Targeting the oxidized form of macrophage migration inhibitory factor (oxMIF) with antibody ON203 activates the tumor microenvironment

AACR American Association for Cancer Research ANNUAL MEETING 2023 # 2974

Alexander Schinagl[1], Barbara Maurer[1], Eric R. Haas[2], Irina Mirkina[1], Julia Mayer[1], Jennifer L. Guerriero[3], Michael Thiele[1]
[1] OncoOne Research & Development GmbH, Vienna, Austria. [2] Ionic Cytometry Solutions, LLC, Cambridge, MA, USA. [3] Brigham and Women's Hospital, Boston, MA, USA.

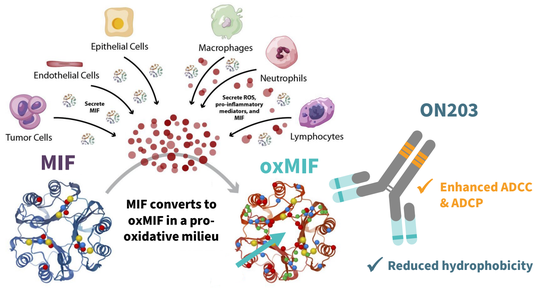
OncoOne

1 Introduction

Activation of the immunosuppressive tumor microenvironment (TMEs) can enhance immunotherapy response rates in solid cancers. One of the key TME regulators is the macrophage migration inhibitory factor (MIF). In addition to MIF's role in tumor cell proliferation, angiogenesis and metastasis, high levels of MIF induce the polarization of macrophages to the tumor enhancing M2-like subtype, suppresses cytotoxic T cells and correlates with poor response to immune checkpoint therapy [1-3]. Therapeutic interventions targeting MIF directly are hampered due to MIF's ubiquitous expression and non-pathological roles.

In contrast, the disease-related structural isoform of MIF [4-6], termed oxMIF, is exclusively present in solid tumor tissue [7] and can be specifically targeted by the novel anti-oxMIF antibody ON203. Neutralizing oxMIF with ON203 blocks the pro-tumorigenic activities historically attributed to MIF [8-9].

oxMIF is the disease-related and druggable isoform of MIF

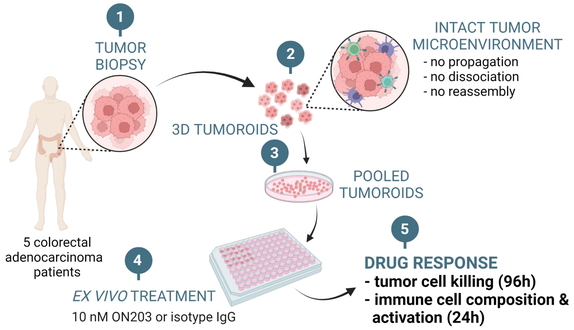


Adapted from Thiele, Donnelly & Mitchell, JITC. 2022 [8]

2 Methods

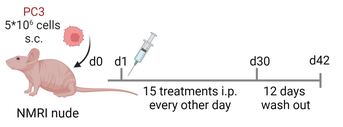
We determined the antitumorigenic and TME-modifying potential of the novel oxMIF-specific antibody ON203 in preclinical models including a xenograft mouse model and freshly isolated tumoroids from colorectal adenocarcinoma (CRC) patients retaining an intact TME. ON203-induced TME modulation and tumor cell killing were assessed by high-content 3D computational bioimaging, secretome analysis and flow cytometry.

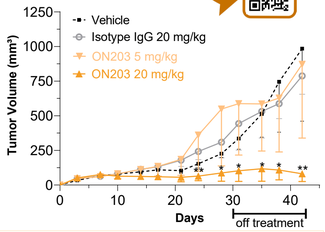
To further characterize the immune population differences between treatment conditions, we employed multi-parameter clustering (FlowSOM/XShift) and compared the abundance of each cluster across conditions. Finally, we used dimensionality reduction (UMAP) to comparatively visualize the clusters in responding vs. non-responding tumoroids.



3 *In vivo* efficacy – PC3 Xenograft Model [9]

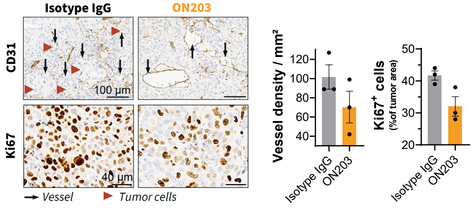
SCAN ME! to check out our MCT First Disclosure Article on ON203 efficacy published today





Median ± interquartile range, n=9 /group, ANOVA with Dunnett's multiple comparisons test on log transformed data
*p ≤0.05; **p ≤0.01, ON203 20mg/kg vs. vs. vehicle + Isotype IgG

ON203 exerts significant tumor suppression upon treatment and maintains a durable effect after treatment ends



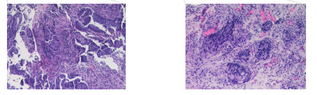
ON203 reduces proliferation and intravasation of tumor cells as well as vascularization, thereby reducing metastases

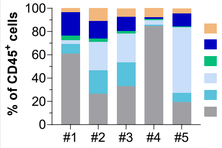
4 *Ex vivo* efficacy – Colorectal Cancer Tumoroids

Colorectal Adenocarcinoma Tumor Biopsies

	#1	#2	#3	#4	#5
Gender	m	m	f	n/a	m
Age	65	65	55	n/a	67
Grade	G3	G2	G2-G3	G2	G2
Stage	pT3pN1b	pT3pN1b	pT3pN2b	pT4apN0	pT3cpNX
IS (%)	40	40	20	20	30

IS…immune score



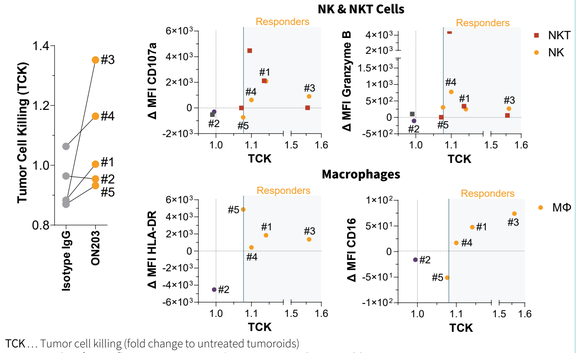


Immune Cell Composition

Determined by flow cytometry; gated on single live CD45⁺ cells (5 - 40 x 10³ cells; CD8⁺ T (CD3⁺CD8⁺), CD4⁺ T (CD3⁺CD8⁻CD56⁻), NK (CD3⁻CD56⁺), NKT (CD3⁺CD56⁺), macrophages MΦ (CD3⁻CD68⁺), other CD45⁺ cells containing e.g. myeloid cells and B cells.

3D primary human cancer models capture the full heterogeneity of individual tumors enabling a more accurate understanding of therapeutic response

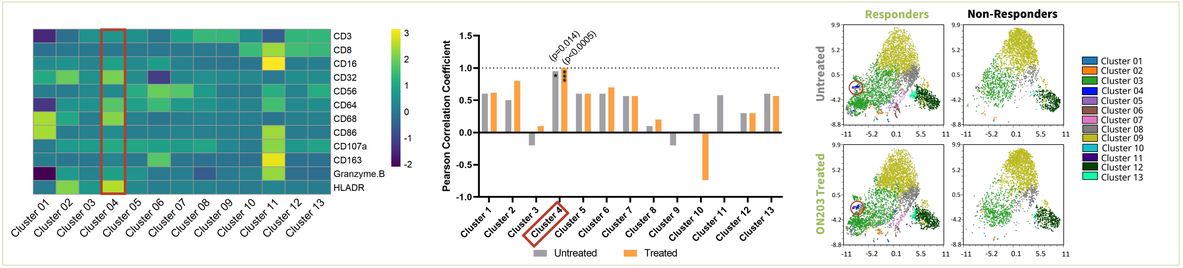
Tumor Cell Killing & Effector Cell Activation



TCK … Tumor cell killing (fold change to untreated tumoroids)
Δ MFI… Delta of mean fluorescence intensity (ON203-untreated tumoroids)

4 out of 5 CRC tumoroids responded with tumor cell killing (TCK) and TCK correlates with effector cell activation

Multi-parameter Clustering & Correlation with Tumor Cell Killing



5 Conclusions

- ✓ **ON203 significantly inhibited tumor growth and reduced proliferation and intravasation of tumor cells in this mouse xenograft model**
- ✓ **Four out of five CRC tumoroids responded to single agent ON203 with enhanced tumor cell killing (TCK) and immune cell activation**
  - **ON203 activated NK and NKT cells through the upregulation of Granzyme B and CD107a)**
  - **ON203 supported macrophage activation and repolarization to the anti-tumor M1-like phenotype via the upregulation of CD16 and HLA-DR, which was confirmed by unbiased multi-parameter cluster analysis**
- ✓ **These findings point to ON203 having a high potential for enhancing efficacy in combination with angiogenesis and checkpoint-inhibitors**

SCAN ME! to download poster

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] Noe JT and Mitchell RA, Front Immunol 2020;11:609948.
[4] Thiele et al., J Immunol. 2015 Sep 1;195(5):2343-52.
[5] Schinagl et al., Biochemistry. 2018 Mar 6;57(9):1523-1532.
[6] Skeens *et al*., Structure. 2022 Mar 22;S0969-2126(22)00088-0
[7] Schinagl et al., Oncotarget. 2016 Nov 8;7(45):73486-73496.
[8] Thiele, Donnelly & Mitchell, JITC. 2022; 10:e005475
[9] Rossmueller G and Mirkina I et al. Mol Cancer Ther. 2023,
Some images were created with biorender.com