A novel diagnostic radioimmunoconjugate targeting oxidized macrophage migration inhibitory factor (oxMIF)

6th Theranostics World Congress · June 24–26, 2022 · RheinMain Congress Center Wiesbaden, Germany

 # 125

Friedmund Bachmann, Alejandro Puchol Tarazona, Christine Landlinger, Randolf J. Kerschbaumer, Michael Thiele, Alexander Schinagl
OncoOne Research & Development GmbH, Karl-Farkas-Gasse 22, A-1030 Vienna, Austria

OncoOne

1 Introduction

Macrophage migration inhibitory factor (MIF) is a pleiotropic, pro-inflammatory cytokine that promotes tumorigenesis and is able to modulate the tumor microenvironment (TME) to immune evasive, and immune tolerant phenotypes. Overexpression of MIF in tumor tissue is associated with poor prognosis. MIF is distinguished from other cytokines and chemokines by its constitutive expression and high presence in circulation of healthy subjects at levels of ~6 ng/ml [1-10].

MIF has proven undruggable by antibodies and small molecules

The founders of OncoOne discovered that MIF occurs in two immunologically distinct conformational isoforms, termed reduced MIF (redMIF) and oxidized MIF (oxMIF) [11]. RedMIF is the abundantly expressed isoform of MIF[11-13]. In contrast, oxMIF is the disease-related isoform that was specifically detected in solid tumors and sites of inflamation[11, 13] (**Fig. 1**). A first generation IgG1 anti-oxMIF antibody (mAb), imalumab, was investigated in Phase 1 (NCT01765790) and Phase 2 studies, in patients with CRC (NCT02540356) and ovarian cancers (NCT02448810) revealing that imalumab was well tolerated and showed signs of efficacy. However, these studies were terminated prematurely[14].

oxMIF – the disease-related and druggable isoform of MIF

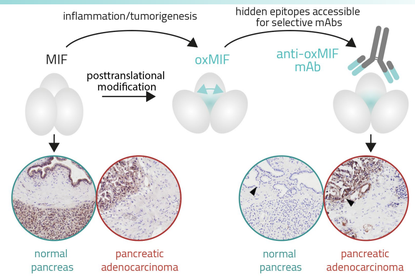


Fig. 1 oxMIF is generated by a posttranslational modification of MIF in inflammatory processes and tumorigenesis[11, 13]

2 Methods

OncoOne designed bioengineered second generation anti-oxMIF mAb **ON102Ab** with highly improved biochemical and biological properties (**Fig. 2**). The diagnostic mAb ON102Ab is an IgG1 bearing Fc mutations abolishing the binding to FcγRs and point mutations in the variable domain to improve physicochemical properties. ON102Ab was labelled with ^{89}Zr resulting in the radiodiagnostic mAb ^{89}Zr-ON102 to enable the detection of solid tumors by positron emission tomography (PET). The anti-oxMIF mAb was compared to the first generation anti-oxMIF mAb imalumab (C0008 internal designation) *in vitro* and *in vivo*. Hydrophobicity and aggregate content were determined by hydrophobic interaction chromatography (HIC), whereas specificity and affinity were determined by ELISA and Surface Plasmon Resonance (SPR), respectively (**Fig. 3**). *In-vitro* safety (**Fig. 4**) was investigated by antibody-dependent cell-mediated cytotoxicity (ADCC) reporter assays and PMBC based cell assays. Tumor penetration/retention and PK were assessed using IRDye 800CW labeled ON102Ab or ^{89}Zr-ON102 in CT26 or HCT116 tumor-bearing Balb/c or Balb/c nude mice, respectively (**Fig. 5**).

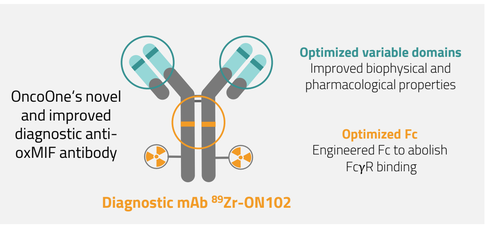
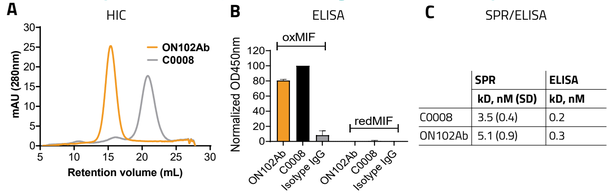


Fig. 2 Bioengineered second generation anti-oxMIF mAb with optimized variable domains and optimized Fc portions for diagnostic purposes

3 Results *in vitro*

ON102Ab demonstrated highly improved biochemical properties compared to C0008, while retaining the low nM affinity to oxMIF



	SPR kD, nM (SD)	ELISA kD, nM
C0008	3.5 (0.4)	0.2
ON102Ab	5.1 (0.9)	0.3

Fig. 3 (**A**) Hydrophobicity and aggregate content were analyzed by HIC. (**B**) The specific binding of ON102Ab to soluble human oxMIF and redMIF was analyzed by ELISA using TNB-MIF as an oxMIF surrogate[11]. (**C**) Affinities for human oxMIF were in the low nM range by SPR analysis and in the pM range by ELISA.

Increased safety through reduced ADCC activity compared to C0008, with no observed unspecific cytokine release

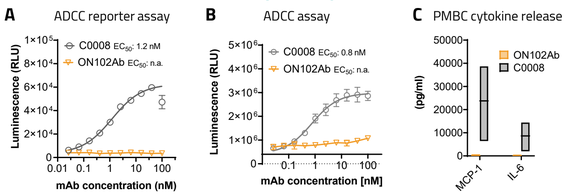
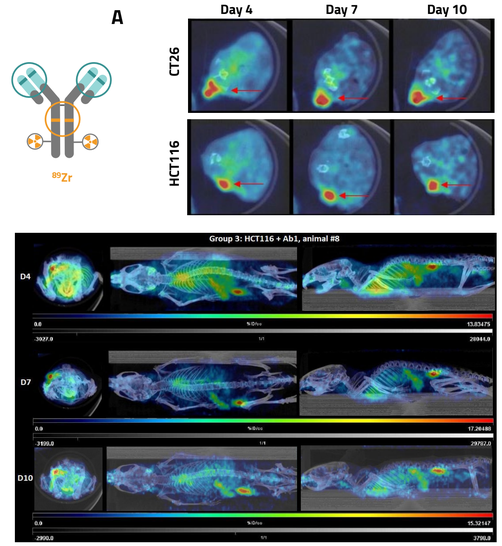


Fig. 4. ADCC was investigated by a reporter bioassay utilizing engineered reporter cells expressing FcγRIIIA (Promega) (**A**) or PBMC-mediated cell killing assay (**B**). Cytokine release (**C**) was studied upon incubation of human PBMCs with anti-oxMIF mAbs by a cytometric bead assays (BioLegend). Mean ± SEM or range is shown (n=2-4)

4 Results *in vivo*

ON102 shows improved biodistribution and >3-fold tumor accumulation and retention in mouse models of colon cancer compared to C0008



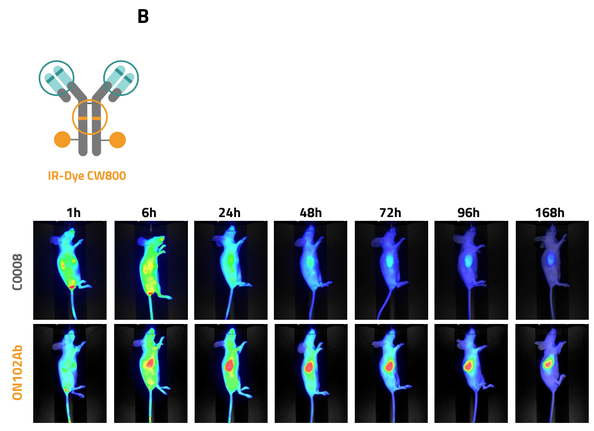


Fig.5 (**A**) HCT116 or CT26-bearing tumor-bearing Balb/c nude or Balb/c mice (tumor volume ~250 mm^3), respectively, were injected i.v. with a single dose ~10 MBq of ^{89}Zr-ON102. Mice were imaged up to 10 days at the time points indicated using a BioPET small animal PET/CT (Sedecal) device. Images were reconstructed with 2DOSEM algorithm with CT-based attenuation correction. (**B**). HCT116 tumor-bearing Balb/c nude mice (tumor volume ~300 mm^3) were injected i.v. with a single dose (5mg/kg) of IR-DyeCW800-labelled ON102Ab or C0008. Mice were imaged up to 7 days at the time points indicated using a LI-COR imaging device. One representative mouse per mAb is shown (n=3-4)

5 Conclusions

OxMIF, the disease-related and druggable isoform of MIF, is a novel target with broad applications in cancer and inflammation therapy and diagnosis. OncoOne's bioengineering efforts significantly improved the physicochemical and biological properties of ON102Ab compared to first generation anti-oxMIF mAb imalumab, while the low affinity for oxMIF was retained. Improvements include increased safety due to a strongly reduced binding to FcγRs as well as improved biodistribution and >3-fold enhanced tumor retention when compared to imalumab. Administration of ^{89}Zr-ON102 allowed the detection of solid tumors by PET imaging in syngrafted and xenografted colorectal tumors in mice. This highlights the potential of ^{89}Zr-ON102 as a safe diagnostic tool for the detection of malignant solid tumors and metastasis in humans and the utility of oxMIF as a tumor-specific target for theranostic applications.

References

[1] Calandra and Roger, Nat Rev Immunol. 2003 Oct;3(10):791-800.
[2] Mitchell et al., Proc Natl Acad Sci U S A. 2002 Jan 8;99(1):345-50.
[3] Ospiyan et al., Drug Discovery Today. 2021 Jul; 26(7), 1728–1734.
[4] Funamizu et al., Int J Cancer. 2013 Feb 15;132(4):785-94.
[5] He et al., Mol Med. 2009 Jan-Feb;15(1-2):1-10.
[6] Krockenberger et al., Anticancer Res. 2012 Dec;32(12):5233-8.
[7] Meyer-Siegler et al., BMC Cancer. 2005 Jul 6;5:73.
[8] Ren et al., Ann Surg. 2005 Jul;242(1):55-63.
[9] Tomiyasu et al., Clin Cancer Res. 2002 Dec;8(12):3755-60.
[10] Roger et al., Front Immunol. 2017 Jan 25;8:26.
[11] Thiele et al., J Immunol. 2015 Sep 1;195(5):2343-52.
[12] Schinagl et al., Biochemistry. 2018 Mar 6;57(9):1523-1532.
[13] Schinagl et al., Oncotarget. 2016 Nov 8;7(45):73486-73496.
[14] Mahalingam et al., Br J Clin Pharmacol. 2020 Sep;86(9):1836-1848.