Preclinical characterization of a novel therapeutic antibody targeting LILRB2

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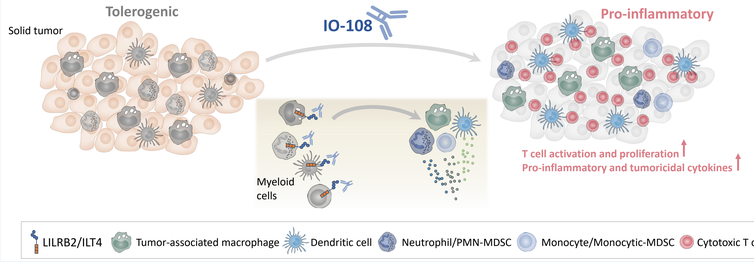
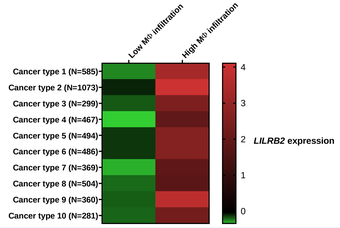
1 Background and rationale

Myeloid-derived suppressor cells, tolerogenic dendritic cells and tumor-associated macrophages inhibit anti-cancer immune responses systemically and in the tumor microenvironment (TME), thereby limiting the efficacy of T cell checkpoint inhibitors. However, the plasticity of myeloid cells may enable therapeutic intervention. The inhibitory receptor LILRB2 (also known as ILT4) is expressed in myeloid cells (monocytes, macrophages, dendritic cells and neutrophils) and is emerging as a key immune checkpoint mediating the tolerogenic activity of myeloid cells associated with cancer. LILRB2 has several ligands (classical MHC-I, HLA-G, ANGPTL2/5, SEMA4A and CD1c/d) and most of these are known to contribute to immune suppression in the TME. The wide expression of LILRB2 in myeloid cells makes it an ideal target to specifically modulate multiple aspects of myeloid cells' activity in the TME and periphery, in order to overcome their pro-tumor effect and enhance efficacy of T cell checkpoint inhibitors. IO-108 is a fully human IgG4 (S228P) therapeutic antibody that binds LILRB2 with high affinity and specificity and blocks LILRB2 ligand binding and receptor activation.

2 IO-108 mechanism of action



3 *LILRB2* expression in tumors associated with high macrophage infiltration



Macrophages are the most abundant immune cell type in many solid tumor types (Cassetta, L., & Pollard, J. W., 2018, *Nature Reviews Drug Discovery*).

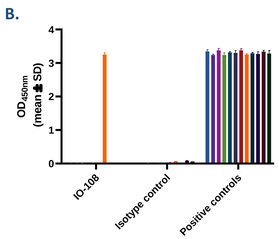
By applying a macrophage gene expression "signature" analysis to solid tumors of TCGA database, we demonstrate increased expression of LILRB2 in tumor samples with high macrophage infiltration.

4 IO-108 is a high affinity antibody specific to LILRB2

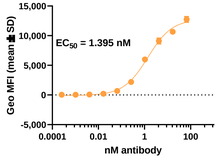
A. Binding kinetics

k_a (1/Ms)	k_d (1/s)	K_D (nM)
2.1E+05	4.3E-04	2.10

Binding specificity



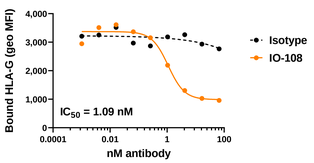
Binding potency on primary classical monocytes



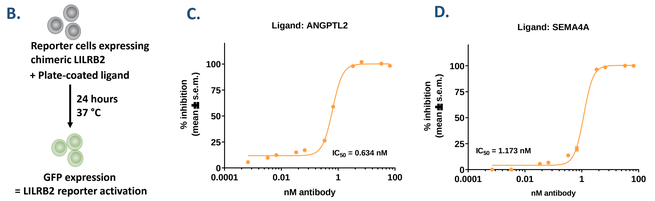
A. Binding kinetics parameters and affinity results from biolayer interferometry. B. ELISA on coated LILR/LAIR1 proteins. C. Dose-response measured by flow cytometry (representative data from one donor).

5 IO-108 is a potent antagonist of LILRB2

Competitive binding with HLA-G



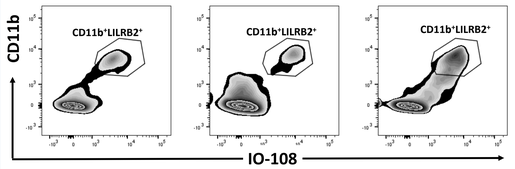
Inhibition of LILRB2 reporter cell activation



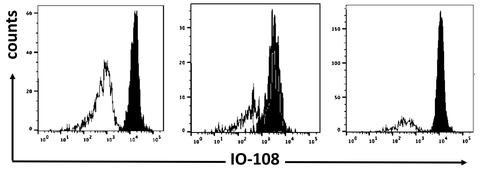
A, C-D. Dose-response of IO-108 inhibitory activity against various LILRB2 ligands. HLA-G at binding concentration = EC_{80} was co-incubated with titrated IO-108 on HEK293 expressing LILRB2 at 4 °C and bound HLA-G measured by flow cytometry. B-D. Measurement of LILRB2 activation (GFP expression) in LILRB2 reporter cells by flow cytometry. C-D. Ligands were plate-coated at 10 µg/mL and cells pre-incubated with titrated IO-108.

6 IO-108 binds all myeloid cells in solid tumor microenvironment and periphery

A. Tumor microenvironment (gated in CD45⁺)

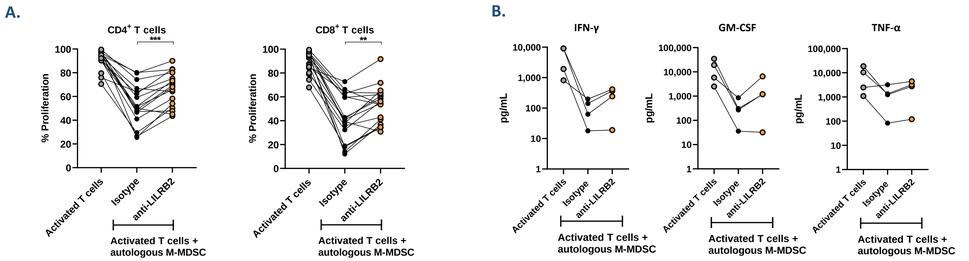


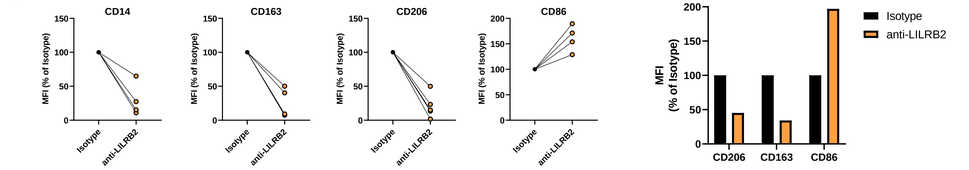
B. Blood (Patient #3)



A. Flow cytometric analysis of IO-108 binding in tumor tissue sample from 3 different patients. CD11b is used as pan-myeloid cell marker and CD45 is used as pan-tumor-infiltrating leukocytes marker.
B. Flow cytometric analysis of IO-108 binding (black-filled histogram) on myeloid cells in peripheral blood from patient #3. White filled histogram corresponds to IgG4 isotype control.

7 The antibody precursor to IO-108 promotes pro-inflammatory phenotype and decreases suppressive activity in patient-derived monocytic cells *ex vivo*

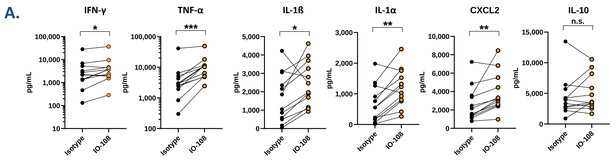




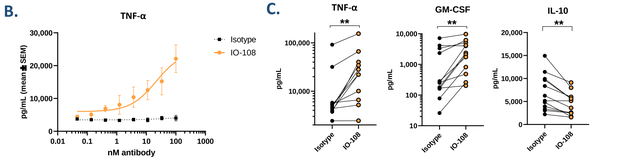
A-D. The *ex vivo* activity of the antibody precursor to IO-108 was tested (at 10 µg/mL) in cells isolated from cancer patient-derived PBMC and ascites. IO-108 is a modified version of this antibody precursor optimized for developability while maintaining the same binding affinity, selectivity and potent biologic activity as its precursor (not shown). A-C. Each line represents result from a different patient. Monocytic (M)-MDSC and autologous T cells isolated from PBMC were co-cultured for 5 days. T cells were activated with soluble tetrameric anti-CD3/CD28 antibody complexes. A. Proliferation of T cells (alone and in co-culture with M-MDSC) measured by flow cytometry (CFSE dilution). ANOVA: ***P= 0.0002, **P= 0.0052. B. Cytokine levels in culture media supernatants. C. Flow cytometric analysis of M-MDSC phenotype at the end of co-culture with autologous T cells. D. $CD14^+$ cells were isolated from an ovarian cancer ascites sample, incubated with antibodies for 7 days and their phenotype analyzed by flow cytometry.

8 IO-108 enhances pro-inflammatory activation of immune cells

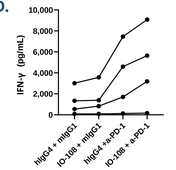
PBMC stimulated with sub-optimal [anti-CD3 mAb]



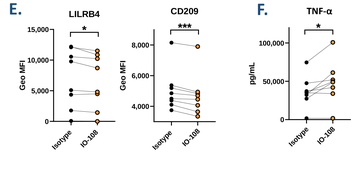
PBMC stimulated with low [LPS]



Allogeneic macrophage/CD4⁺ T cell co-cultures treated with anti-PD-1

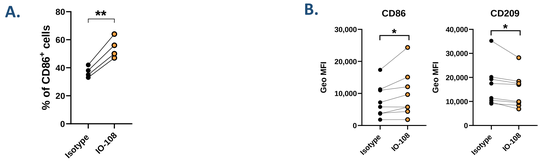


Dendritic cells stimulated with LPS



A, C-F. Each line represents result from a different healthy donor or allogeneic co-culture, as indicated. Antibodies were tested at 100 nM. Paired t test: *P<0.05, **P<0.01, ***P=0.0001, n.s.= non-significant. A. PBMC stimulated with sub-optimal [anti-CD3 mAb] for 3 days and cytokine levels measured in culture media supernatant. B-C. PBMC stimulated with low [LPS] for 3 days and cytokine levels measured in media culture supernatants. B. Dose response of IO-108 activity (representative data from one donor). D. M-CSF -induced monocyte-derived macrophages were co-cultured for 6 days with allogeneic $CD4^+$ T cells and IFN-γ levels measured in culture media supernatants. A mouse anti-PD-1 blocking antibody, or its isotype (mIgG1), was used in combination with IO-108 or its isotype (hIgG4). E-F. Immature monocyte-derived dendritic cells were stimulated with LPS for 2 days. E. Expression of tolerogenic markers analyzed by flow cytometry. F. TNF-α levels in culture media supernatant.

9 IO-108 induces differentiation of monocytic cells into activated dendritic cells



A-B. Each line represents result from a different healthy donor. Antibodies were tested at 100 nM. Paired t test: *P<0.05, **P=0.002. A. Classical monocytes incubated with antibodies in the presence of GM-CSF and IL-4. At the end of 6 days, CD86 expression was measured by flow cytometry in resultant dendritic cells (gated in $CD11b^+CD14^-CD11c^+$). B. Immature monocyte-derived dendritic cells were incubated with antibodies in the absence of any other stimulus and their phenotype analyzed by flow cytometry after 2 days.

10 Summary

- ***LILRB2* mRNA expression is associated with macrophage infiltration in many solid tumor types from TCGA.**
- **IO-108 is a fully human IgG4 (S228P) anti-LILRB2 antibody displaying high affinity, specificity and potent antagonistic activity.**
- **IO-108 binds all myeloid cells in tumor microenvironment and periphery.**
- **IO-108 and its precursor antibody promote pro-inflammatory phenotype and differentiation of myeloid cells, enhance immune cell activation and alleviate myeloid cell suppressive activity in various *ex vivo* systems, including samples from solid tumor patients.**
- **The *in vivo* efficacy of IO-108 is currently being evaluated in mouse models.**
- **Preclinical characterization of IO-108 suggests potential therapeutic benefit in solid tumors unresponsive to T cell checkpoint inhibitors.**
- **IO-108 has favorable pharmacokinetics profile.**
- **IO-108 IND filing planned for 1H 2021.**