# Harnessing oxidized macrophage migration inhibitory factor (oxMIF) as a druggable isoform of MIF for targeted anticancer therapies

OncoOne

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## Introduction

Macrophage migration inhibitory factor (MIF) is a pleiotropic, pro-inflammatory cytokine that promotes tumorigenesis via activation of anti-apoptotic, pro-angiogenic and pro-proliferative pathways [1, 2] (**Fig. 1**). Patients with cancer can have overexpressed MIF in tumor tissue, which has been associated with high tumor burden and grade, increased metastasis risk, and poor prognosis [3-8]. MIF has further been described as counter-regulator of endogenous and exogenous corticoids [1] (**Fig. 2**). MIF is markedly different from other cytokines and chemokines because it is constitutively expressed, stored in the cytoplasm and is present in the circulation of healthy subjects at high levels of ~6 ng/ml [9]. Due to its ubiquitous nature, MIF can be considered as an inappropriate target for therapeutic intervention.

However, the founders of OncoOne have discovered that MIF occurs in two immunologically distinct conformational isoforms, termed reduced MIF (redMIF) and oxidized MIF (oxMIF) [10] (**Fig. 3-5**). RedMIF is an abundantly expressed isoform of MIF circulating in bloodstream of healthy individuals and it seems to represent a latent non-active storage form [11]. In contrast, oxMIF is the disease related isoform that was detected in patients with acute and chronic inflammatory diseases (**Fig. 6**) and is specifically expressed in solid tumors [10, 12] (**Fig. 7**) which renders oxMIF an attractive target for therapeutic intervention . A first generation fully human IgG1 anti-oxMIF monoclonal antibody (mAb), imalumab, was investigated in a Phase 1 study in patients with CRC, NSCLC and ovarian cancers. The Phase 1 study revealed no safety concern in patients with advanced solid tumors at doses up to 37.5 mg/kg Q2W and tumor biopsies showed reasonable tissue penetration of Imalumab (**Fig. 8**). The Phase I investigators concluded that further clinical investigation is warranted to assess the role of oxMIF as a therapeutic target in humans based on preclinical evidence suggesting that oxMIF may play a role in carcinogenesis and cancer-associated inflammation [13].

To overcome limitations of the first generation anti-oxMIF antibodies, OncoOne is focused on developing multiple proprietary, second generation anti-oxMIF antibody modalities by combining oxMIF specificity with cytotoxic function. As anti-oxMIF mAbs have been shown to sensitize human cancer cell lines to the action of cytotoxic drugs *in vitro* and *in vivo* [12], we anticipate that our novel therapeutic anti-oxMIF antibody ON103 can be combined with SoC chemotherapeutics. We have started IND-enabling preclinical evaluation for our lead therapeutic candidate ON103, a novel bioengineered mAb targeting oxMIF with enhanced effector functions and improved biophysical properties. We plan to combine our lead therapeutic candidate with a radiolabeled anti-oxMIF antibody (ON102) as a companion diagnostic. This co-development will guide clinical drug development and enable the targeted treatment of patients harboring oxMIF-positive tumors (**Fig.A**).

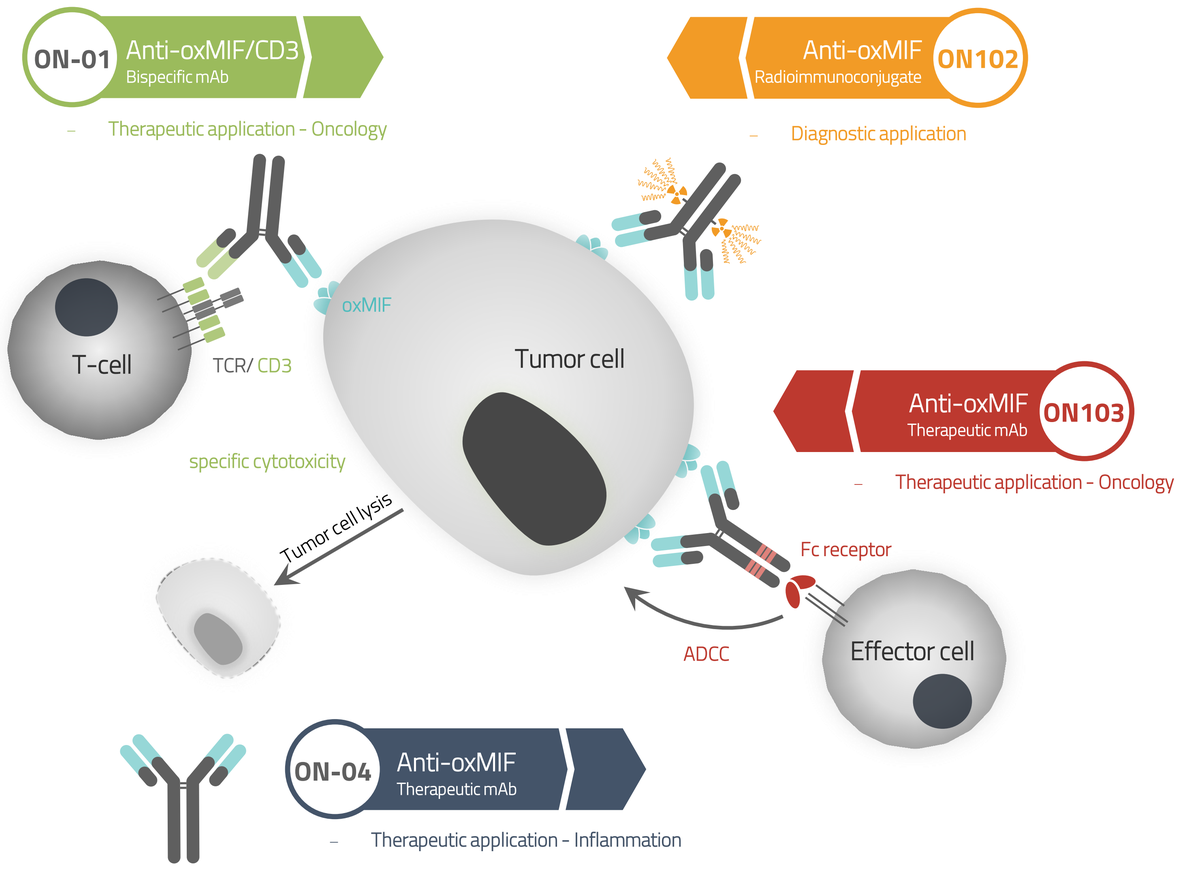


**Fig. A. OncoOne is developing multiple proprietary drug modalities targeting oxMIF.**
We created an antibody with enhanced effector functions that has already entered IND enabling studies and will be developed in parallel with a diagnostic anti-oxMIF radioimmunoconjugate, designated as projects ON103 and ON102, respectively. This strategy will allow targeted treatment of patients harboring oxMIF-positive tumors with ON103, potentially combined with standard of care chemotherapeutics. In addition, project ON-01 deals with anti-oxMIF/CD3 bispecific antibodies which recruit and activate t cells for targeted elimination of tumor cells by cytotoxic t cells. OncoOne's newest ox-MIF program, ON-04, is an anti-oxMIF antibody in fast-track development for the treatment of chronic inflammatory diseases.

## Research on MIF and development of anti-oxMIF antibodies

### MIF is an inflammation- and tumorigenesis-promoting cytokine which can override anti-inflammatory effects of glucocorticoids

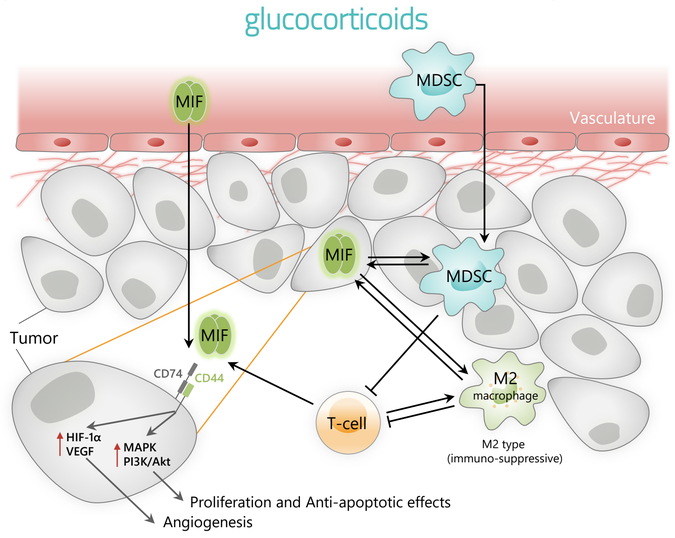


**Fig. 1. Role of MIF in activation of anti-apoptotic, pro-angiogenic and pro-proliferative pathways.** MIF is secreted into extracellular space, where it can interact with membrane receptor CD74, expressed on the surface of tumor cells. This interaction affects several proliferation and angiogenesis pathways, and can also modulate the anti-tumor immune response by upregulating the expression of specific immunosuppressor cells. MAPK = mitogen-activated protein kinase; PI3K = phosphoinositide 3-kinase; Akt = protein kinase B; HIF-1α = hypoxia-induced factor 1α; VEGF = vascular epithelial growth factor; MDSC = myeloid-derived suppressor cell.

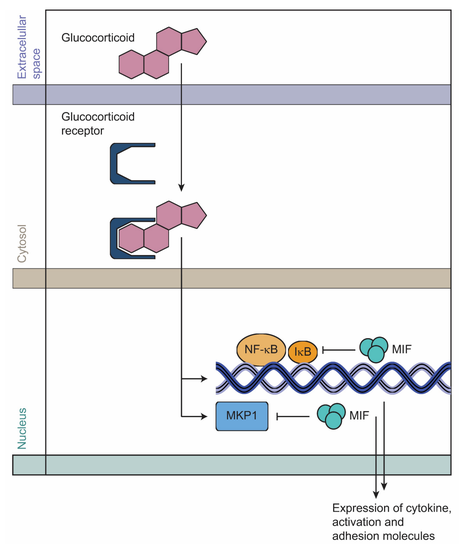


**Fig. 2. The physiological role of MIF is to counterbalance the inhibitory effects of steroids on the inflammation and immune responses.** MIF transcriptionally suppresses glucocorticoid induction of the nuclear factor-κB (NF-κB) inhibitor IκB and consequently acts as an antagonist of glucocorticoid-mediated immune suppression. Thus, MIF inhibition has been proposed as a potentially useful pharmacological strategy for the treatment of inflammatory and autoimmune diseases, especially conditions characterized by resistance to glucocorticoid therapy or by glucocorticoid dependence [14].

### Molecular switch between redMIF and oxMIF is crucial for antibody binding

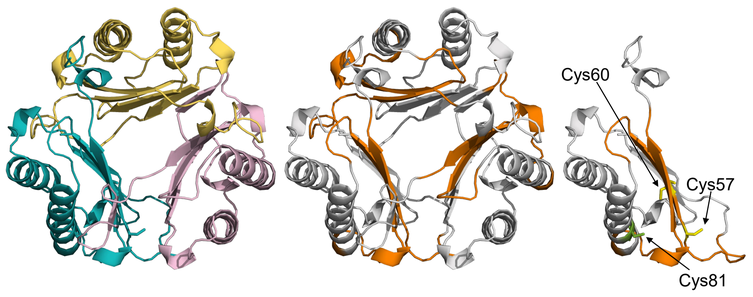


**Fig. 3. Crystal structure of MIF trimer with a 2.6 Å resolution. Left**: each subunit accounts for 12.5 kDa resulting in a total molecular weight of 37.5 kDa. **Middle**: Anti-oxMIF antibody-binding regions comprising aa 50-68 and 86-102 are depicted in orange. **Right**: Anti-oxMIF antibody-binding region of a single MIF subunit. Highlighted residues $Cys_{57}$-Ala-Leu-$Cys_{60}$ (yellow) present a core of MIF´s thiol protein oxidoreductase motif. Oxidation of $Cys_{81}$ (green) results in a conformational change and transition of redMIF to oxMIF (right) [15, 16, PDB entry 1MIF].

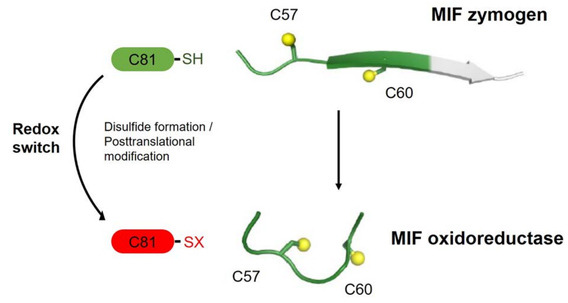


**Fig. 4. RedMIF might reflect a latent zymogenic form of MIF, and formation of oxMIF leads to a physiologically relevant, i.e., enzymatically active, state.** Crystal structure analysis of the human MIF-derived EPCALCS peptide bound in its oxMIF-like conformation by the Fab fragment of an anti-oxMIF antibody revealed that this peptide adopts a curved conformation, making the central thiol protein oxidoreductase motif competent to undergo disulfide shuffling. This conformational change in the MIF molecule enables binding of oxMIF-specific antibodies. Reprinted with permission from [11], Copyright © 2018 American Chemical Society.

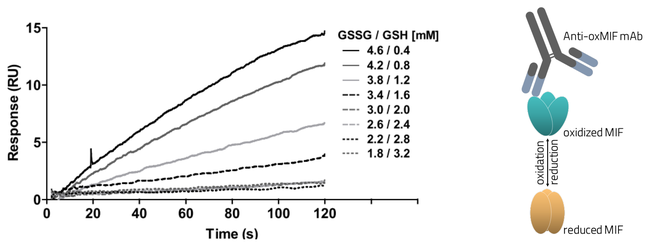


**Fig. 5. Redox-dependent binding of anti-oxMIF Abs to recombinant human MIF. Left:** Surface plasmon resonance studies using immobilized anti-oxMIF antibodies showed redox- dependent binding of human MIF in different redox buffers. Higher concentrations of oxidized glutathione (GSSG) versus reduced glutathione (GSH) resulted in a structural change within MIF that enabled binding of MIF to the anti-oxMIF antibody (illustrated schematically on the left) [10]. Recently, redox-dependent structural flexibility of MIF/oxMIF was confirmed by NMR- studies [17]. Fig. 5 left originally published in The Journal of Immunology, [10], Copyright © 2015 The American Association of Immunologists, Inc.

### Anti-oxMIF antibody excellently colocalizes with oxMIF at the sites of inflammation and tumors

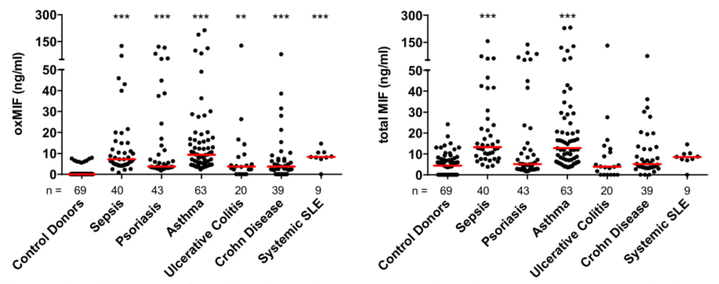


**Fig. 6. OxMIF is significantly elevated in plasma of patients with acute and chronic inflammatory diseases** [10]. **Left:** Plasma levels of oxMIF were significantly elevated in samples from patients with acute and chronic inflammatory diseases (up to 250 ng/ml) whereas healthy individuals did not contain detectable oxMIF. **Right**: Plasma samples from healthy individuals showed a baseline level of ~5ng/ml total MIF (total MIF = redMIF + oxMIF) and significant elevation was only detected in samples from patients with septicemia and asthma. Fig. 6 originally published in The Journal of Immunology, [10], Copyright © 2015 The American Association of Immunologists, Inc.

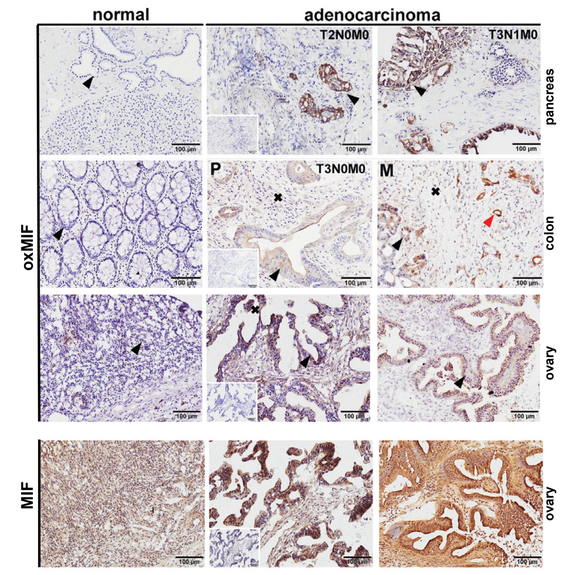


**Fig. 7. oxMIF occurs in malignant tissue and can be detected in primary tumors and metastases of different solid tumors by IHC.** Samples were obtained from adenocarcinomas of pancreas, colon and ovary, and from respective healthy organs. Samples were stained with monoclonal anti-oxMIF Abs or polyclonal anti-MIF Abs followed by DAB and hematoxylin [12].

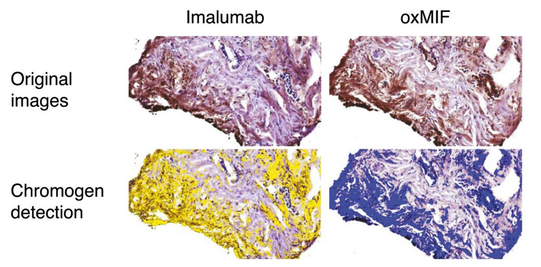


**Fig. 8. Overlap of anti-oxMIF Ab imalumab (yellow) and oxMIF (blue).** Anti-oxMIF antibody well penetrated mCRC tumor and co-localized with oxMIF [13].

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