IO-108, a Fully Human Therapeutic Antibody Blocking the Myeloid Checkpoint LILRB2/ILT4, Promotes Innate and Adaptive Anti-Cancer Immunity in Preclinical Studies

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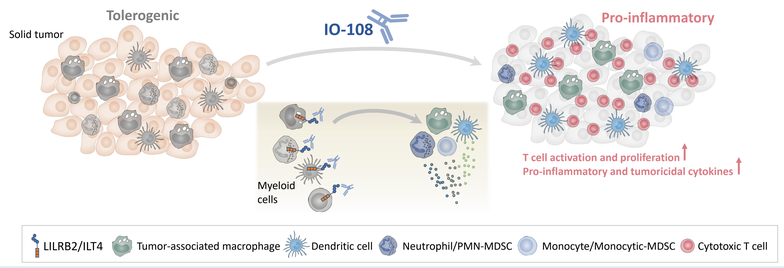
1 Abstract

Tumor-associated myeloid cells 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 immune inhibitory receptor LILRB2 (also known as ILT4) is expressed primarily by myeloid cells (monocytes, macrophages, dendritic cells and neutrophils), and has emerged as a key myeloid checkpoint contributing to the tolerogenic activity of myeloid cells associated with cancer. LILRB2 has several ligands (classical and non-classical MHC-I, ANGPTL2/5, SEMA4A and CD1) and most of these are known to contribute to immune suppression in the TME. Using computational biology approaches applied to the RNA-seq dataset of TCGA, we found that *LILRB2* expression is associated with the gene expression "signature" of macrophages infiltrating solid tumors. Therefore, LILRB2 is a compelling target to overcome immune suppressive activity of cancer-associated myeloid cells.

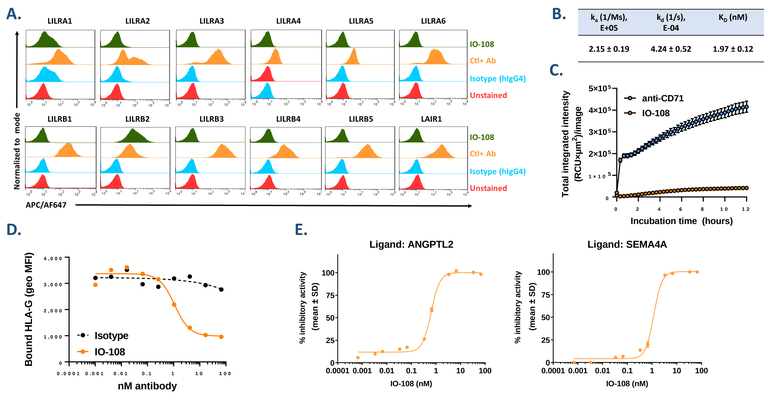
IO-108 is a fully human IgG4 therapeutic antibody that binds to LILRB2 with high affinity and specificity. IO-108 binds to all myeloid cells in the solid TME and periphery. In vitro studies support that blockade of LILRB2 interaction with its various ligands is the anticipated mechanism of action of IO-108. The LILRB2 antagonist activity of IO-108 produces the desired pro-inflammatory (re)polarization of myeloid cells. IO-108 treatment results in increased pro-inflammatory responses and an enhanced antigen-presenting cell (APC) phenotype to multiple stimuli (e.g., T cell activators, STING and TLR agonists) in ex vivo assays. In addition, IO-108 precludes the anti-inflammatory myeloid cell phenotype resulting from "tumor conditioning" and promotes the differentiation of monocytes and immature dendritic cells (DC) into pro-inflammatory DC. IO-108 enhances the effect of PD-1 blocking antibodies in allogeneic mixed leukocyte reactions of CD4⁺ T cells and macrophages. Moreover, IO-108 polarized primary myeloid cells isolated from solid tumor patient blood and ovarian cancer-associated ascites towards a pro-inflammatory phenotype and attenuated their suppressive effect on autologous T cell proliferation and production of pro-inflammatory cytokines. In vivo, IO-108 inhibits tumor growth in mouse models, which is associated with immune cell activation. Importantly, IO-108 presents a favorable pharmacokinetic and safety profile in preclinical models.

Collectively, the preclinical characterization of IO-108 enabled a comprehensive clinical biomarker plan and lends rationale to the clinical study (NCT05054348) of IO-108 as a novel immunotherapy for multiple solid tumor types, including those relapsed/refractory to standard of care therapies.

2 Therapeutic hypothesis

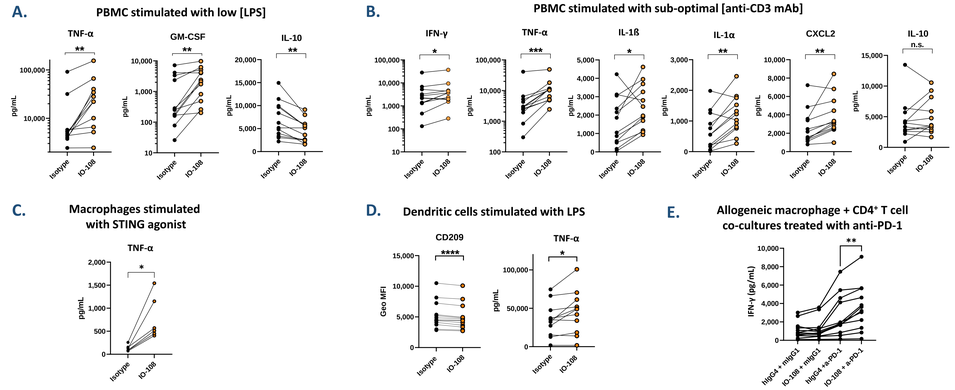


3 IO-108 is a selective and potent LILRB2 ligand-blocking antibody



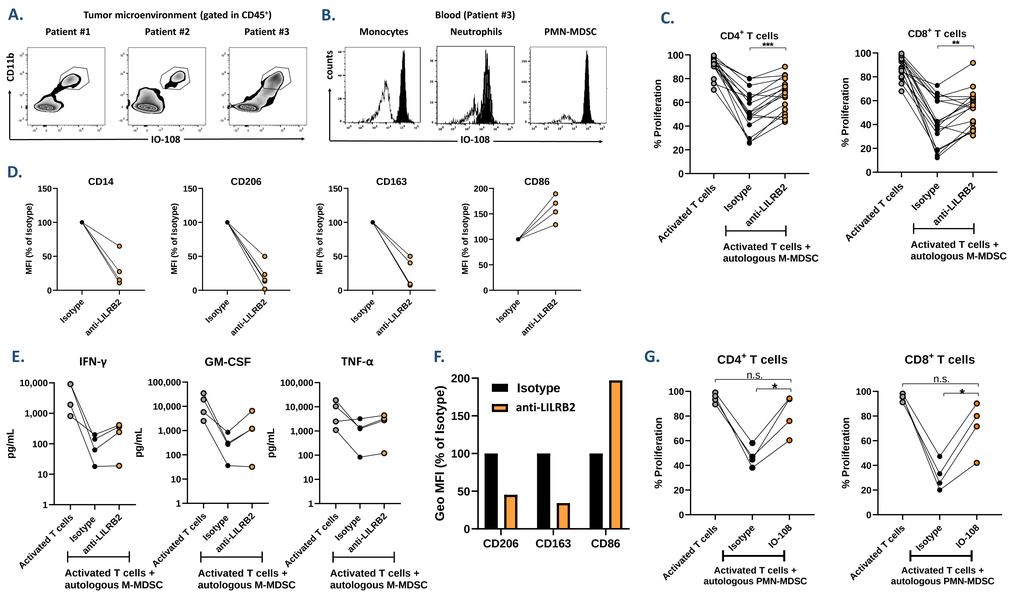
A. Flow cytometric data showing binding specificity of IO-108 to LILRB2+ reporter cells. B. IO-108 binding kinetics parameters and affinity results (biolayer interferometry). Data shown are mean ± SD. C. Representative data of real-time monitoring of target:antibody complex levels in lysosome of monocyte-derived macrophages showing that IO-108 does not internalize upon LILRB2 binding (anti-CD71 = positive control). Data shown are mean ± SEM. D-E. Dose-response of IO-108 blocking activity against LILRB2 ligands. D. HLA-G at binding concentration = EC_{80} was co-incubated with titrated IO-108 on HEK293 expressing LILRB2 and bound HLA-G measured by flow cytometry. E. IO-108 inhibits LILRB2 reporter cell activation by plate-coated (10 μg/mL) ligands.

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



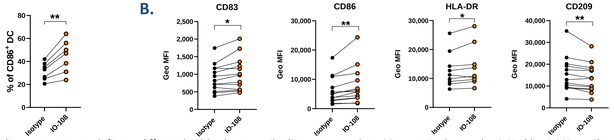
A-E. Each line represents result from a different healthy donor or allogeneic co-culture, as indicated. Antibodies were tested at 100 nM. *p<0.05, **p<0.01, ***p=0.0001, ****p <0.0001, n.s.= non-significant (paired *t* test or ANOVA, as appropriate). A-D. Cytokine levels determined by Luminex assay. A. PBMC stimulated with 50 ng/mL LPS for 3 days and cytokine levels measured in media culture supernatants. B. PBMC stimulated with 10 ng/mL anti-CD3 mAb for 3 days and cytokine levels measured in culture media supernatant. C. Monocyte-derived macrophages stimulated with 5 μg/mL 2'3'-cGAMP in the presence of IO-108 or isotype control and TNF-α measured in media culture supernatants. D. Immature monocyte-derived dendritic cells were stimulated with 100 ng/mL LPS for 2 days. Expression of tolerogenic marker CD209 was analyzed by flow cytometry and TNF-α levels in culture media supernatant. E. 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 (ELISA). A mouse anti-PD-1 blocking antibody, or its isotype (mIgG1), was used in combination with IO-108 or its isotype (hIgG4).

5 IO-108 decreases the immune suppressive activity and promotes a pro-inflammatory phenotype in cancer patient-derived MDSC *ex vivo*



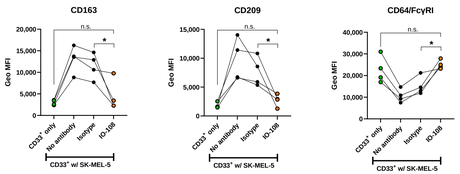
A. Flow cytometric analysis of IO-108 binding in tumor tissue samples from 3 different patients. CD11b is used as pan-myeloid cell marker and CD45 is used as pan-tumor-infiltrating leukocyte marker. B. Flow cytometric analysis of IO-108 binding (black-filled histogram) on myeloid cells in peripheral blood from patient #3. White filled histogram corresponds to IgG4 isotype control. C-F. 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). C-E. Each line represents results 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. C. 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. D. Flow cytometric analysis of M-MDSC phenotype at the end of co-culture with autologous T cells. E. Cytokine levels in culture media supernatants. F. CD14⁺ cells were isolated from an ovarian cancer ascites sample, incubated with anti-LILRB2 (IO-108 precursor) or isotype control antibodies for 7 days and their phenotype analyzed by flow cytometry. G. Proliferation of patient PBMC-derived T cells (alone and in co-culture with autologous PMN-MDSC) measured by flow cytometry (CFSE dilution). ANOVA: *p<0.05, n.s. = non-significant.

6 IO-108 induces differentiation of monocytic myeloid cells into stimulatory dendritic cells



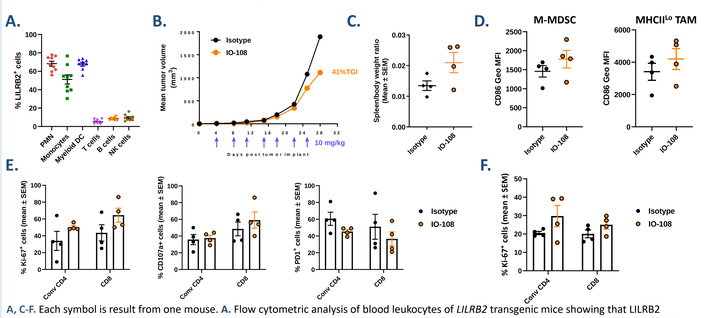
A-B. Each line represents result from a different healthy donor. Antibodies were tested at 100 nM. Paired t test: *p<0.05, **p=0.002. A. Classical monocytes incubated with antibodies in the presence of GM-CSF and IL-4. At the end of 6 days, CD86 expression was measured by flow cytometry in resultant 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.

7 IO-108 counteracts "tumor-conditioning" of myeloid cells



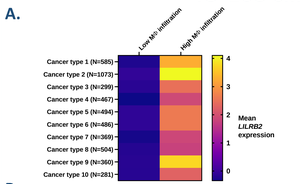
Myeloid cells (CD33+ PBMC) were left untreated or co-cultured with SK-MEL-5 melanoma cells. Each line represents the resulting expression levels of anti-inflammatory (CD163 and CD209) and pro-inflammatory (CD64/FcγRI) cell surface markers from a different donor at the end of the 5-day culture. Similar results were obtained using A549 lung carcinoma cells. *p<0.05, n.s.= non-significant (ANOVA).

8 IO-108 inhibits tumor growth in a syngeneic mouse model by promoting innate and adaptive immunity

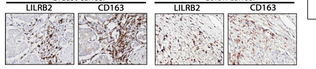


A, C-F. Each symbol is result from one mouse. A. Flow cytometric analysis of blood leukocytes of *LILRB2* transgenic mice showing that LILRB2 expression (mean ± SEM) is restricted to myeloid cells. B. IO-108 caused 41% (average) tumor growth inhibition (TGI) of LLC in syngeneic *LILRB2* transgenic mice. C. Increased spleen size in IO-108-treated mice in B (study day 28) suggests enhanced immune activation. D-F. Immune cell profiling from mice in B (study day 28). D. IO-108 caused increased CD86 expression in tumor-infiltrating M-MDSC and MHCII^Lo TAM. E. IO-108 promoted activation, proliferation and cytotoxic activity in tumor-infiltrating T cells. F. IO-108 promoted T cell proliferation of peripheral (splenic) T cells.

9 IO-108 clinical biomarker plan



	Pre-treatment	On-treatment *vs* pre-treatment
Peripheral blood	• Myeloid cell phenotype • T cell activation markers • Immune cell counts • Soluble HLA-G levels	• Receptor occupancy • LILRB2 expression • Myeloid cell phenotype • T cell activation markers • Immune cell counts • Cytokine & chemokine levels
Tumor	LILRB2 protein expression Gene expression studies	Gene expression studies
	Patient segmentation	Confirmation of MoA and therapeutic hypothesis



A. Analysis of TCGA RNA-seq database shows that *LILRB2* mRNA expression in solid tumors is associated with a high macrophage infiltration gene expression "signature". B. IHC with a proprietary LILRB2 antibody confirms LILRB2 expression in tumor-associated macrophages (CD163+).