



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

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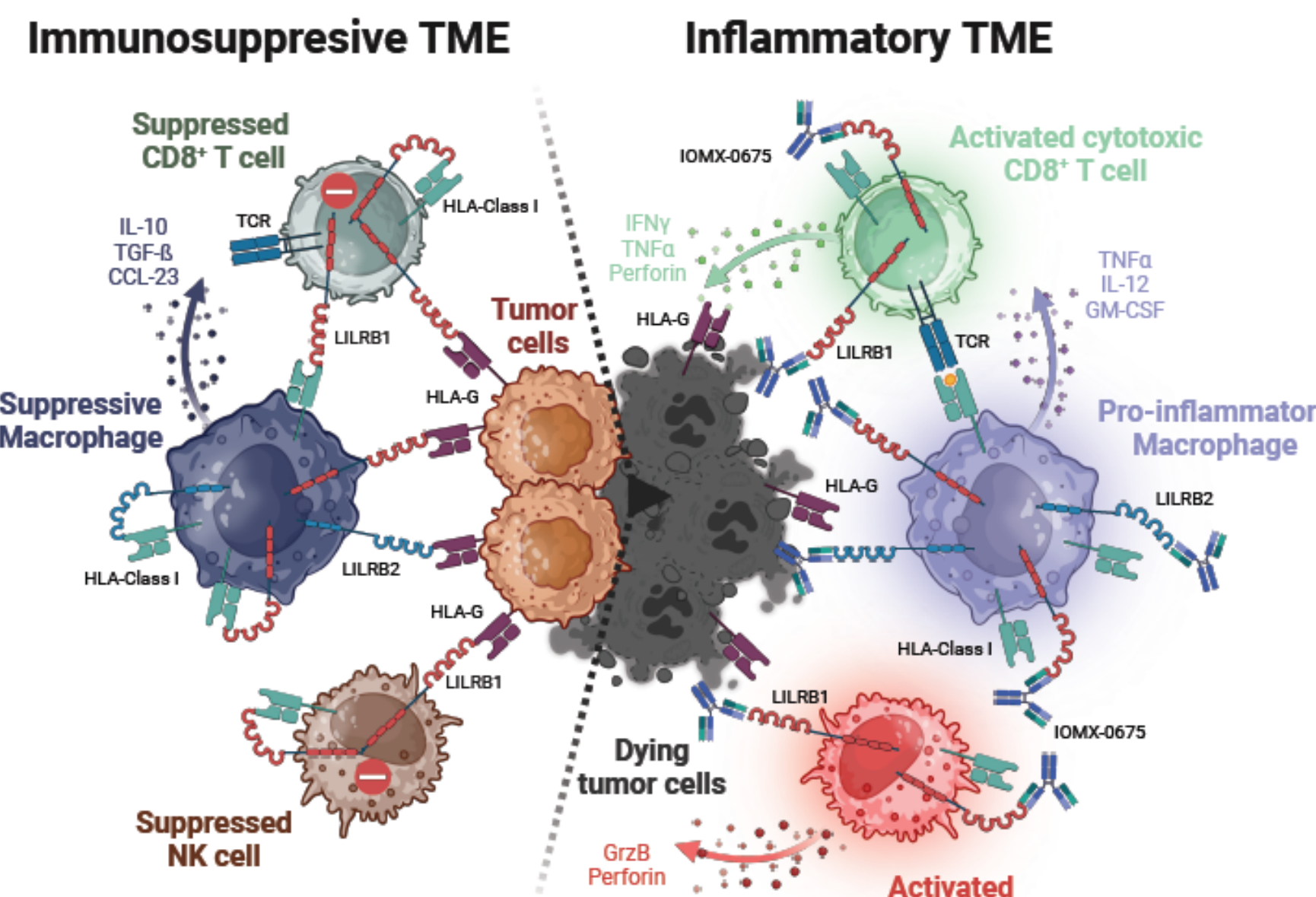
Introduction

Myeloid checkpoints in the tumor microenvironment have gained increased attention in the context of tumor immunity with a key role of these receptors in tumor resistance mechanisms. 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). While tumor-infiltrating myeloid cells express both LILRB1 and LILRB2, lymphoid cells are restricted to LILRB1 expression. LILRB1 and LILRB2 are frequently upregulated in patients non-responsive to T cell checkpoint blockade, implicating a key role of these receptors in immune evasion.

IOMX-0675 is a fully human, Fc-silenced monoclonal immunoglobulin G1 (IgG1) antibody, identified from iOmx' proprietary phage display library. It displays a highly differentiated binding profile, with selective, high-affinity binding to the inhibitory receptors LILRB1 and LILRB2, while binding with only negligible affinity to the closely related immune-activating LILR family members LILRA1 and LILRA3.

IOMX-0675 efficiently leads to macrophage repolarization and enhanced phagocytosis correlating with IOMX-0675 receptor occupancy in a flow-based assay. In autologous immune cell co-culture assays, IOMX-0675 demonstrates high potency in reprogramming immunosuppressive macrophages and restoring cytotoxic T cell activity. Additionally, in a CD34⁺ stem cell-engrafted humanized mouse xenograft melanoma model, IOMX-0675 demonstrates potent anti-tumor activity and pharmacodynamic modulation of tumor-associated macrophages, mirroring our *in vitro* findings.

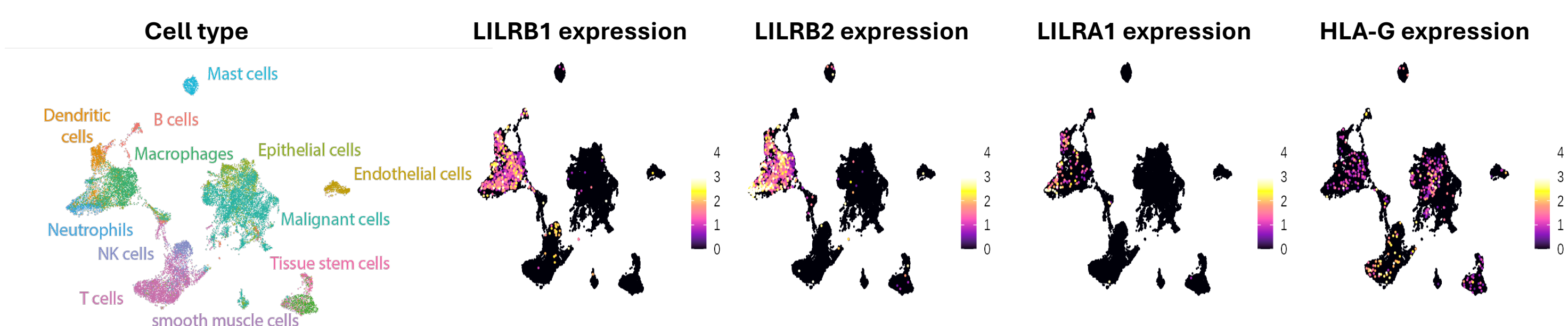
In summary, we discovered IOMX-0675, a cross-specific antibody antagonizing both LILRB1 and LILRB2 with high selectivity. Due to its highly differentiated binding profile which translates into superior potency, IOMX-0675 shows best-in-class potential for a dual-targeting myeloid checkpoint inhibitor that may maximize efficacy in patients across a variety of solid tumor indications.



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
- 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 immuno-activating LILRA1/3

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



B) Differential binding profile of IOMX-0675

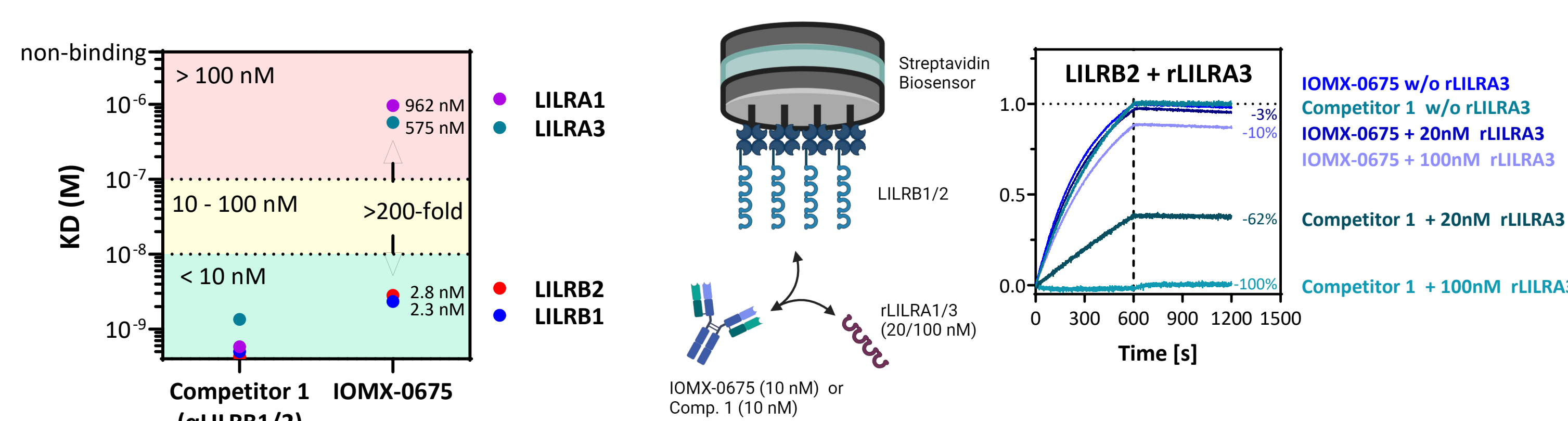


FIGURE I
A) Single-cell RNA sequencing (scRNAseq) datasets from Barkley et. al. 2022 Nat Genet, Lin et al 2020 Genome Med and Kim et al 2020 Nat Com. were analyzed using the Seurat R package. The extracted and quality-filtered RNAseq data from breast cancer (n=4), colorectal cancer (1), lung adenocarcinoma (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) Differential binding profile of IOMX-0675 against LILRB1/2 and LILRA1/3 compared to clinical competitor antibody (Competitor 1). C) Binding kinetics of IOMX-0675 or competitor 1 antibody at 10 nM to LILRB2 in the presence of 20 nM or 100 nM recombinant LILRA3 (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

A) LILRB1 / LILRB2 positive immune cell populations

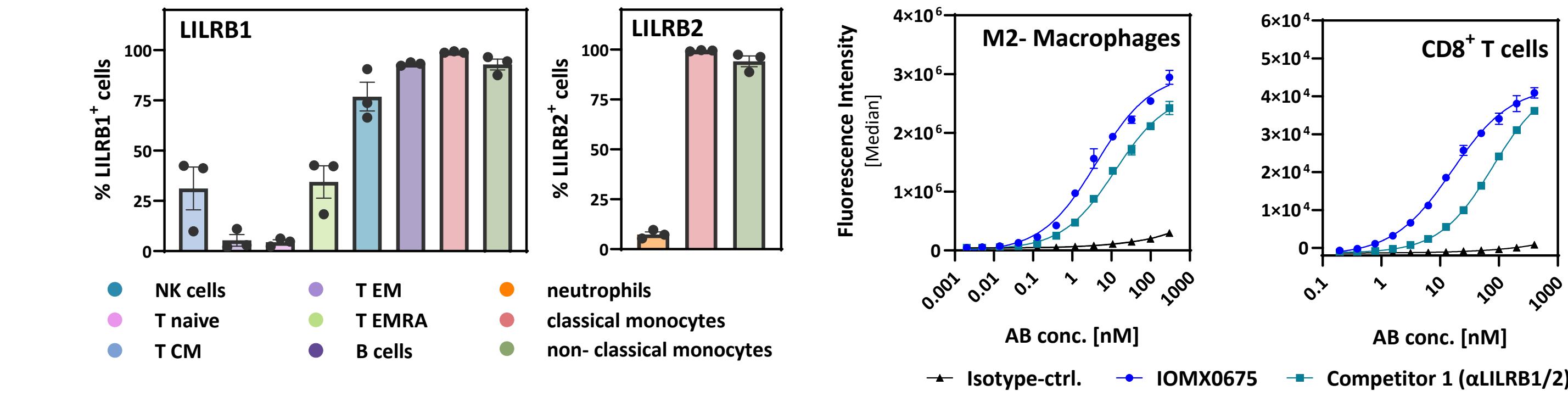
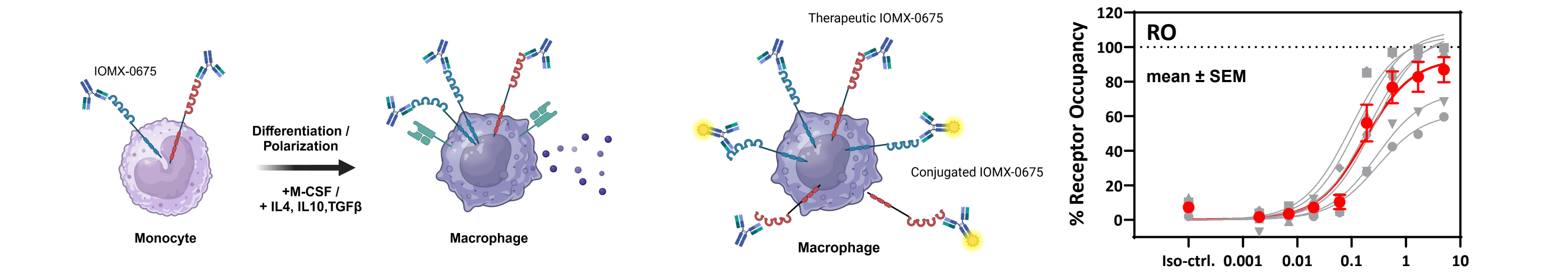


FIGURE II
A) Whole blood from healthy donors (n=3) was analyzed by flow cytometry regarding LILRB1 & LILRB2 target expression on relevant immune cell populations (NK = natural killer, CM = central memory; EM = effector memory, EMRA = effector memory cells re-expressing CD45RA). Data is depicted as mean $\pm$ SEM and is representative for 3 donors. B) IOMX-0675, competitor 1 and isotype-control antibodies were labelled with Alexa Fluor 647 and titrated on primary CD8⁺ T cells from healthy PBMC donors or *in vitro* differentiated M2-like macrophages according to the protocol depicted in Figure III A. Median fluorescence intensity was analyzed by flow cytometry and is depicted as mean $\pm$ SD. Data are representative of 3 donors.

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

- Across multiple donors, IOMX-0675 consistently demonstrates robust reprogramming of immunosuppressive macrophages
- Macrophage repolarization correlates with IOMX-0675 receptor occupancy

A) Macrophage generation



C) Consistent repolarization of M2-like macrophages across multiple donors

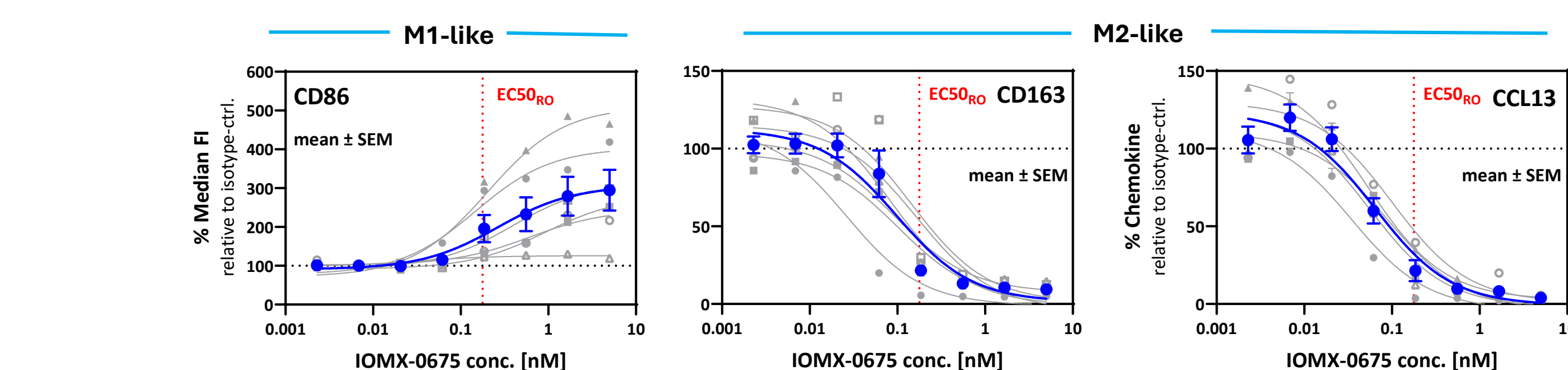


FIGURE III
A) Differentiation of monocytes from healthy PBMC donors and polarization towards M2-like macrophages according to the depicted protocol. B / C) Treatment with IOMX-0675 or corresponding isotype ctrl. antibody throughout the differentiation/polarization period. LILRB1/2 receptor occupancy as well as surface receptors CD86 and CD163 were analyzed by flow cytometry on day 8. CCL-13 was analyzed by a bead-based multiplex immunoassays from the supernatants on day 8. Results are shown as mean $\pm$ SEM of all donors (red / blue line) and each donor individually (grey lines) (B+C).

IOMX-0675 reprograms macrophages in a LILRA3 dominated environment

- Even in LILRA3-dominated environments, IOMX-0675 enhances the pro-inflammatory phenotype of M1-like macrophages

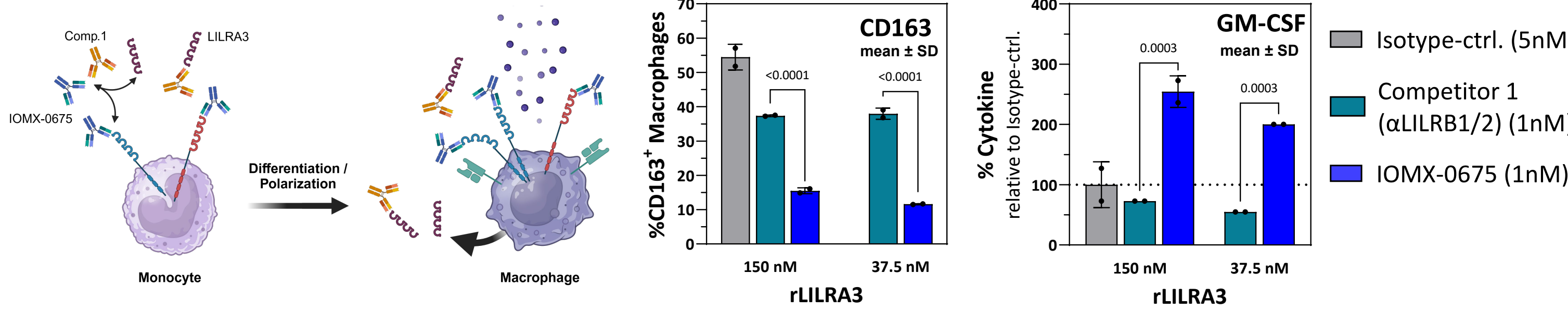
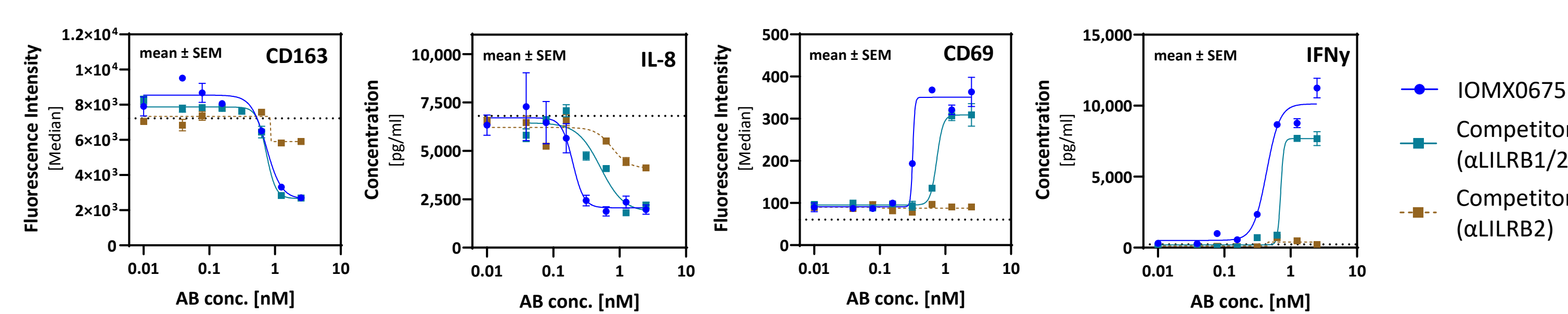


FIGURE IV
Differentiation of monocytes from healthy PBMC donors and polarization towards M1-like macrophages according to the depicted protocol. Treatment with IOMX-0675, competitor 1 or corresponding isotype control antibody in the presence of 37.5 nM or 150 nM recombinant LILRA3 (rLILRA3). Surface receptor CD163 were analyzed by flow cytometry on day 8 and GM-CSF was analyzed by bead-based multiplex immunoassays from the supernatants on day 8. Results are shown as the average for a concentration of 1 nM (IOMX-0675 and Competitor 1) and 5 nM (Isotype-ctrl.), respectively.

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 enhances tumor cell phagocytosis and correlates with receptor occupancy

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



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

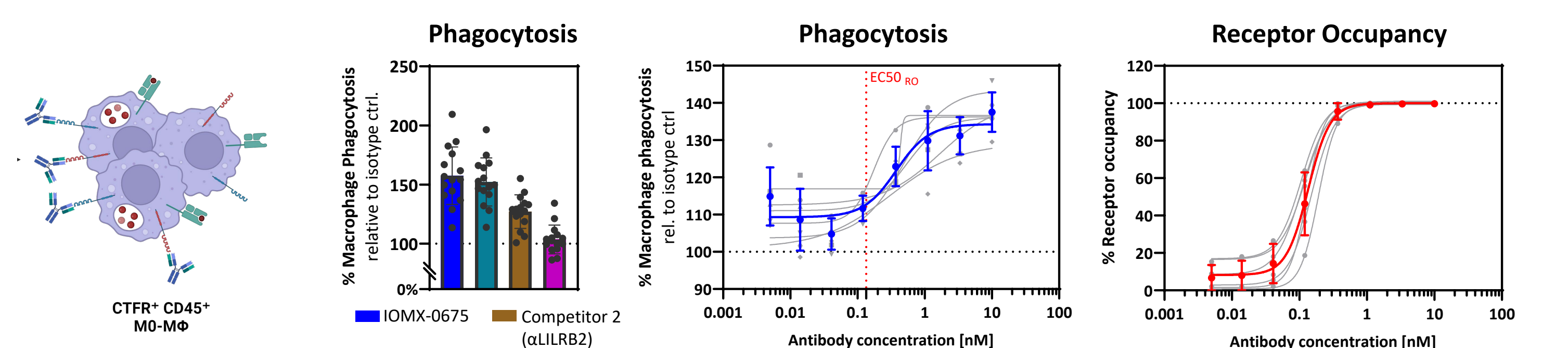


FIGURE V
A) M2-like macrophages were co-cultured with autologous T cells for 3 days. Treatment with IOMX-0675, competitor 1/2 or appropriate isotype control antibody was performed throughout the assay. Flow cytometry (CD163, CD69) and supernatant analysis by bead-based multiplex immunoassays were done 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) labelled A375 tumor cells were incubated with M0-like macrophages for 2h and treated with IOMX-0675, competitor molecules and corresponding isotype ctrl. Phagocytic activity was measured by flow cytometry. Phagocytosis induction upon treatment with IOMX-0675 and competitor molecules (10 nM) is shown across 13 donors as mean $\pm$ SD (left graph). Dose-dependent phagocytic activity (middle graph) as well as LILRB1/ LILRB2 receptor occupancy (right graph) was analyzed for n= 6 donors by flow cytometry. Results are shown as mean $\pm$ SD of n=6 donors (blue / red line) and each donor individually (grey lines).

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

- 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 competitor

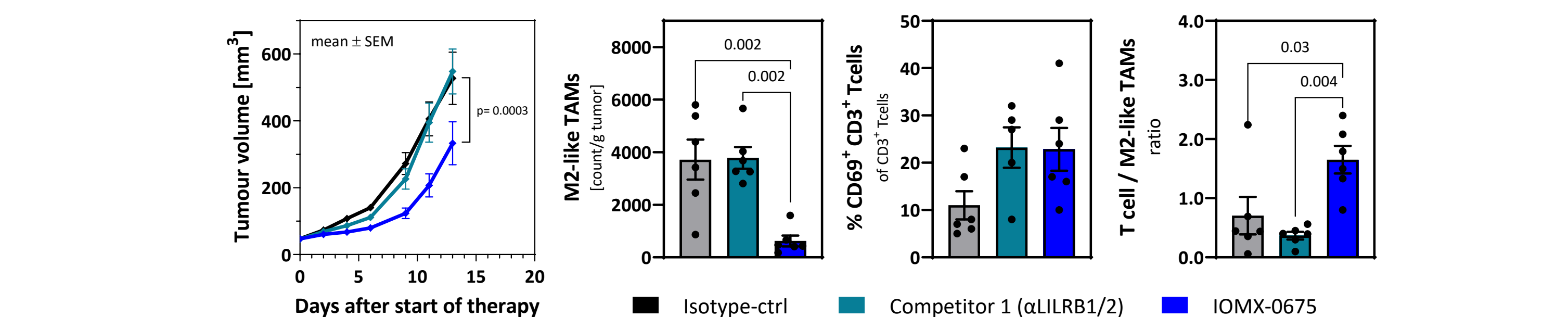


FIGURE VI
A375 melanoma tumor cells were implanted subcutaneously into NOG-EXL mice, fully humanized with CD34⁺ stem cells from two donors. Randomized animals were treated twice weekly with 20 mg/kg IOMX-0675, competitor 1, or corresponding isotype control antibody. Tumor infiltrating immune cells were analyzed by flow cytometry. Activated CD69⁺ CD3⁺ T cells, CD68⁺CD11c-HLA-DR- M2-like macrophages and the ratio of CD3⁺ T cells versus M2-like macrophages are shown as mean $\pm$ SEM.

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 immuno-activating LILR family members LILRA1/3.
- The highly differentiated binding profile of IOMX-0675 translates into remarkably superior potency in various binding and *in vitro* functional assays of macrophage repolarization, T cell suppression and tumor cell phagocytosis as compared to mono- or dual-targeting antibodies currently in clinical development.
- IOMX-0675 demonstrates its best-in-class potential by repolarizing the immunosuppressive tumor microenvironment *in vivo* and thereby inhibiting tumor growth in a humanized, myeloid engrafted melanoma model.
- CTA/IND-enabling studies for IOMX-0675 completed with CTA submission planned for Q4 2024.