ON203: A new antibody targeting the oxidized form of macrophage migration inhibitory factor (oxMIF) exerts antitumorigenic activity and modulates the tumor microenvironment

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1 Introduction

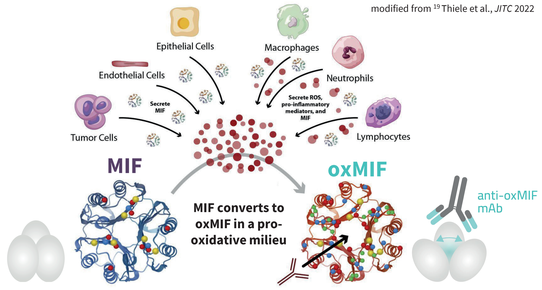
Macrophage migration inhibitory factor (MIF) is a pleiotropic, pro-inflammatory cytokine promoting tumorigenesis and modulating the tumor microenvironment (TME) to immune evasive and immune tolerant phenotypes. Overexpression of MIF in tumor tissue is associated with poor prognosis. MIF distinguishes from other cytokines and chemokines due to its constitutive expression and high presence in circulation of healthy subjects.[1-12]

➡ **MIF 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).[13-15] redMIF is the abundantly expressed isoform,[13, 16-17] whereas oxMIF is the disease-related isoform specifically detected in solid tumors.[13, 17] A first generation IgG1 anti-oxMIF antibody, imalumab (in house C0008), was investigated in Phase 1 (NCT01765790) and Phase 2 studies demonstrating safety and signs of efficacy. These studies were terminated prematurely.[18]

➡ **oxMIF – the disease-related and druggable isoform of MIF**

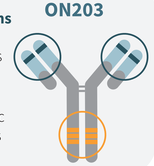
modified from [19] Thiele et al., *JITC* 2022



2 ON203 – Optimized Targeting of oxMIF

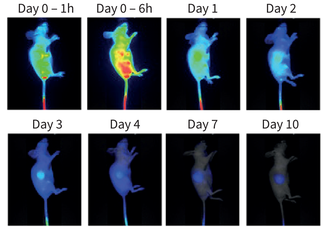
Optimized variable domains
Improved biophysical and pharmacological properties

Optimized Fc
Engineered Fc to enhance Fc mediated effector functions



✓ highly specific
✓ reduced aggregation
✓ enhanced ADCC

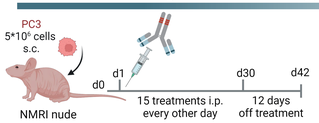
3 Biodistribution in Xenograft Tumor Model



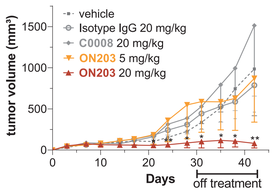
HCT116 tumor-bearing BALB/c nude mice were injected i.v. with a single dose (5mg/kg) of IRDye 800CW-labeled ON203

→ tumor retention up to 10 days
→ circulation half-life ~24h

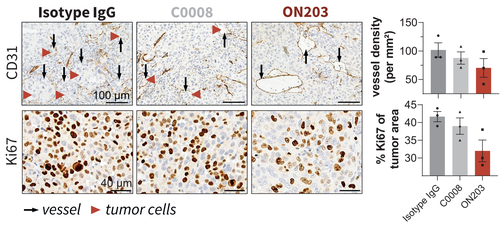
4 *In vivo* efficacy – PC3 Xenograft Tumor Model



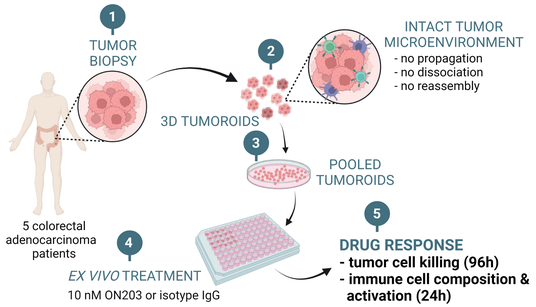
PC3 tumors were analyzed 12 days post last treatment. CD31⁺ vessel density and Ki67⁺ proliferation marker were reduced in ON203-treated tumors.

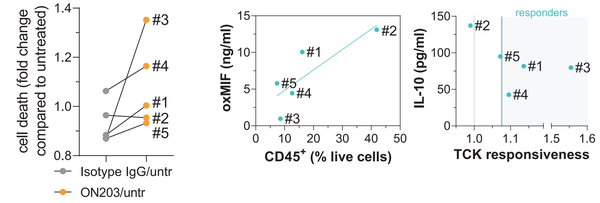


ON203 exerts **superior *in vivo* efficacy** in a prophylactic **mouse model of prostate cancer** compared to C0008



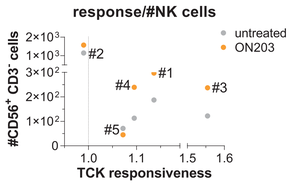
5 *Ex vivo* efficacy – Colorectal Cancer Tumoroids



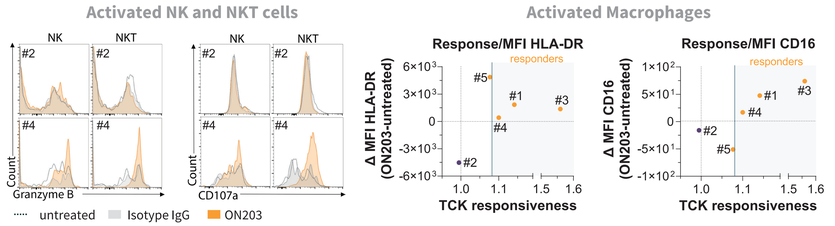


Left: Cell viability of untreated, 10 nM isotype IgG- or 10 nM ON203-treated tumor cells compared 96h post-treatment by high-content 3D computational bioimaging.
Right: oxMIF levels correlate positively with the number of immune cells in tumoroids. The non-responder tumoroid #2 showed the highest IL-10 level.

6 ON203 activates effector cells in Colorectal Cancer Tumoroids



Left: ON203 induced an increase in NK cell numbers in ON203-responder tumoroids (gated on live single CD45⁺ cells).
Bottom left: The expression of the activation marker Granzyme B and the degranulation marker CD107a increased in ON203 responders on NK and NKT cells, whereas the non-responder #2 showed no expression changes.
Bottom: ON203 led to a polarization of macrophages towards an anti-tumor phenotype in responders, visualized by an increase in HLA-DR and FcγRIII CD16 on macrophages.
Determined by flow cytometry 24 hours post treatment.



4 out of 5 CRC tumoroids responded with **tumor cell killing**
Tumor cell killing correlates with **activated effector cells** in ON203 responders
→ NK/NKT (↑ activation and degranulation markers Granzyme B and CD107a)
→ Macrophages (↑ HLA-DR and CD16)

7 Conclusions

oxMIF, the disease-related and druggable isoform of MIF, is a novel target with broad applications in cancer therapy. Our results demonstrate direct antitumorigenic effects of the anti-oxMIF antibody ON203 (i) by blocking the biologic function of oxMIF thereby reducing tumor cell proliferation and angiogenesis and (ii) by immunomodulation of the tumor microenvironment. In the upcoming Phase 1 trial we will evaluate ON203's safety, tolerability, pharmacokinetics, and pharmacodynamics in patients with solid tumors. ON203 bears a high potential as a standalone therapy or in rational combinations with immune checkpoint inhibitors or antiangiogenic agents in the treatment of solid tumors.

References

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