Preclinical PET imaging with ^{89}Zr-labelled oxMIF-specific antibody delineates subcutaneous tumors in colorectal murine models

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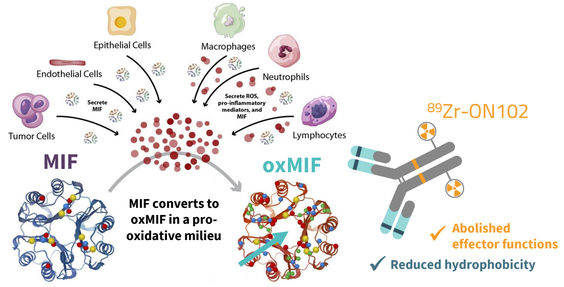
1 Aim/ Introduction

The oxidized macrophage migration inhibitory factor (oxMIF) is the disease-related and structurally distinct isoform of the well-known pleiotropic cytokine MIF [1-6]. It arises as a result of post-translational modification within the pro-oxidative environment of the tumor microenvironment in solid tumors, contributing to inflammatory processes and tumorigenesis. As a consequence, oxMIF is exclusively localized in the tumor microenvironment (TME) of solid tumors. The specific oxidation of MIF exposes novel epitopes in the central channel of the MIF trimer, making them accessible targets for anti-oxMIF antibodies [7-8].

ON102Ab, a bioengineered anti-oxMIF monoclonal antibody, offers improved pharmacokinetics, biodistribution, and safety compared to a previously tested anti-oxMIF antibody [9]. Its potential for tumor detection using positron emission tomography (PET) was evaluated, showing promising results for precision imaging and targeted therapy.

oxMIF is the disease-related and druggable isoform of MIF



Adapted from Thiele, Donnelly & Mitchell, JITC. 2022 [8]

2 Material and Methods

We developed a second generation anti-oxMIF monoclonal IgG1 antibody, ON102Ab, with significantly improved biochemical and biological properties and point mutations abolishing the binding to FcγRs.

As part of this study, we compared ON102Ab to the first-generation mAb imalumab (C0008) using various physicochemical and biological tests including hydrophobic interaction chromatography (HIC) to analyze hydrophobicity and aggregation, while ELISA and Surface Plasmon Resonance (SPR) were employed to assess specificity and affinity. In-vitro safety was examined through antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) reporter assays, ADCC assay using human PBMCs, and cytokine release measurement from human PBMCs.

To investigate tumor penetration and retention, as well as pharmacokinetics (PK), we conducted experiments in Balb/c nude mice with subcutaneous HCT116 colorectal xenograft tumors. IRDye 800CW-labeled ON102Ab was intravenously injected, and mice were monitored for up to 7 days. Additionally, we radiolabeled ON102Ab with ^{89}Zr after conjugation with the chelator DFO*. This ^{89}Zr-ON102Ab was administered into Balb/c or Balb/c nude mice bearing CT26 or HCT116 colorectal tumors, respectively, and whole-body PET images were taken at 4, 7, and 10 days post-injection.

OncoOne's novel and improved diagnostic anti-oxMIF antibody

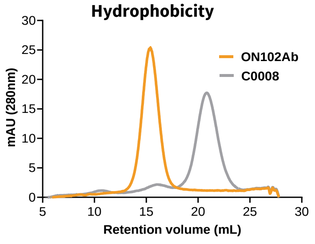
Diagnostic mAb ^{89}Zr-ON102

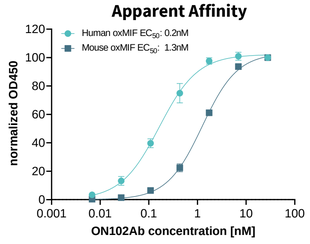
Optimized variable domains
Improved biophysical and pharmacological properties

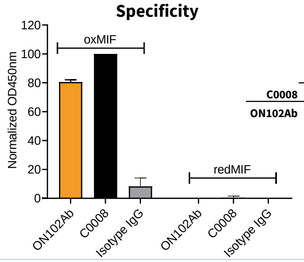
Optimized Fc
Engineered Fc to abolish FcγR binding

Optimized bioengineered second-generation anti-oxMIF mAb for solid tumor diagnosis using positron emission tomography (PET)

3 *In vitro* physicochemical Results





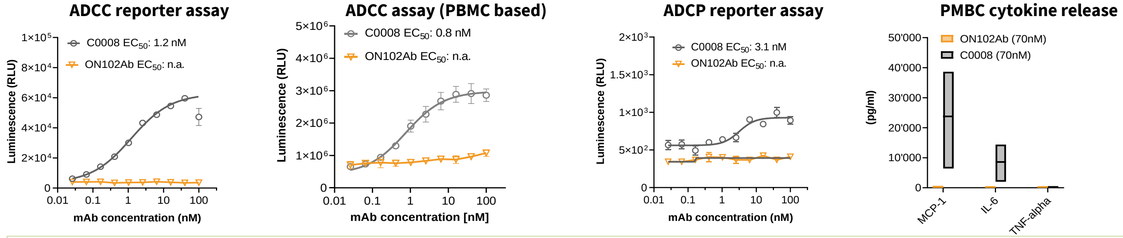


	SPR K_D, nM (SD)	ELISA EC_{50}, nM
C0008	3.5 (0.4)	0.2
ON102Ab	5.1 (0.9)	0.3

ON102Ab shows enhanced physicochemical properties compared to the first generation anti-oxMIF mAb.

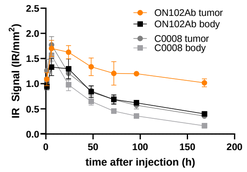
ON102Ab exhibits excellent specificity and affinity to oxMIF, with binding in the low nM range. Importantly, it does not interact with the reduced and abundant MIF isoform.

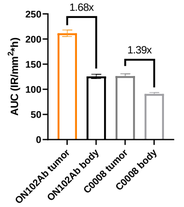
4 *In vitro* safety evaluation Results

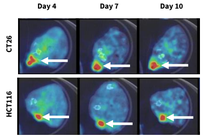


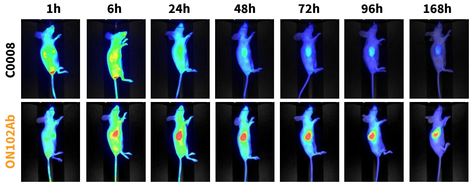
ON102Ab is expected to have a favorable safety profile as it does not induce ADCC or ADCP and does not trigger unspecific cytokine release from human PBMCs.

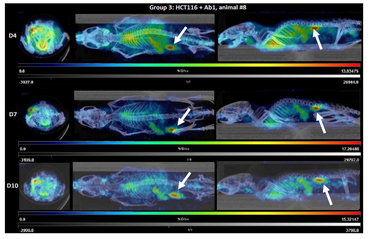
5 *In vivo* Results











C0008	
Terminal half life (tumor)	**3.5 days**
Terminal half life (body)	**2.4 days**

ON102Ab	
Terminal half life (tumor)	**9.5 days**
Terminal half life (body)	**5.4 days**

In a HCT116 xenograft model, IR-dye labeled ON102Ab demonstrated tumor accumulation and retention, with a terminal tumor half-life of 9.5 days and a promising tumor-to-body ratio.

Long-lasting tumor retention was observed for ^{89}Zr-ON102 in xenograft (HCT116) and syngraft (CT26) colon cancer models.

6 Conclusions

- ✓ **ON102Ab is an optimized monoclonal antibody targeting the oxidized macrophage migration inhibitory factor (oxMIF) with :**
  - Improved physicochemical and biological properties and high specificity and affinity for oxMIF in the low nM range.
  - An advantageous in vitro safety profile including reduced binding to FcγRs and no unspecific cytokine release from human PBMCs.
- ✓ **^{89}Zr-ON102 shows promising tumor accumulation and retention for >10 days in murine colorectal cancer models.**
- ✓ **These findings emphasize the potential of oxMIF as a tumor-specific target for theranostic intervention, and demonstrate the safety and diagnostic utility of ^{89}Zr-ON102 in detecting malignant solid tumors in humans.**

References

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