

Discovery of MACO355: a first in mechanism ligand-blocking independent LILRB1/2/3 antibody for cancer therapy

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Highlights of MACO-355

- A novel, non ligand blocking, fully human anti LILRB1/2/3 monoclonal antibody
- amplifies production of TNF α by macrophages in a dose dependent manner
- reverts M2 (TGF β /IL-10/IL-4) macrophage mediated suppression of T cell activity *in vitro*
- Slows tumour growth *in vivo*
- binds to selected members of LILRB and LILRA families
- targets unique, membrane proximal epitope
- requires engagement of Fc receptor(s) for full activity
- rewires macrophage kinase network in a novel MoA
- favorable developability and safety profile
- currently in CMC and IND enabling studies
- novel soluble biomarker hypothesis to guide patient selection
- novel PD markers identified which are associated with MoA

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INTRODUCTION

Macrophages populate most solid tumors in large numbers and limit effective anti-tumoral immune responses [1]. The immunoreceptor tyrosine-based inhibitory motifs ('ITIMs') containing leukocyte immunoglobulin-like receptors (LILR) B1 and LILRB2 are expressed on tumour associated macrophages [2]. Despite sharing multiple ligands such as major histocompatibility complex class I G (HLA-G), LILRB1 regulates phagocytosis, whereas blocking of LILRB2 ligand binding was shown to enhance cytokine release [3]. To date, therapeutic approaches to targeting LILRB1 and LILRB2 have blocked receptor – ligand interactions to relieve ligand-mediated immune suppression. To obtain antibodies with novel and superior LILRB1/LILRB2 modulating activity, we investigated both ligand blocking and non ligand-blocking clones.

DISCOVERY OF MACO355

We identified MACO355 from a panel of fully human antibodies binding to both LILRB1 and LILRB2 and which were able to enhance secretion of TNF α by human monocyte-derived macrophages (MDMs) upon treatment with low levels of TLR agonists.

Surprisingly, only MACO355, unlike all other antibodies from the lead panel or competitor molecules, was able to stimulate secretion of pro-inflammatory cytokines by MDMs polarized under strong immunosuppressive conditions (CSF1/TGF β /IL-10) – **FIGURE 1**.

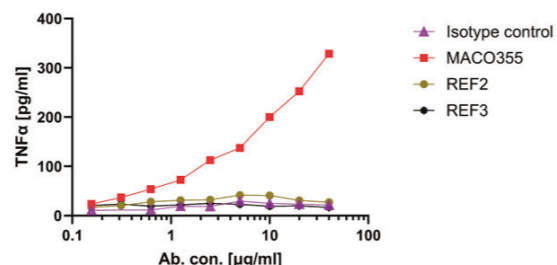


Figure 1. MACO355 stimulates TLR-agonist treated monocyte-derived macrophages (MDMs) to produce TNF α under strong immunosuppressive (CSF1/IL-10/TGF β) conditions in a dose dependent manner, unlike reference antibodies; REF2 – LILRB2 specific and REF3 – LILRB1/2 specific. The proinflammatory reprogramming of MDMs was observed for multiple donors and under different immunosuppressive conditions (data not shown).

MACO355 reprogramming relies on LILRB2 but not on LILRB1

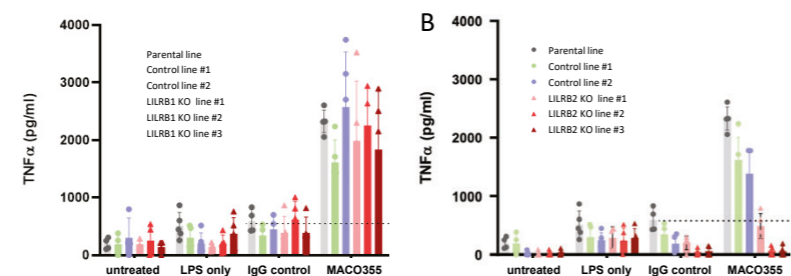
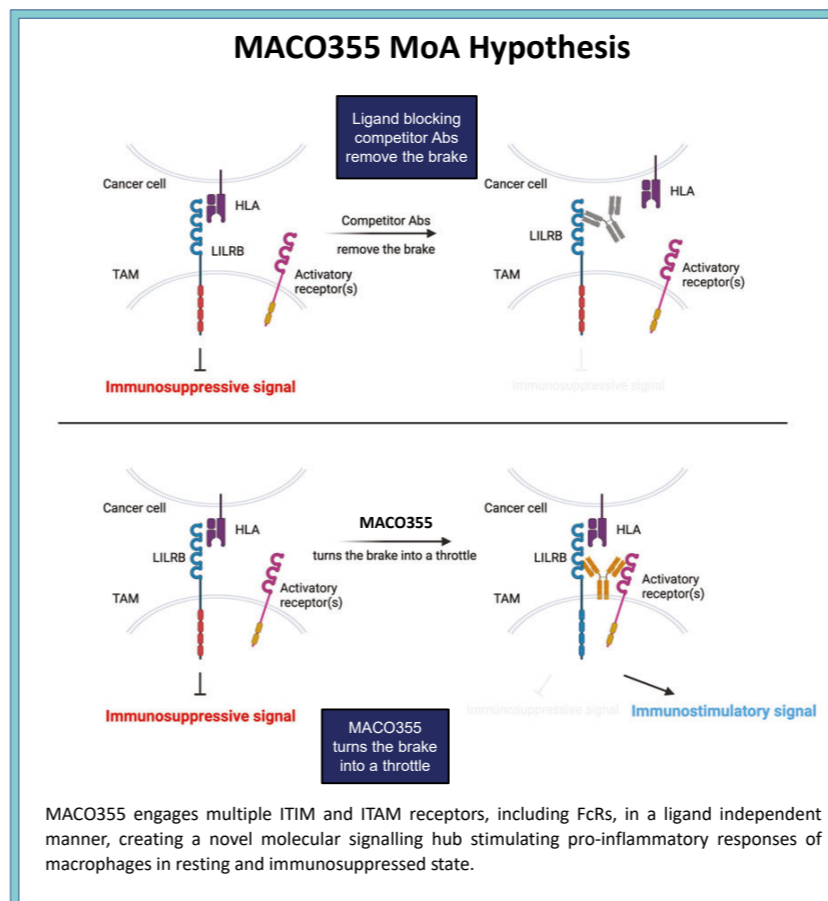


Figure 2. Macrophages derived from human iPSCs genetically engineered to suppress expression of either LILRB1 (A) or LILRB2 (B), were stimulated with LPS in the presence of MACO355 or isotype control. Production of TNF α was measured by ELISA. Three different KO lines were used for each receptor. Dotted line represents the isotype control level of LPS stimulated TNF α production. **NB:** KO of LILRB2 but not LILRB1 abolishes MACO355 stimulated cytokine production.

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MACO355 MoA Hypothesis



MACO355 engages multiple ITIM and ITAM receptors, including FcRs, in a ligand independent manner, creating a novel molecular signalling hub stimulating pro-inflammatory responses of macrophages in resting and immunosuppressed state.

MACO355 relieves M2 (TGF β /IL-10/IL4) macrophage induced T-cell suppression

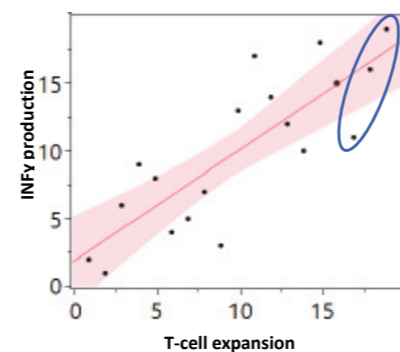


Figure 3. MACO355 reinvigorates T-cells immunosuppressed by co-culture with M2 macrophages. T-cell activation has been determined by cell proliferation and INF γ release. The ranked results for MACO355 are indicated in blue.

MACO355 binds multiple human LILRs

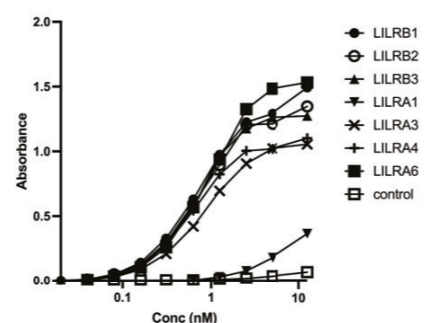


Figure 4. MACO355 binds to LILRB1, LILRB2, LILRB3, LILRA3, LILRA4, and LILRA6 with sub/low nM EC50 in ELISA. Weak binding was additionally observed to LILRA1, and no binding to LILRB4, LILRB5, LILRA2, and LILRA5 was detected (data not shown).

MACO355 does not compete with LILRB:ligand binding

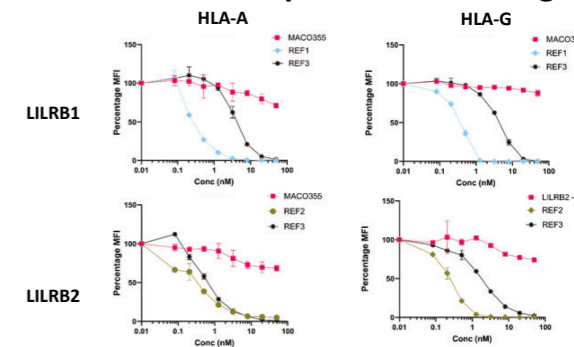


Figure 5. Binding of fluorescently labelled oligomeric HLA-A or of HLA-G to human LILRB1 or human LILRB2 over-expressing cells was analysed by flow cytometry. **NB:** Lack of inhibition of ligand-receptor binding by MACO355, unlike reference antibodies used (REF1 – LILRB1 specific, REF2 – LILRB2 specific, REF3 – LILRB1/2 dual binder).

MACO355 suppresses tumour growth *in vivo*

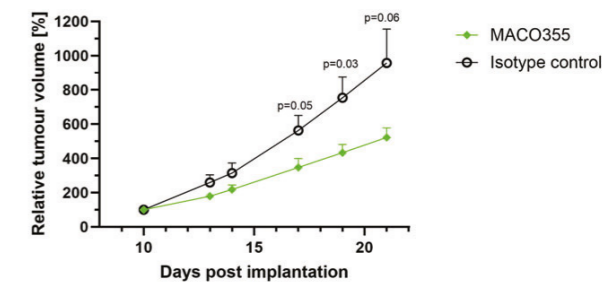


Figure 6. Human CD34⁺ cord blood haematopoietic stem cells humanized BRGSF mice (N=9) were implanted with A375 cancer cells and treated with antibodies over three weeks. Tumour growth was significantly (t-test) slowed in MACO355 group in comparison to the isotype control group.

MACO355 activity depends on binding to FcRs

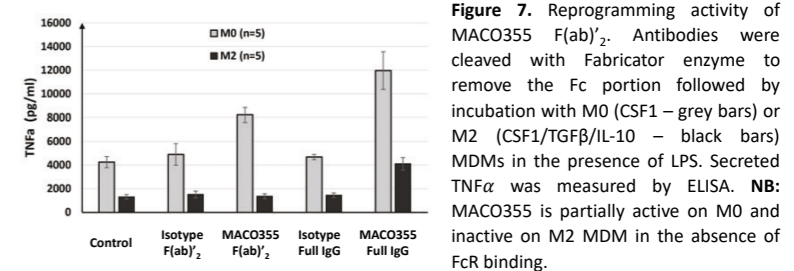


Figure 7. Reprogramming activity of MACO355 F(ab)². Antibodies were cleaved with Fabricator enzyme to remove the Fc portion followed by incubation with M0 (CSF1 – grey bars) or M2 (CSF1/TGF β /IL-10 – black bars) MDMs in the presence of LPS. Secreted TNF α was measured by ELISA. **NB:** MACO355 is partially active on M0 and inactive on M2 MDM in the absence of FcR binding.

Unique cell membrane proximal epitope and MoA

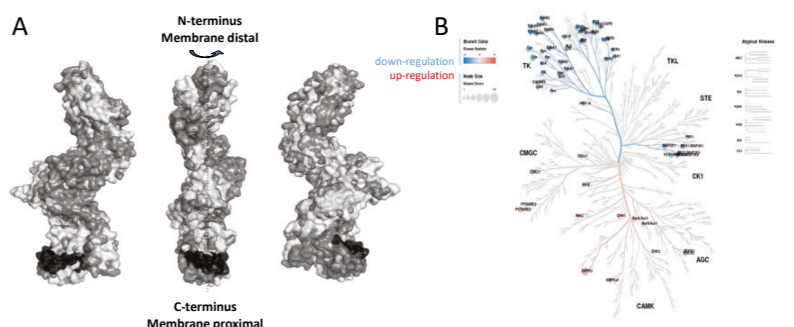


Figure 8. B. Overlay of 3D crystal structures of human LILRB1 (light grey) and LILRB2 (dark grey) ectodomains. MACO355 binds a unique epitope located in the cell membrane proximal domain (black) as determined by hydrogen-deuterium exchange method. **B.** MACO355 rewires intracellular kinase activity network as determined by the pan-kinase phosphorylation activity assay.