Barnes K, Girgis R, Mathai SC, Zaiman A, Damico RL, and Hassoun PM.** Serum Macrophage Migration Inhibitory Factor (MIF) Is Increased In Patients With Pulmonary Arterial Hypertension. *American journal of respiratory and critical care medicine* 183: A1990, 2011.
36. **Kolb TM, Varney J, Hassoun PM, and Damico RL.** Macrophage Migration Inhibitory Factor (MIF)-Deficient Mice Are Protected From Right Ventricular Remodeling In A Model Of

- Chronic Hypoxia-Induced Pulmonary Hypertension. *American journal of respiratory and critical care medicine* 183: A4971, 2011.
37. **Lam CS, Roger VL, Rodeheffer RJ, Borlaug BA, Enders FT, and Redfield MM.** Pulmonary hypertension in heart failure with preserved ejection fraction: a community-based study. *Journal of the American College of Cardiology* 53: 1119-1126, 2009.
 38. **Le Hiress M, Akagah B, Bernadat G, Tu L, Thuillet R, Huertas A, Phan C, Fadel E, Simonneau G, Humbert M, Jalce G, and Guignabert C.** Design, Synthesis, and Biological Activity of New N-(Phenylmethyl)-benzoxazol-2-thiones as Macrophage Migration Inhibitory Factor (MIF) Antagonists: Efficacies in Experimental Pulmonary Hypertension. *J Med Chem* 61: 2725-2736, 2018.
 39. **Le Hiress M, Tu L, Ricard N, Phan C, Thuillet R, Fadel E, Dorfmueller P, Montani D, de Man F, Humbert M, Huertas A, and Guignabert C.** Proinflammatory Signature of the Dysfunctional Endothelium in Pulmonary Hypertension. Role of the Macrophage Migration Inhibitory Factor/CD74 Complex. *American journal of respiratory and critical care medicine* 192: 983-997, 2015.
 40. **Li M, Riddle SR, Frid MG, El Kasmi KC, McKinsey TA, Sokol RJ, Strassheim D, Meyrick B, Yeager ME, Flockton AR, McKeon BA, Lemon DD, Horn TR, Anwar A, Barajas C, and Stenmark KR.** Emergence of fibroblasts with a proinflammatory epigenetically altered phenotype in severe hypoxic pulmonary hypertension. *J Immunol* 187: 2711-2722, 2011.
 41. **Luedike P, Alatzides G, Papathanasiou M, Heisler M, Pohl J, Lehmann N, and Rassaf T.** Circulating macrophage migration inhibitory factor (MIF) in patients with heart failure. *Cytokine* 110: 104-109, 2018.
 42. **Luedike P, Alatzides G, Papathanasiou M, Heisler M, Pohl J, Lehmann N, and Rassaf T.** Predictive potential of macrophage migration inhibitory factor (MIF) in patients with heart failure with preserved ejection fraction (HFpEF). *Eur J Med Res* 23: 22, 2018.
 43. **Maeda NY, Aiello VD, Santos PC, Thomaz AM, Kajita LJ, Bydlowski SP, and Lopes AA.** Relation of Macrophage Migration Inhibitory Factor to Pulmonary Hemodynamics and Vascular Structure and Carbamyl-Phosphate Synthetase I Genetic Variations in Pediatric Patients with Congenital Cardiac Shunts. *Mediators Inflamm* 2019: 7305028, 2019.
 44. **Marshall JD, Sauler M, Tonelli A, Rao Y, Bucala R, Lee PJ, and Fares WH.** Complexity of macrophage migration inhibitory factor (MIF) and other angiogenic biomarkers profiling in pulmonary arterial hypertension. *Pulm Circ* 7: 730-733, 2017.
 45. **Montani D, Perros F, Gambaryan N, Girerd B, Dorfmueller P, Price LC, Huertas A, Hammad H, Lambrecht B, Simonneau G, Launay JM, Cohen-Kaminsky S, and Humbert M.** C-kit-positive cells accumulate in remodeled vessels of idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 184: 116-123, 2011.
 46. **Morrell NW, Aldred MA, Chung WK, Elliott CG, Nichols WC, Soubrier F, Trembath RC, and Loyd JE.** Genetics and genomics of pulmonary arterial hypertension. *The European respiratory journal* 53: 2019.
 47. **Perveen S, Ayasolla K, Zagloul N, Patel H, Ochani K, Orner D, Benveniste H, Salerno M, Vaska P, Zuo Z, Alabed Y, Nasim M, Miller EJ, and Ahmed M.** MIF inhibition enhances pulmonary angiogenesis and lung development in congenital diaphragmatic hernia. *Pediatric research* 85: 711-718, 2019.
 48. **Rabinovitch M, Guignabert C, Humbert M, and Nicolls MR.** Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res* 115: 165-175, 2014.

49. **Rajasekaran D, Groning S, Schmitz C, Zierow S, Drucker N, Bakou M, Kohl K, Mertens A, Lue H, Weber C, Xiao A, Luker G, Kapurniotu A, Lolis E, and Bernhagen J.** Macrophage Migration Inhibitory Factor-CXCR4 Receptor Interactions: EVIDENCE FOR PARTIAL ALLOSTERIC AGONISM IN COMPARISON WITH CXCL12 CHEMOKINE. *J Biol Chem* 291: 15881-15895, 2016.
50. **Roger T, Schneider A, Weier M, Sweep FC, Le Roy D, Bernhagen J, Calandra T, and Giannoni E.** High expression levels of macrophage migration inhibitory factor sustain the innate immune responses of neonates. *Proc Natl Acad Sci U S A* 113: E997-1005, 2016.
51. **Russell KE, Chung KF, Clarke CJ, Durham AL, Mallia P, Footitt J, Johnston SL, Barnes PJ, Hall SR, Simpson KD, Starkey MR, Hansbro PM, Adcock IM, and Wiegman CH.** The MIF Antagonist ISO-1 Attenuates Corticosteroid-Insensitive Inflammation and Airways Hyperresponsiveness in an Ozone-Induced Model of COPD. *PLoS One* 11: e0146102, 2016.
52. **Sartina E, Suguihara C, Ramchandran S, Nwajei P, Rodriguez M, Torres E, Hehre D, Devia C, Walters MJ, Penfold ME, and Young KC.** Antagonism of CXCR7 attenuates chronic hypoxia-induced pulmonary hypertension. *Pediatr Res* 71: 682-688, 2012.
53. **Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, and Souza R.** Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 53: 2019.
54. **Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, Trembath RC, Jennings S, Barker L, Nicklin P, Walker C, Budd DC, Pepke-Zaba J, and Morrell NW.** Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. *Circulation* 122: 920-927, 2010.
55. **Stefanantoni K, Sciarra I, Vasile M, Badagliacca R, Poscia R, Pendolino M, Alessandri C, Vizza CD, Valesini G, and Ricciari V.** Elevated serum levels of macrophage migration inhibitory factor and stem cell growth factor beta in patients with idiopathic and systemic sclerosis associated pulmonary arterial hypertension. *Reumatismo* 66: 270-276, 2015.
56. **Summar ML, Hall LD, Eeds AM, Hutcheson HB, Kuo AN, Willis AS, Rubio V, Arvin MK, Schofield JP, and Dawson EP.** Characterization of genomic structure and polymorphisms in the human carbamyl phosphate synthetase I gene. *Gene* 311: 51-57, 2003.
57. **Sweatt AJ, Hedlin HK, Balasubramanian V, Hsi A, Blum LK, Robinson WH, Haddad F, Hickey PM, Condliffe R, Lawrie A, Nicolls MR, Rabinovitch M, Khatri P, and Zamanian RT.** Discovery of Distinct Immune Phenotypes Using Machine Learning in Pulmonary Arterial Hypertension. *Circulation research* 124: 904-919, 2019.
58. **Tamura Y, Phan C, Tu L, Le Hiress M, Thuillet R, Jutant EM, Fadel E, Savale L, Huertas A, Humbert M, and Guignabert C.** Ectopic upregulation of membrane-bound IL6R drives vascular remodeling in pulmonary arterial hypertension. *The Journal of clinical investigation* 128: 1956-1970, 2018.
59. **Tilstam PV, Qi D, Leng L, Young L, and Bucala R.** MIF family cytokines in cardiovascular diseases and prospects for precision-based therapeutics. *Expert Opin Ther Targets* 21: 671-683, 2017.
60. **van Riel AC, Schuurin MJ, van Hessen ID, Zwinderman AH, Cozijnsen L, Reichert CL, Hoorntje JC, Wagenaar LJ, Post MC, van Dijk AP, Hoendermis ES, Mulder BJ, and Bouma BJ.** Contemporary prevalence of pulmonary arterial hypertension in adult congenital heart disease following the updated clinical classification. *Int J Cardiol* 174: 299-305, 2014.

61. **Voelkel NF, Gomez-Arroyo J, Abbate A, Bogaard HJ, and Nicolls MR.** Pathobiology of pulmonary arterial hypertension and right ventricular failure. *The European respiratory journal* 40: 1555-1565, 2012.
62. **White DA, Fang L, Chan W, Morand EF, Kiriazis H, Duffy SJ, Taylor AJ, Dart AM, Du XJ, and Gao XM.** Pro-inflammatory action of MIF in acute myocardial infarction via activation of peripheral blood mononuclear cells. *PLoS One* 8: e76206, 2013.
63. **Zhang B, Luo Y, Liu ML, Wang J, Xu DQ, Dong MQ, Liu Y, Xu M, Dong HY, Zhao PT, Gao YQ, and Li ZC.** Macrophage migration inhibitory factor contributes to hypoxic pulmonary vasoconstriction in rats. *Microvasc Res* 83: 205-212, 2012.
64. **Zhang B, Shen M, Xu M, Liu LL, Luo Y, Xu DQ, Wang YX, Liu ML, Liu Y, Dong HY, Zhao PT, and Li ZC.** Role of macrophage migration inhibitory factor in the proliferation of smooth muscle cell in pulmonary hypertension. *Mediators Inflamm* 2012: 840737, 2012.
65. **Zhang T, Kawaguchi N, Hayama E, Furutani Y, and Nakanishi T.** High expression of CXCR4 and stem cell markers in a monocrotaline and chronic hypoxia-induced rat model of pulmonary arterial hypertension. *Exp Ther Med* 15: 4615-4622, 2018.
66. **Zhang Y, Talwar A, Tsang D, Bruchfeld A, Sadoughi A, Hu M, Omonuwa K, Cheng KF, Al-Abed Y, and Miller EJ.** Macrophage migration inhibitory factor mediates hypoxia-induced pulmonary hypertension. *Mol Med* 18: 215-223, 2012.
67. **Zorzaneli L, Maeda NY, Clave MM, Aiello VD, Rabinovitch M, and Lopes AA.** Serum Cytokines in Young Pediatric Patients with Congenital Cardiac Shunts and Altered Pulmonary Hemodynamics. *Mediators Inflamm* 2016: 7672048, 2016.