

Novel bioengineered monoclonal antibodies targeting oxidized macrophage migration inhibitory factor (oxMIF) as anti-cancer therapeutics and diagnostics

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 distinguishes from other cytokines and chemokines due to its constitutive expression and high presence in circulation of healthy subjects at levels of ~6 ng/ml¹⁻¹⁰.

➡ 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 MIF¹¹⁻¹³. In contrast, oxMIF is the disease-related isoform that was specifically detected in solid tumors^{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 prematurely¹⁴.

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

2 Methods

OncoOne designed two bioengineered second generation anti-oxMIF mAbs (ON203 & ON102Ab) with highly improved biochemical and biological properties (Fig. 2). The novel anti-oxMIF mAbs share the same point mutations in the variable domains to improve physicochemical properties. The therapeutic mAb ON203 comprises an IgG1/2 hybrid constant heavy chain region including mutations to enhance effector functions. The diagnostic mAb ON102Ab comprises an IgG1 constant heavy chain region with mutations to abolish the binding to FcγRs. ON102Ab was labelled with ⁸⁹Zr resulting in the radiodiagnostic mAb ⁸⁹Zr-ON102. The novel anti-oxMIF mAbs were compared to the first generation anti-oxMIF mAb imalumab (C0008 internal designation) *in vitro* and *in vivo*. Hydrophobicity and aggregation 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* efficacy (Fig. 4) and *in-vitro* safety (Fig. 4) were 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 ⁸⁹Zr-ON102 in CT26 or HCT116 tumor-bearing Balb/c or Balb/c nude mice, respectively (Fig. 5). *In-vivo* efficacy was determined in two PC3 xenograft models in NMRI nude mice (Fig. 6-7).

3 Results *in vitro*

ON203 and ON102Ab demonstrated highly improved biochemical properties compared to C0008, while retaining the low nM affinity to oxMIF

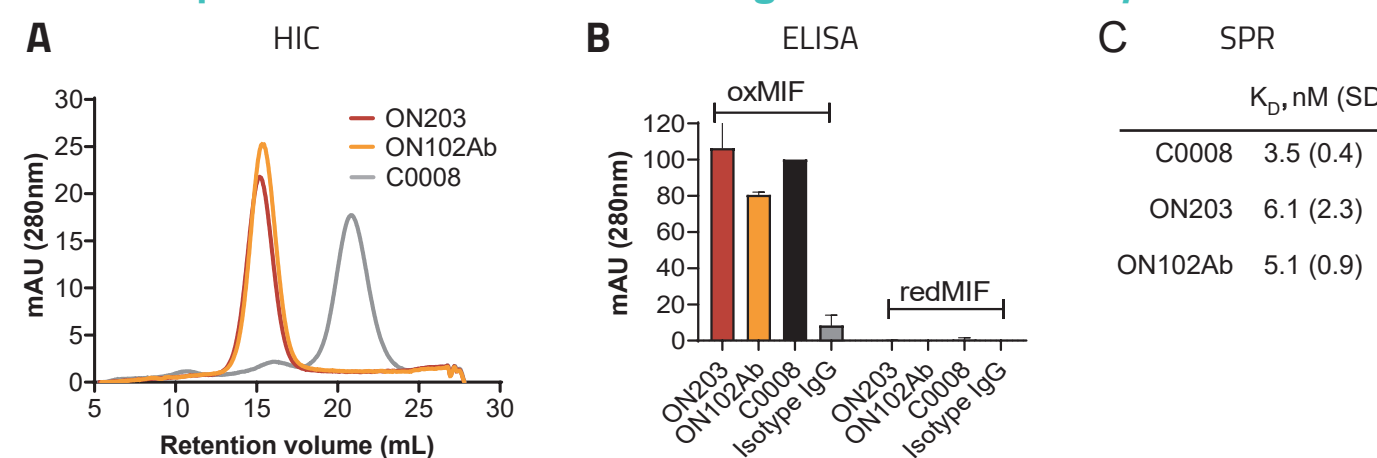


Fig. 3 (A) Hydrophobicity and aggregate content were analyzed by HIC. (B) The specific binding of ON203 and ON102Ab towards soluble human oxMIF and redMIF was analyzed by ELISA using TNB-MIF as an oxMIF surrogate¹¹. (C) SPR analysis revealed affinities in the low nM range for human oxMIF.

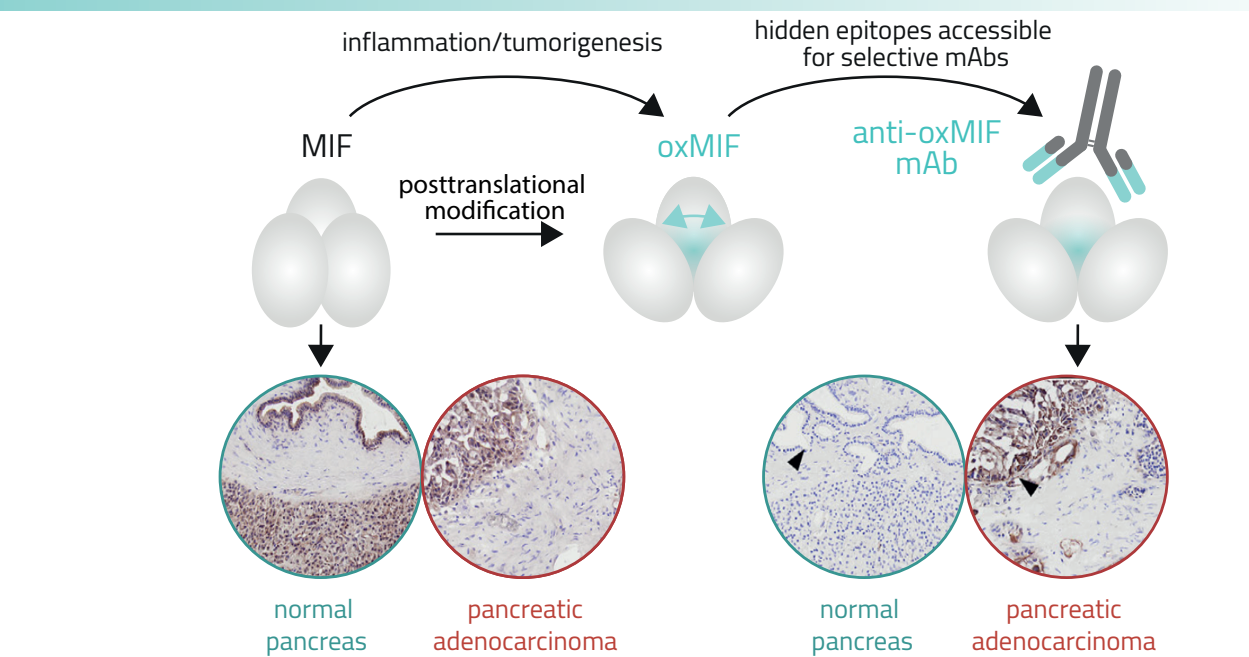


Fig. 1 OxMIF is generated by a posttranslational modification of MIF in inflammatory processes and tumorigenesis^{11,13}

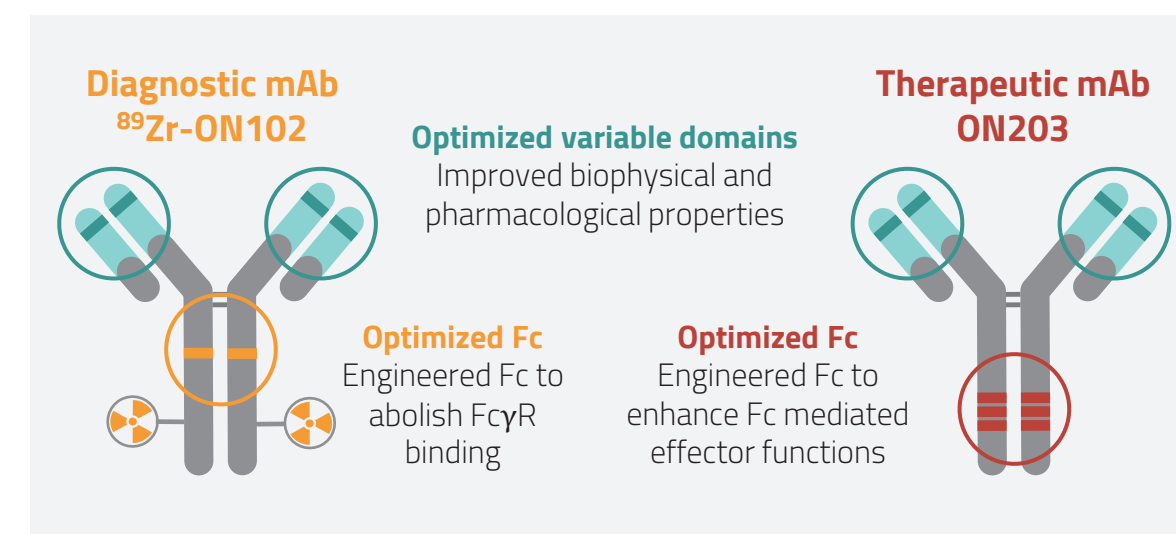


Fig. 2 Bioengineered second generation anti-oxMIF mAbs with optimized variable domains and optimized Fc portions for the intended purpose

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

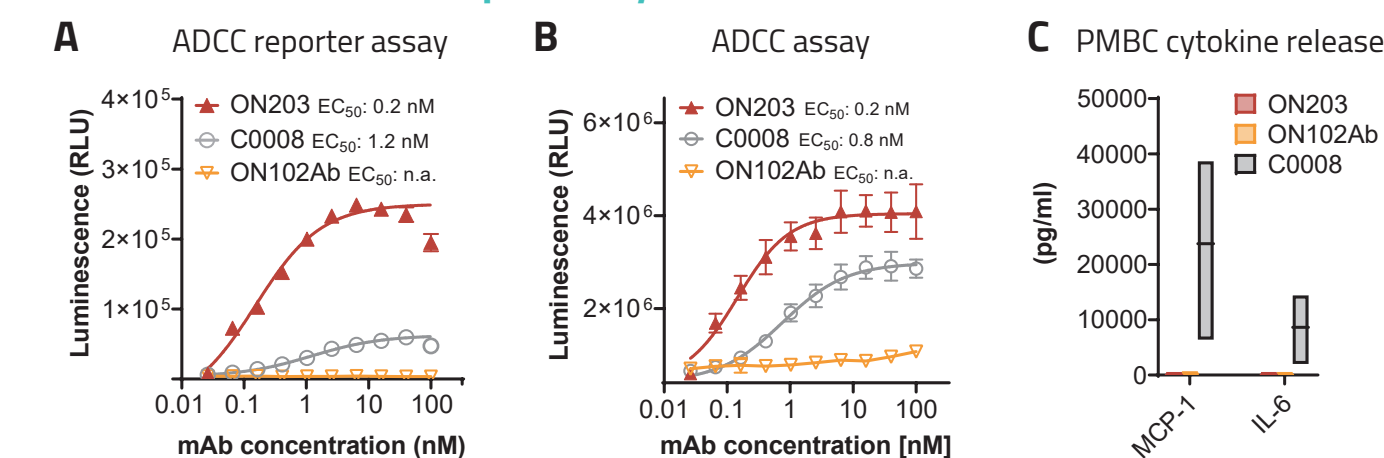


Fig. 4 ADCC was investigated by a reporter bioassay (A) utilizing engineered reporter cells expressing FcγRIIIa (Promega) or PBMC-mediated cell killing assays (B). Cytokine release (C) was studied upon incubation of human PBMCs with anti-oxMIF mAbs by a cytometric bead assays (BioLegend). Mean ± SEM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

4 Results *in vivo*

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

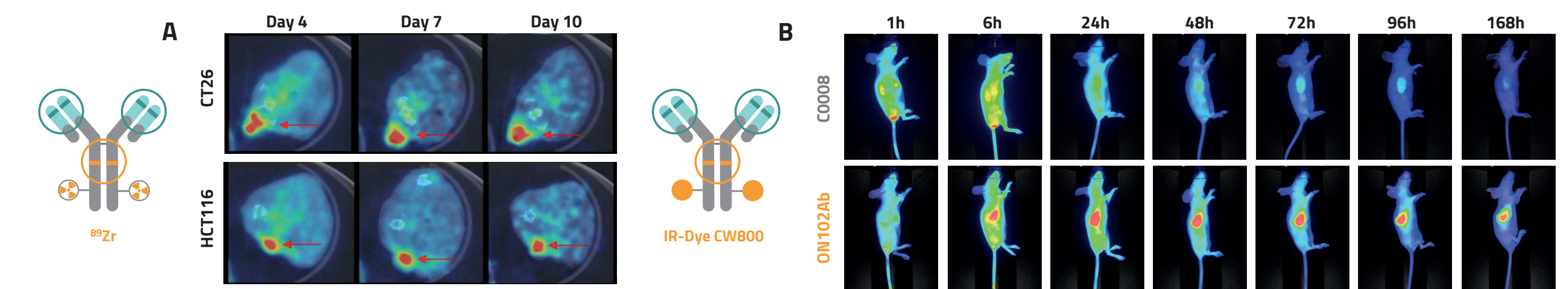


Fig.5 (A) HCT116 or CT26-bearing tumor-bearing Balb/c nude or Balb/c mice (tumor volume ~250 mm³), respectively, were injected i.v. with a single dose ~10 MBq of ⁸⁹Zr-ON102. Mice were imaged up to 10 days at the time points indicated using a BioPET small animal PET/CT (Sedecal) device. Images were reconstructed with 2DOSEM algorithm with CT-based attenuation correction. (B). HCT116 tumor-bearing Balb/c nude mice (tumor volume ~300 mm³) 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)

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

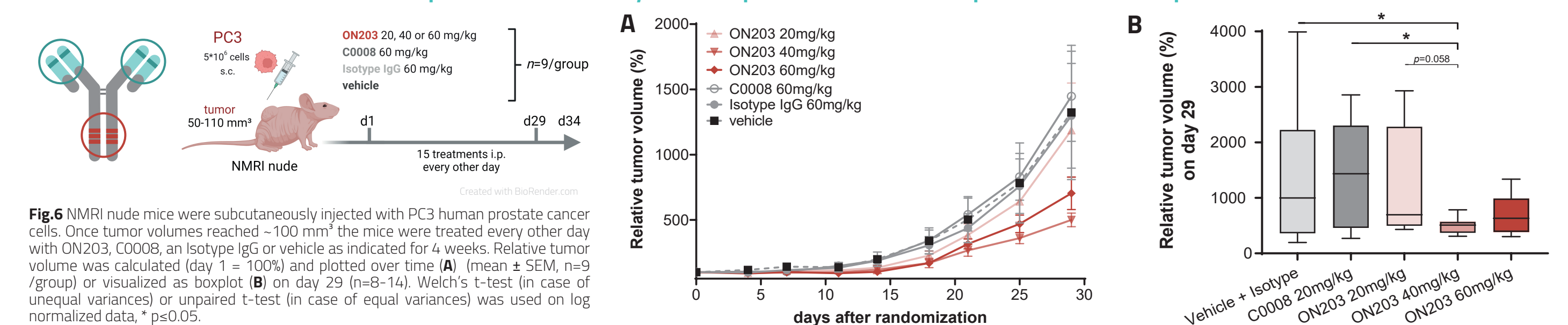


Fig.6 NMRI nude mice were subcutaneously injected with PC3 human prostate cancer cells. Once tumor volumes reached ~100 mm³ the mice were treated every other day with ON203, C0008, an Isotype IgG or vehicle as indicated for 4 weeks. Relative tumor volume was calculated (day 1 = 100%) and plotted over time (A) (mean ± SEM, n=9 /group) or visualized as boxplot (B) on day 29 (n=8-14). Welch's t-test (in case of unequal variances) or unpaired t-test (in case of equal variances) was used on log normalized data, * p<0.05.

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

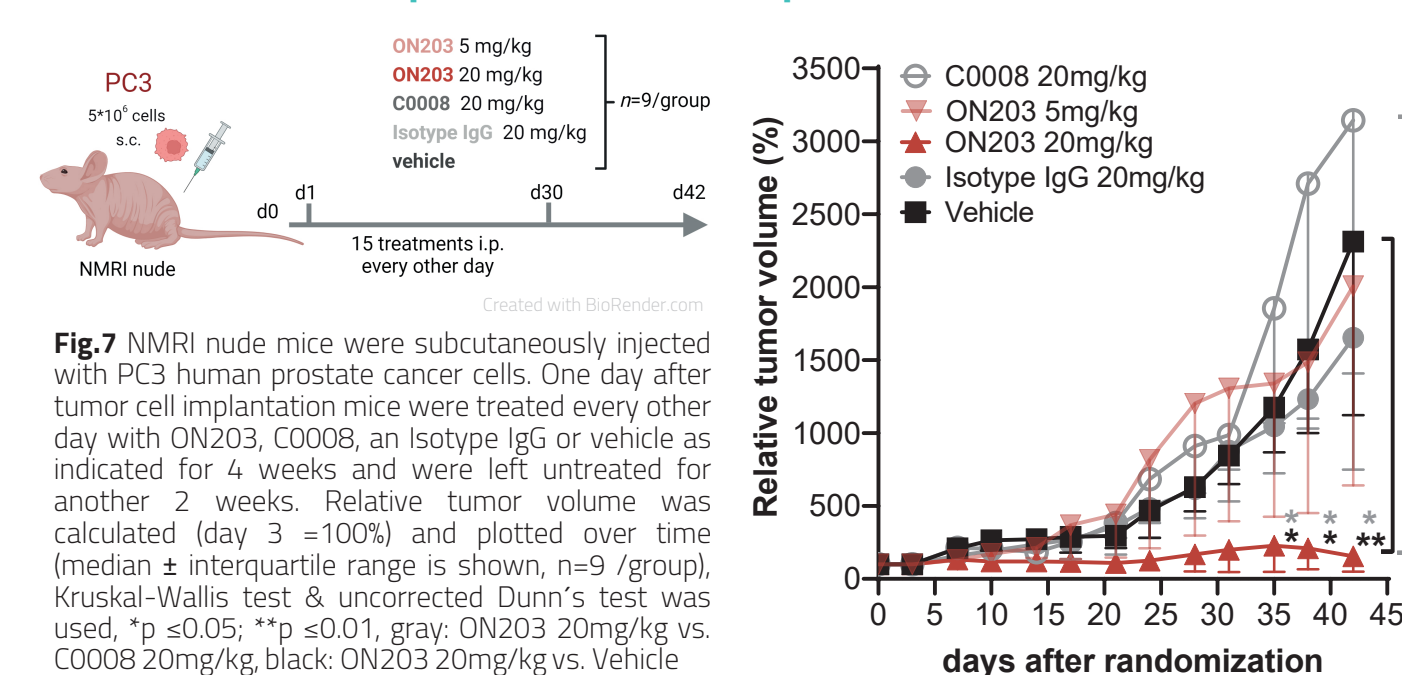


Fig.7 NMRI nude mice were subcutaneously injected with PC3 human prostate cancer cells. One day after tumor cell implantation mice were treated every other day with ON203, C0008, an Isotype IgG or vehicle as indicated for 4 weeks and were left untreated for another 2 weeks. Relative tumor volume was calculated (day 3 =100%) and plotted over time (median ± interquartile range is shown, n=9 /group), Kruskal-Wallis test & uncorrected Dunn's test was used, *p ≤0.05; **p ≤0.01, gray: ON203 20mg/kg vs. C0008 20mg/kg, black: ON203 20mg/kg vs. Vehicle

5 Conclusions

oxMIF, the disease-related and druggable isoform of MIF, is a novel target with broad applications in cancer therapy. OncoOne's bioengineering efforts significantly improved the physicochemical and biological properties of both ON203 and ON102Ab compared to first generation anti-oxMIF mAb imalumab, while the low nM affinity for oxMIF was retained. Improvements include a 10-fold enhanced Fc-mediated effector functions (ON203), as well as improved biodistribution and >3-fold enhanced tumor retention (ON102Ab) when compared to imalumab. OncoOne has started IND-enabling preclinical evaluation for our lead candidate ON203, with an anticipated IND end of 2022. ON203 has therapeutic potential as monotherapy or in combination with standard of care chemotherapy regimens¹³ or checkpoint inhibitors¹⁵. A first-in-human phase I study in patients with solid tumors is being planned with an anticipated start in the first quarter of 2023.

