ON203: A novel bioengineered anti-oxMIF antibody with improved biophysicochemical properties and antitumorigenic activity

Gregor Rossmueller, Irina Mirkina, Verena Hoeld, Mert Basaran, Barbara Maurer, Alexander Schinagl
OncoOne Research & Development GmbH, Karl-Farkas-Gasse 22, A-1030 Vienna, Austria

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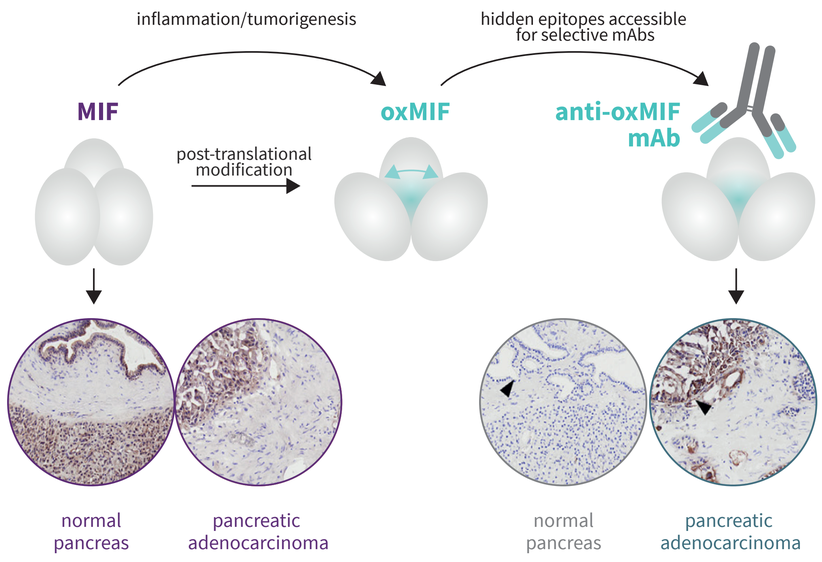
1 Introduction

Macrophage migration inhibitory factor (MIF) is a pleiotropic, pro-inflammatory cytokine which can directly promote 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 distinguishes from other cytokines and chemokines due to its constitutive expression and high presence in circulation of healthy subjects.[1-12]

MIF is considered undruggable by antibodies and small molecules

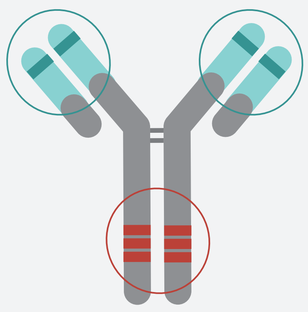
The founders of OncoOne discovered that MIF occurs in two immunologically distinct conformational isoforms, termed reduced MIF (redMIF) and oxidized MIF (oxMIF).[13] The redox-dependent MIF structure modifications affect enzymatic and biological functions.[14] RedMIF is the abundantly expressed isoform,[13, 15-16] whereas oxMIF is the disease-related isoform specifically detected in solid tumors.[13, 16, 17] A first generation IgG1 anti-oxMIF antibody, imalumab, was investigated in Phase 1 (NCT01765790) and Phase 2 studies in patients with CRC (NCT02448810) and ovarian cancers (NCT02540356) demonstrating that imalumab was well tolerated and showed signs of efficacy. These studies were terminated prematurely.[18]

oxMIF – the disease-related and druggable isoform of MIF



2 ON203 – Design of Optimized oxMIF Targeting

Therapeutic mAb ON203



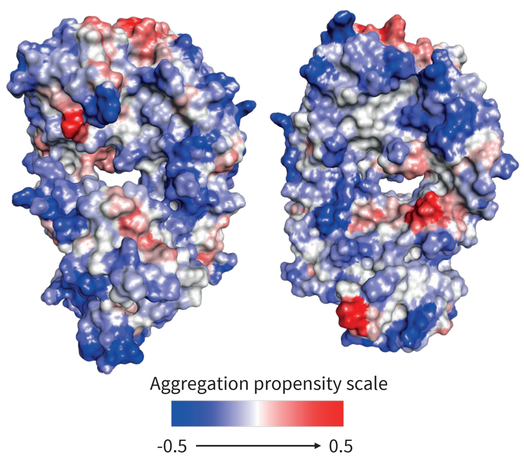
Optimized variable domains
Improved biophysical and pharmacological properties

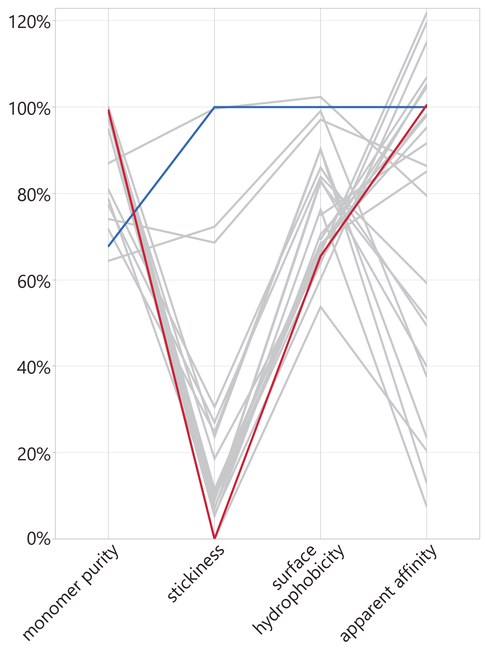
Optimized Fc
Engineered Fc to enhance Fc mediated effector functions

The bioengineered second-generation anti-oxMIF antibody ON203 is less hydrophobic and demonstrated reduced aggregation, while retaining the low nM affinity of imalumab. The Fc of ON203 carries a IgG1/2 hybrid constant heavy chain region with mutations to enhance effector functions.

3 Physicochemical Screening of Lead Precursor mAb

First generation anti-oxMIF antibody BaxB01 Fab structure (PDB:6FOE) with indicated aggregation hotspots modeled with Discovery Studio in VH-VL (left) and VL-VH (right) orientation. 100% sequence identity with imalumab Fab.

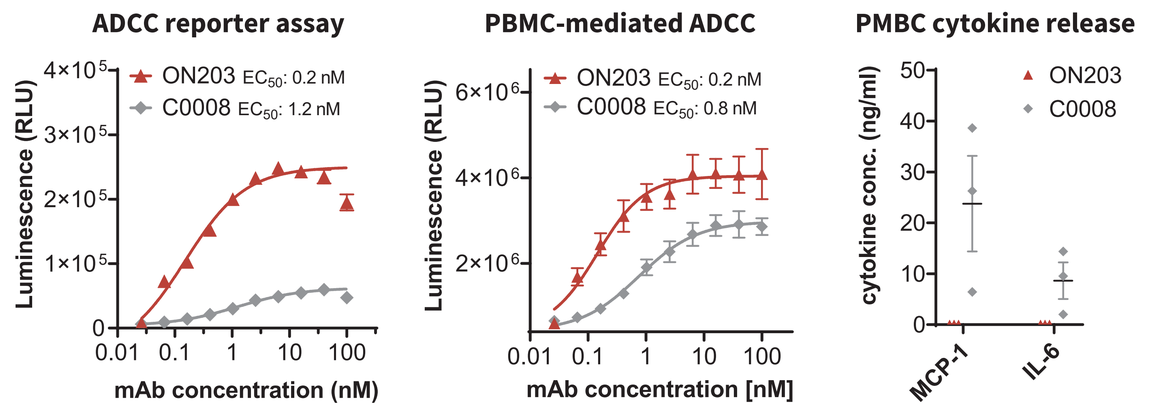




The right figure demonstrates the biophysical screening process of the generated mutants. The blue line represents C0008 (imalumab without the C-terminal lysine), the parental antibody for optimization. Screening variants with mutated VH/VL domains are shown in grey. The monomeric purity was determined by SEC and antibody stickiness is indicated by the delay in the elution from the SEC column. Surface hydrophobicity was evaluated by HIC and apparent binding affinity to oxMIF was analyzed by direct-binding ELISA to immobilized oxMIF. Data were normalized to C0008, except for monomer purity.

The V_H/V_L of ON203 (red line) demonstrated reduced aggregation, stickiness and hydrophobicity while maintaining the affinity to oxMIF.

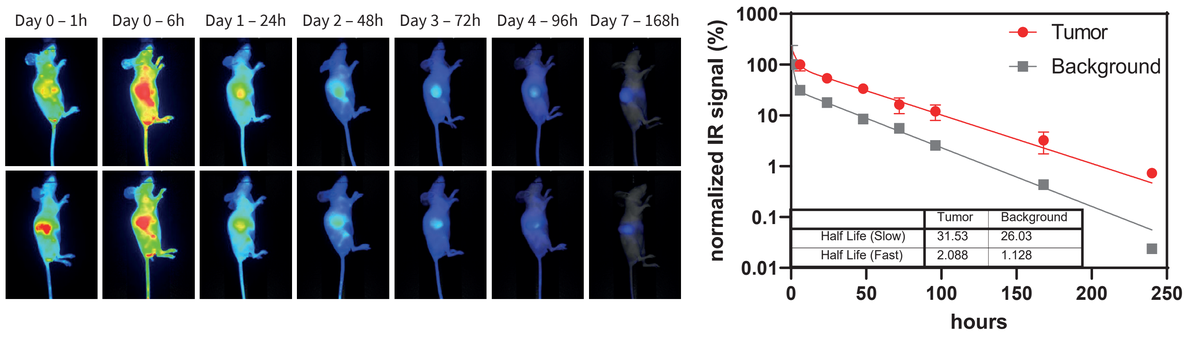
4 *In vitro* assessment of ON203's efficacy and safety



ADCC activity assessed in a reporter bioassay utilizing engineered reporter cells expressing FcγRIIIA (Promega) or in a PBMC-mediated target cell killing assay. Cytokine release was studied upon incubation of human PBMCs with anti-oxMIF mAbs (70nM) by a cytometric bead assays (BioLegend). Mean ± SEM or range is shown (n=2-4).

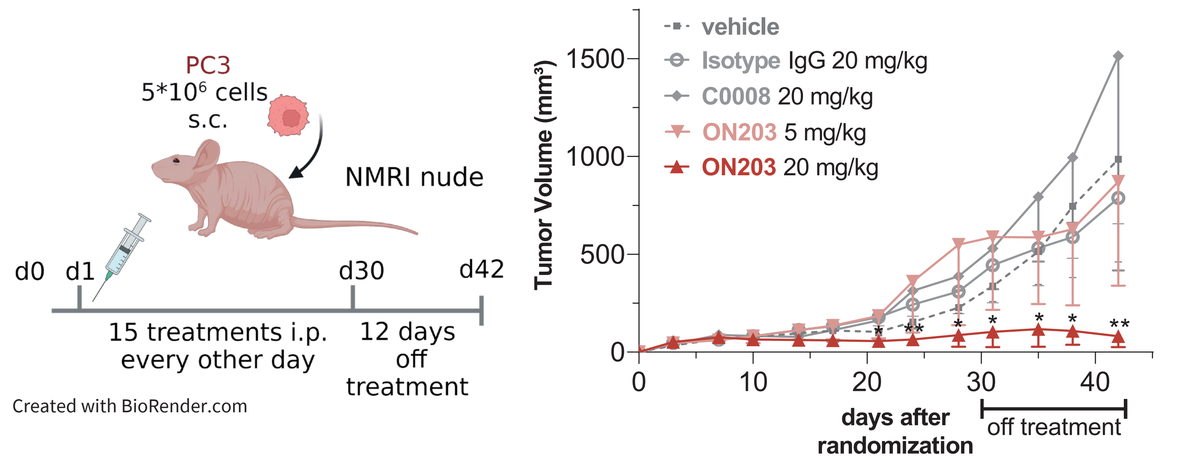
In vitro efficacy is increased up to 10-fold for ON203 compared to C0008, but no unspecific cytokine release is observed

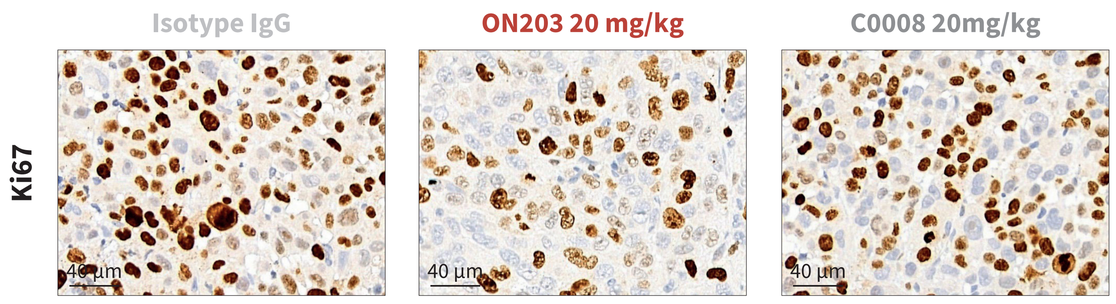
5 Biodistribution of ON203 in a Xenograft Tumor Model



Tumor accumulation and retention of ON203 >7 days in a xenograft mouse model (HCT116 colon cells in BALB/c nude mice) at 5 mg/kg single i.v. dose

6 Efficacy in a Prophylactic Xenograft PC3 Tumor Model





ON203 suppresses tumor growth and reduces expression of proliferation marker Ki67 compared to C0008 (imalumab).

7 Conclusions & Outlook

Bioengineering of the first generation anti-oxMIF mAb imalumab led to **ON203**

- Improved physicochemical properties (hydrophobicity ↓, aggregation ↓)
- Enhanced *in vitro* efficacy (ADCC & ADCP)
- Improved *in vitro* safety (unspecific cytokine release ↓)
- Improved efficacy in *in vivo* cancer model
- Maintaining nM affinity to oxMIF

Lead candidate **ON203** is on track to enter **clinical phase I by** 2023

References

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