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IOMX-0675, a LILRB1 and LILRB2 cross-specific antibody, effectively repolarizes immunosuppressive myeloid cells and activates T cells leading to potent tumor cell killing

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Introduction

Myeloid checkpoints have recently gained increasing attention in the context of tumor immune evasion. LILRB1 (ILT2) and LILRB2 (ILT4) are immunosuppressive receptors of the leukocyte immunoglobulin-like receptor (LILR) family that recognize both classical and non-classical MHC-I molecules (e.g., HLA-G). LILRB1 and LILRB2 are expressed across tumor-infiltrating myeloid and lymphoid cells, are frequently co-expressed with immune-activating LILR family members LILRA1 and LILRA3 in various tumor indications and are upregulated in patients non-responsive to T cell checkpoint blockade. In addition, the non-classical MHC-I molecule HLA-G, a major ligand of LILRB1 and LILRB2, is overexpressed and associated with poor prognosis in several solid tumor types.

IOMX-0675, a fully human, Fc-silenced, immunoglobulin G1 (IgG1) monoclonal antibody, that selectively binds with high affinity to the inhibitory receptors LILRB1 and LILRB2, while only weakly binding to the closely related immunoactivating LILR family members LILRA1 and LILRA3, was identified from iOmx's proprietary phage display library. The antibody was evaluated in various biochemical assays to demonstrate its potency to bind LILRB1 and LILRB2 even in a LILRA1/LILRA3 enriched environment and thereby blocking the interaction with their ligands, such as HLA-G. IOMX-0675 promotes the phagocytic and pro-inflammatory activity of various macrophage subtypes and rescues the activity of the lymphoid immune system in co-cultures of M2-like macrophages with autologous T cells, superior to other clinical competitors targeting this pathway. In addition, dual-targeting LILRB1/2 by IOMX-0675 harnesses both innate as well as adaptive immunity and thereby significantly inhibits tumor growth in a melanoma tumor xenograft model within fully humanized NOG-EXL mice.

In summary, IOMX-0675, a cross-specific antibody that antagonizes both LILRB1 and LILRB2 with high selectivity, while sparing the closely related immuno-activating LILR family members LILRA1/3, effectively reprograms the immunosuppressive myeloid compartment and restores the cytotoxic T cell activity in the tumor microenvironment. The differential binding profile of IOMX-0675 offers best-in-class potential and may maximize anti-tumor efficacy for the benefit of patients with high unmet medical need, who are resistant to T cell checkpoint blockade.

Immunosuppressive TME — Inflammatory TME

Conclusion

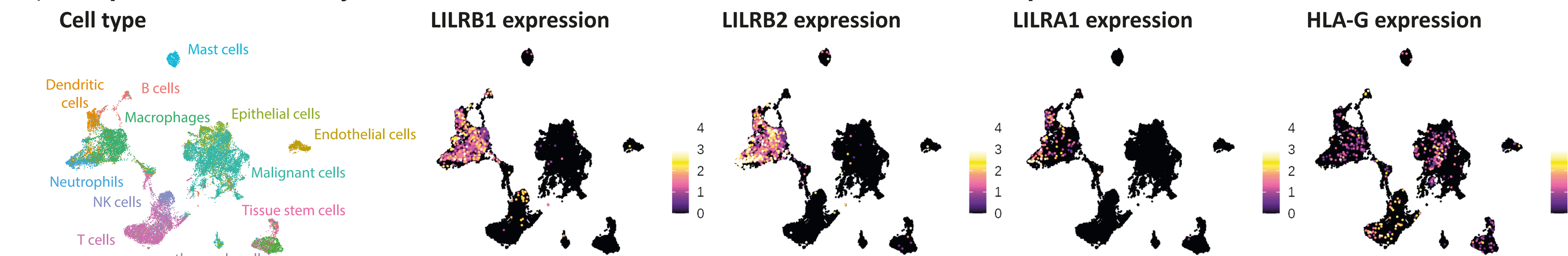
- IOMX-0675 is a fully human, Fc-silenced cross-specific antibody binding with high affinity to the immunosuppressive receptors LILRB1 and LILRB2 while sparing their closely related immunoactivating LILR family members LILRA1 and LILRA3.
- The differential binding profile of IOMX-0675 to LILRB1 and LILRB2 leverages remarkably superior potency in various binding and *in vitro* functional assays of macrophage repolarization, T cell suppression and tumor cell phagocytosis compared to clinical competitors on the pathway.
- IOMX-0675 demonstrates its best-in-class potential by repolarizing the immunosuppressive tumor microenvironment *in vivo* and thereby inhibiting tumor growth in a fully humanized, myeloid engrafted melanoma tumor model.
- Fast-track CTA/IND-enabling studies for IOMX-0675 ongoing.

Results

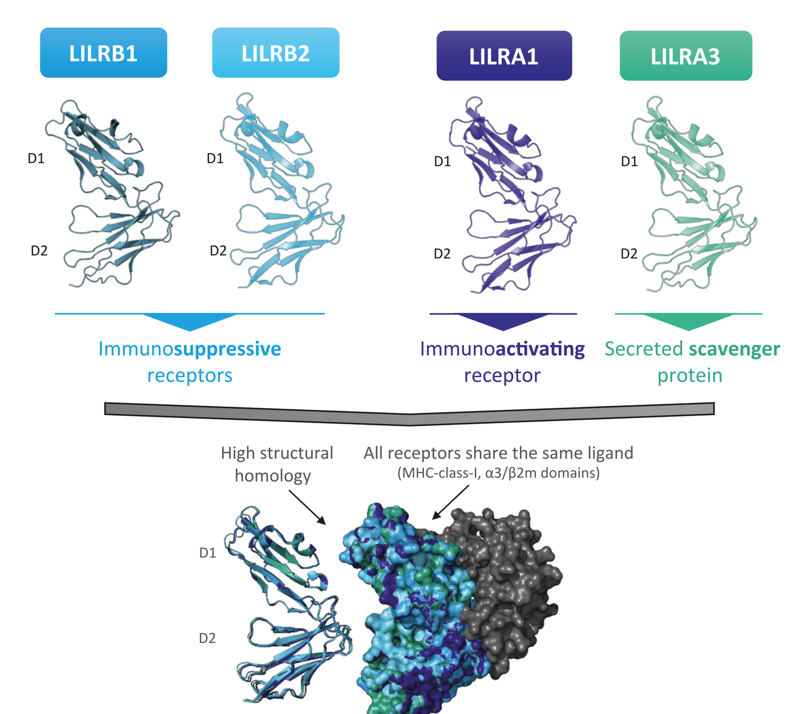
IOMX-0675 selectively modulates the immunomodulatory LILRB1/2 receptors

- Single-cell RNAseq analysis reveals co-expression of LILRB1, LILRB2 and LILRA1 on individual immune cells in tumor biopsy samples
- HLA-G expression demonstrated on myeloid, lymphoid as well as cancer cells
- Close structural and sequence homology of the immunosuppressive LILRB1/2 receptors with the immunoactivating LILRA1/3 receptors
- IOMX-0675 demonstrates high affinity to the immunosuppressive LILRB1 & LILRB2 receptors
- IOMX-0675 shows a superior binding profile over a clinical competitor targeting LILRB1/2 by avoiding binding to immunoactivating LILRA1/3
- IOMX-0675 provides superior blockade of the HLA-G - LILRB1/2 ligand-receptor interaction

A) Co-expression of LILR family members and HLA-G in the tumor microenvironment of cancer patients



C) High structural homology between LILRA- & LILRB-family members



C) Differential binding profile of IOMX-0675

D) Disruption of the HLA-G - LILRB1/2 ligand-receptor interaction

FIGURE I

A) Single-cell RNA sequencing (scRNAseq) datasets from Barkley et. al. 2022 were analyzed using the Seurat R package. The extracted and quality-filtered RNAseq data from breast cancer (n=4), colorectal cancer (1), lung adenocarcinoma (4), ovarian cancer (3) and pancreatic cancer (9) were used to analyze LILRB1, LILRB2, LILRA1 and HLA-G expression in the annotated cell types. LILRA3 was not annotated in the analyzed data set. Cell annotations shown in the UMAP plot are taken from the original publication. **B)** Crystal structure of the two N-terminal Ig-like domains of LILRB1 and AlphaFold predicted structures of the corresponding domains of LILRB2, LILRA1 and LILRA3 demonstrate a high structural homology and matching interaction site between the LILR receptor family and the α3/β2m domains of HLA-G (dark gray). **C)** Differential binding profile of IOMX-0675 against LILRB1/2 and LILRA1/3 compared to clinical competitor antibody (Competitor 1). Binding kinetics of both antibodies to LILRB1/2 and LILRA1/3 were measured by biolayer interferometry (BLI) on an Octet Red96e and fitted using a 1:1 binding model. **D)** Fab fragments from IOMX-0675 or Competitor 1 and assay buffer control were used to examine the displacement of precoated LILRB1/2 from the HLA-G interaction site by BLI as shown in the graphical assay illustration.

IOMX-0675 shows high receptor binding even in a LILRA1/3 dominated environment

- Despite the presence of LILRA1 & LILRA3, IOMX-0675 shows high affinity to LILRB1 & LILRB2

Binding of IOMX-0675 to LILRB1 or LILRB2 in the presence of LILRA1 or LILRA3

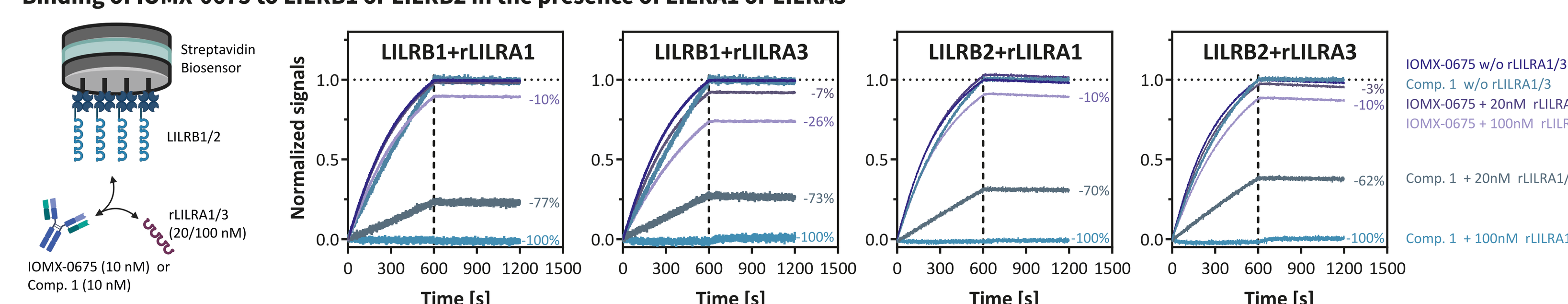


FIGURE II

Binding kinetics of IOMX-0675 or competitor 1 (Comp. 1) antibody at 10 nM to LILRB1 and LILRB2 in the presence of 20 nM or 100 nM recombinant LILRA1 (rLILRA1) or rLILRA3. Interactions were measured by BLI on an Octet Red96e.

IOMX-0675 demonstrates strong binding to primary immune cells

- IOMX-0675 binds to primary cells of the myeloid and lymphoid immune lineage with single digit nM affinity

Binding of IOMX-0675 to primary immune cells

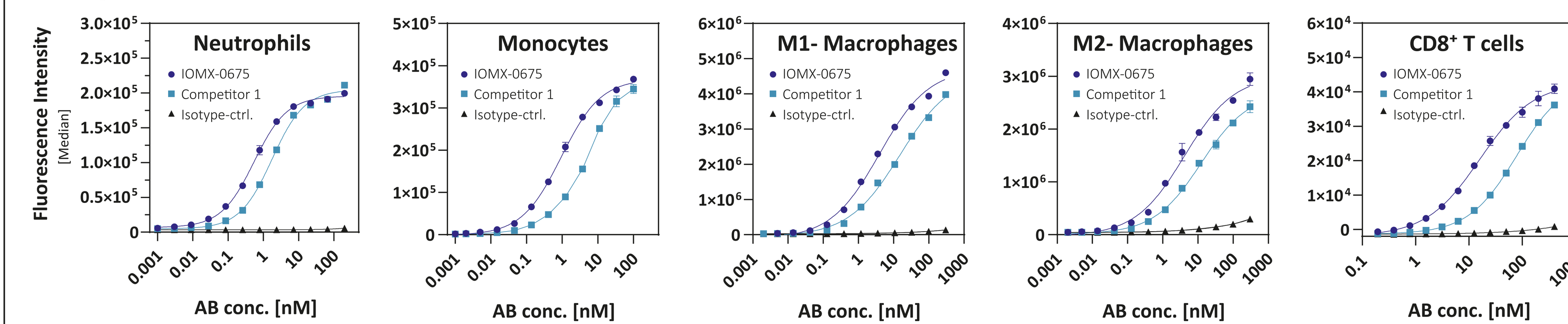


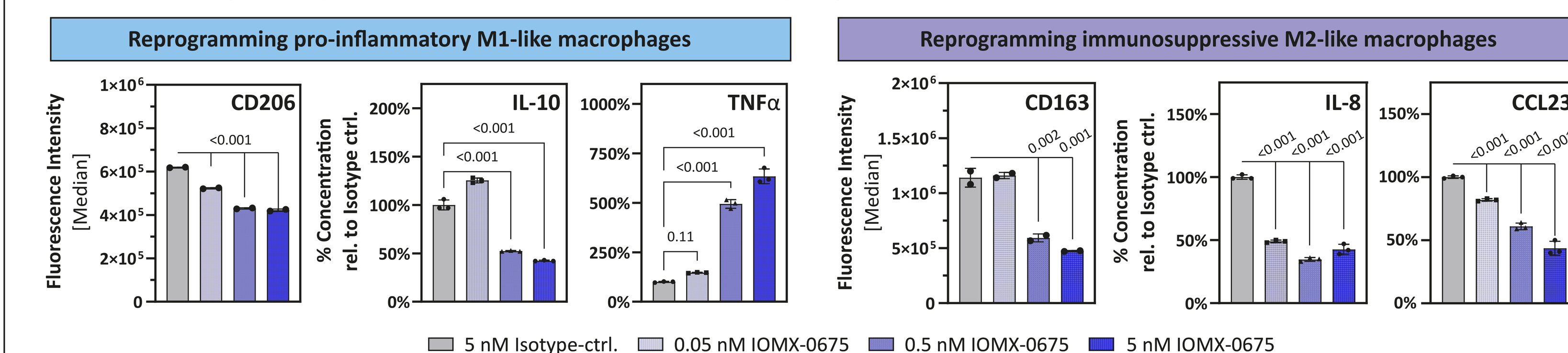
FIGURE III

IOMX-0675, competitor 1 and isotype-ctrl. antibodies were labeled with Alexa Fluor 647 and titrated on primary immune cells from healthy PBMC donors or *in vitro* differentiated M1- and M2-like macrophages according to the protocol depicted in Figure IV B. Median fluorescence intensity was analyzed by flow cytometry and is depicted as mean ± standard deviation (SD). Data are representative of 3 donors.

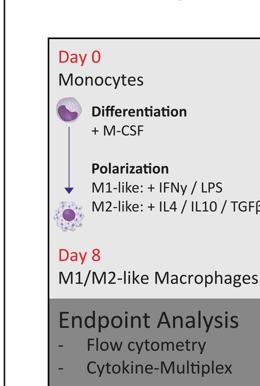
IOMX-0675 reprograms macrophages to a pro-inflammatory M1-like phenotype

- IOMX-0675 promotes a pro-inflammatory M1-like macrophage phenotype in both immunosuppressive and inflammatory conditions
- Compared to clinical competitors, IOMX-0675 demonstrates far superior pharmacodynamic activity in reprogramming of macrophages
- Even in LILRA3-dominated environments, IOMX-0675 enhances the pro-inflammatory phenotype of M1-like macrophages

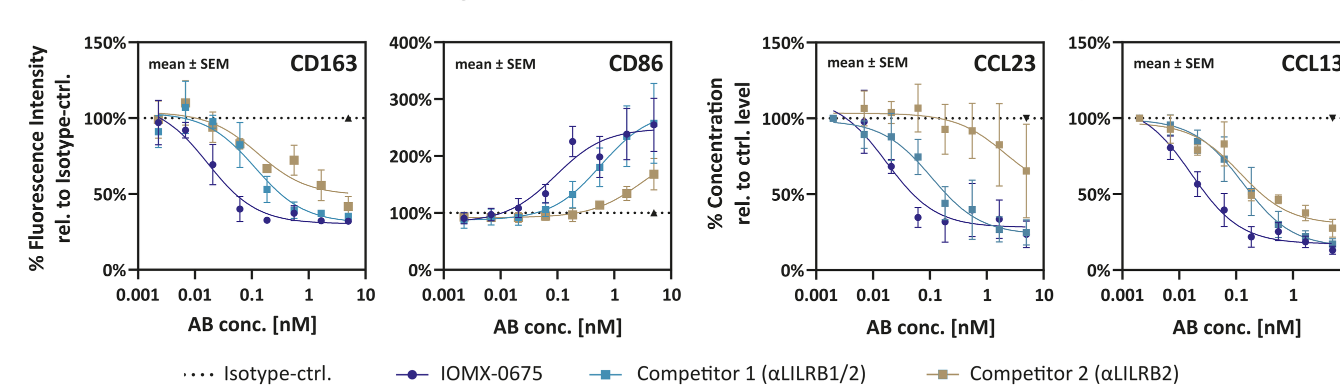
A) Proinflammatory phenotypic switch of M1- and M2-like of macrophages by IOMX-0675-induced LILRB1/2 blockade



B) Study design



C) Repolarization of M2-like macrophages in comparison to clinical competitors on the pathway



D) Enhancement of the M1-like macrophage phenotype in a LILRA3-enriched environment

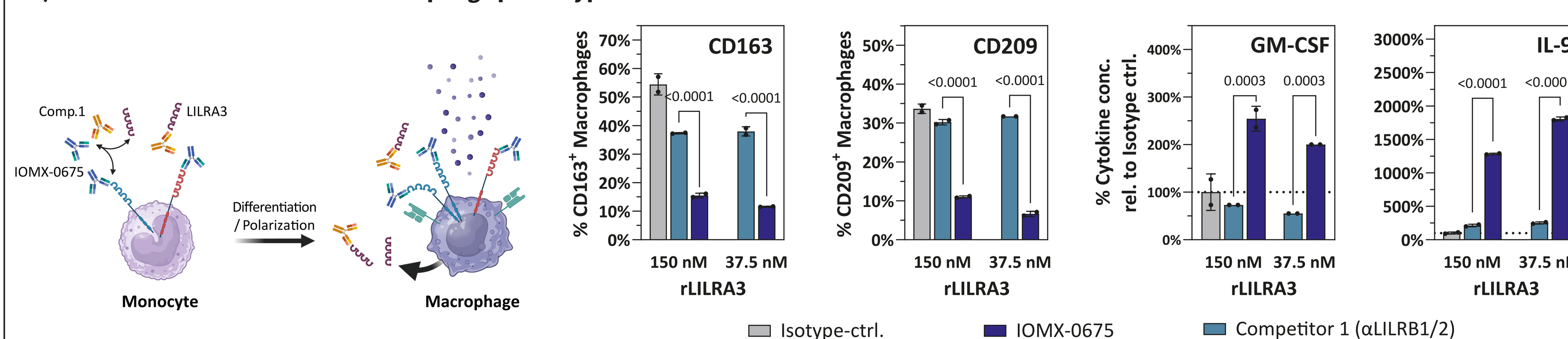


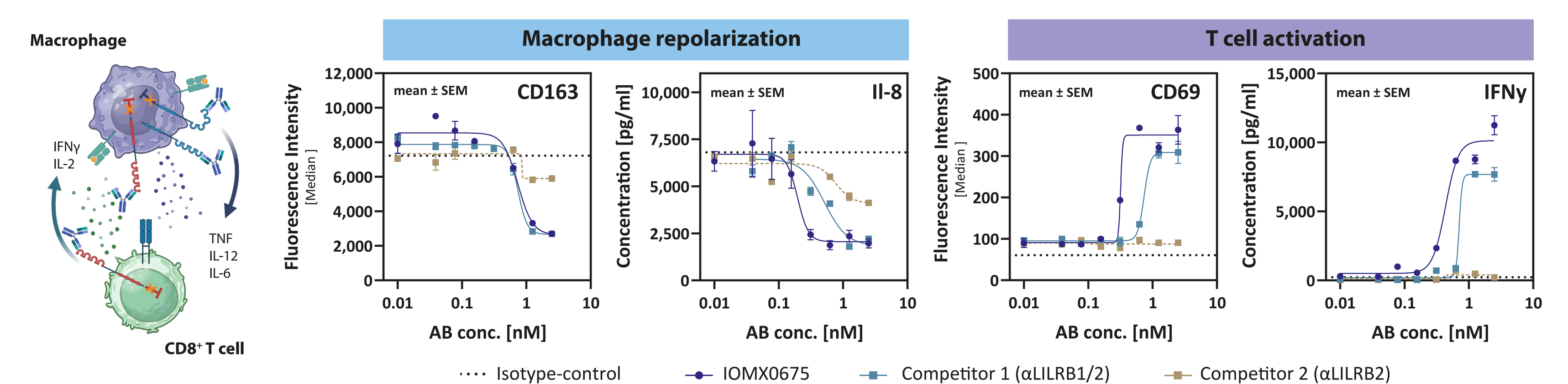
FIGURE IV

Differentiation of monocytes from healthy PBMC donors and polarization towards M1- **(A/D)** and M2-like macrophages **(A/C)** according to the protocol depicted in **B**. Treatment with IOMX-0675, competitor 1, competitor 2 or corresponding isotype control antibody throughout the differentiation/polarization period. Surface receptors CD163, CD209 and CD86 were analyzed by flow cytometry on day 8. The depicted cytokines and chemokines were analyzed by bead-based multiplex immunoassays from the supernatants on day 8. Data are presented as mean ± SD unless otherwise stated.

Macrophage repolarization by IOMX-0675 reactivates immunosuppressed cytotoxic T cells and enhances tumor cell phagocytosis

- Superior repolarization of M2-like macrophages by IOMX-0675 translates into stronger activation of immunosuppressed cytotoxic T cells compared to clinical competitors on the LILRB1/LILRB2 pathways
- Dual targeting of LILRB1 and LILRB2 by IOMX-0675 outperforms monospecific targeting of the receptors with respect to tumor cell phagocytosis

A) Repolarization of M2-like macrophages and activation of effector T cells in an *in vitro* macrophage - T cell co-culture system



B) Enhancement of tumor cell phagocytosis through inhibition of the LILRB1/2 pathway

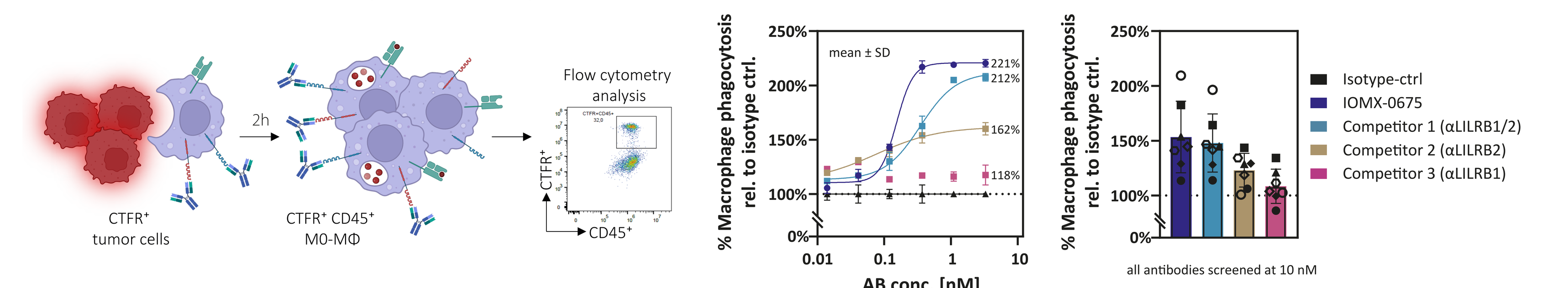


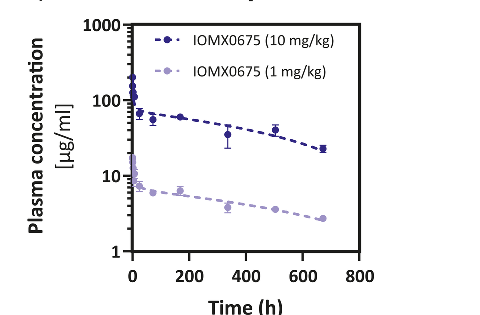
FIGURE V

A) M2-like macrophages were co-cultured with autologous T cells for 3 days. Treatment with IOMX-0675, competitor 1, competitor 2 or appropriate isotype control antibody was performed throughout the assay. Flow cytometry (CD163, CD69) and supernatant analysis by bead-based multiplex immunoassays were done on day 8 (Macrophages) and day 11 (T cells). **B)** M0-like macrophages were generated by differentiation of monocytes for 6 days with M-CSF. Cell Trace Far Red (CTFR) labeled A375 tumor cells were incubated with M0-like macrophages for 2h and treated with IOMX-0675, competitor 1/2/3 or corresponding isotype control. Dose-dependent phagocytic activity was analyzed by flow cytometry and is shown for one representative donor (left graph). Average tumor cell phagocytosis is shown for seven donors (right graph) for IOMX-0675, competitor 1/2/3 and corresponding isotype control antibody.

IOMX-0675 exhibits *in vivo* anti-tumor efficacy in a highly aggressive melanoma model

- IOMX-0675 demonstrates a linear, dose-dependent pharmacokinetic profile
- In the highly aggressive A375 melanoma xenograft model, IOMX-0675 shows significant single-agent activity
- Inhibition of the LILRB1/2 pathway by IOMX-0675 or competitor 1 enhances T cell activation *in vivo*
- IOMX-0675 demonstrates its best-in-class potential by repolarizing the tumor microenvironment in contrast to a clinical comparator

A) Pharmacokinetic profile of IOMX-0675



B) *In vivo* anti-tumor study in a CD34⁺ fully humanized, myeloid engrafted A375 melanoma model

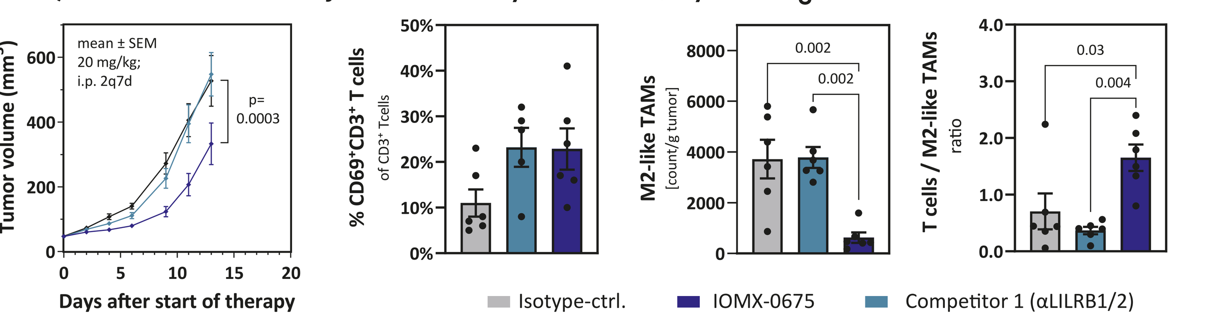


FIGURE VI

A) Pharmacokinetic analysis for single intravenous administration of 1 or 10 mg/kg of IOMX-0675 in C57Bl6 animals. EDTA plasma samples from three animals per time point were analyzed by ELISA. **B)** A375 melanoma tumor cells were implanted subcutaneously into NOG-EXL mice, fully humanized with $CD34^+$ stem cells from two donors. Randomized animals were treated twice weekly with 20 mg/kg IOMX-0675, competitor 1, or corresponding isotype control antibody. Tumor infiltrating immune cells were analyzed by flow cytometry. Activated $CD69^+$ $CD3^+$ T cells, $CD68^+CD11c^-HLA\text{-}DR^-$ M2-like macrophages and the ratio of $CD3^+$ T cells versus M2-like macrophages are shown as mean ± SEM.

Statistical information

Unless otherwise indicated, graphs are representative of data from at least two independent donors. Sigmoidal dose–response curves were fitted to the data using 4-Parameter Logistic (4PL) nonlinear curve models for *in vitro* dose responses. Data points show mean ±SEM, unless otherwise noted. Significance was calculated using one-way ANOVA analysis including Tukey's multiple comparison analysis. Competitor antibodies were produced in-house based on patent derived sequences.

Contact