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In Vitro Characterization of a Dual Antagonistic Anti-LILRB2/LILRB4 Monoclonal Antibody

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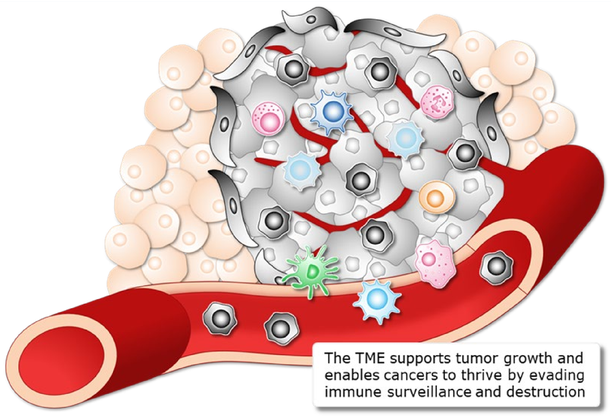
Abstract

Introduction: Myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment are a potential therapeutic target in immune checkpoint cancer therapy, but MDSC-targeted therapies have yet been shown to improve survival. The leukocyte immunoglobulin (Ig)-like receptor B2 is a member of the leukocyte Ig-like receptor (LILR) family and is predominantly expressed on the surface of cells of the myelomonocytic lineage (monocytes, macrophages and dendritic cells). LILRB2 is a negative regulator of myeloid cells and is an immune check-point receptor. We chose to generate mAbs against LILRB2 as a potential immuno-therapeutic for cancer. **Methods:** PentaMice® strains (5 WT mouse strains comprising 9 MHC class II haplotypes to maximize Ab diversity) were immunized with human LILRB2. Lymphocytes were fused with a myeloma partner to generate lead monoclonal hybridomas. 24 purified anti-LILRB2 mAbs were generated and evaluated by arrayed surface plasma resonance (SPR, Carterra® LSA® instrument) to assign epitope bins. mAb humanization was performed in silico to maximize T20 "humanness" scores (algorithm developed by Curia). Humanized mAbs were transiently expressed using Curia's TunaCHO™ platform and tested in in vitro MDSC assays. Autologous co-cultures of magnetic bead enriched-human blood-derived MDSC (HLA-DR-CD14+) model cells and T cells (CD3+) were stimulated with plate-bound anti-CD3/CD28 to induce T cell activation. Cytokine secretion was assessed 4 days later. **Results:** A total of 384 hybridoma supernatants were selected from the primary screen. 245 were confirmed by ELISA and 178 stained HEK293 cells expressing LILRB2 cells by flow cytometry (FACS). 24 hybridomas were further selected, cloned and sequenced. Five epitope bins were identified via Carterra® LSA® instrument. Two clones were chosen for humanization, designated 6-I11A and 4-O19A and were recombinantly expressed with the human IgG4 isotype. FACS analysis on HEK293 cells expressing either huLILRB2 or huLILRB4 revealed that clone hu4-O19A-IgG4 was specific for huLILRB2. Surprisingly, clone hu6-I11A-IgG4 stained both huLILRB2 and huLILRB4. FACS analysis with hu4-O19A-IgG4 on human MDSCs exhibited an EC50 of 11.9 nM and a KD of 1.5 nM in binding to recombinant huLILRB2 antigen, while hu6-I11A-IgG4 had an EC50 of 3.2 nM and a KD of 0.6 nM. In co-cultures with human MDSCs and T cells, both hu6-I11A-IgG4 and hu4-O19A-IgG4 reversed cytokine suppression by the MDSCs by increasing GM-CSF, IL-10, TNF-α, and IL-6, while suppressing IL-13, and had little effect on IL-17A, MIP-3a, IL-4, IL-2 and IFN-γ compared to treatment with the isotype control, with hu6-I11A-IgG4 having a greater effect as an antagonist than hu4-O19A-IgG4. **Conclusions:** We have identified a dual anti-LILRB2/LILRB4 antagonist. Targeting MDSCs with this antibody offers great promise for immunotherapy for cancer as well as its immense potential for synergy with PD-1 blockade.

Introduction

Tumors Create a Toxic, Immunosuppressive Microenvironment (TME)



MDSCs are a Major Therapeutic Target

- Tumors are surrounded by endothelial and stroma cells, and invading immune cells, both innate and adaptive[1,2]
- Complex regulatory network supports tumor growth, enabling cancers to thrive by evading immune surveillance and destruction[2,3]
- The TME sabotages tumor-killing cytotoxic CD8 T cells[1]
- Myeloid-derived suppressor cells (MDSCs) interfere with anticancer immunity[2,3]
- Levels of MDSCs tend to correlate with tumor stage, patient survival, and metastatic burden and may predict poor response to certain cancer treatments[4]
- MDSCs represent a central mechanism of immunosuppression in cancer; targeting these cells could significantly improve our ability to fight cancer[5,6]

Therapeutic Strategies Include[6]:

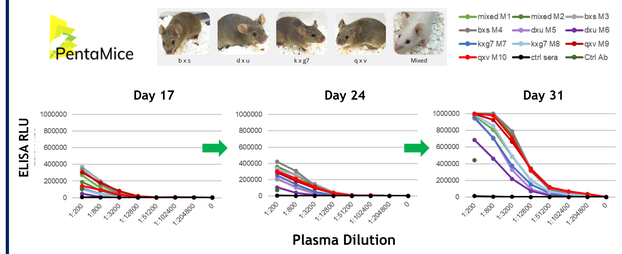
- Promoting differentiation of MDSCs to a non-immunosuppressive cell type
- Blocking MDSC immunosuppressive functions
- Inhibiting MDSC expansion
- Eliminating MDSCs

Targeting LILRB2 in Cancer

- LILRB2 is expressed in various types of solid tumors: colon, breast, lung, hepatocellular, pancreas, and prostate[7]
- Overexpression contributes to metastasis correlating with poor survival[7]
- Expressed on macrophages, fibroblasts, and plasma cells within the TME in breast cancer patients[7]
- Induces M2 macrophage polarization, impairs T-cell function, inhibition reverses immunosuppressive role[7]
- Active area of development with several monoclonal antibodies in the clinic[7]

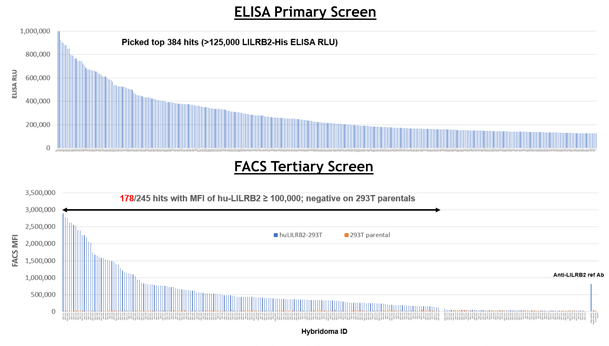
Results

Fig 1: PentaMice Immunization



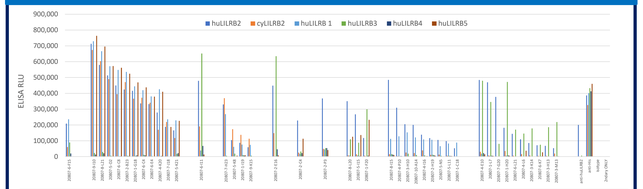
Titer checks guided selection of mice with highest LILRB2 titers

Fig 2: Identification of LILRB2 Binders



ELISA and FACS analysis identified hundreds of LILRB2 binders

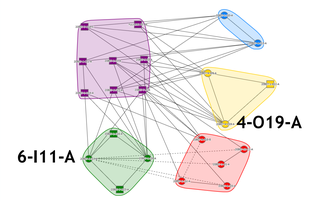
Fig 3: ELISA Specificity Screen



	huLILRB2	cyLILRB2	huLILRB1	huLILRB3	huLILRB4	huLILRB5	# of Hits
Profile 1	+	+	+	+	-	-	1
Profile 2	+	+	+	-	-	+	11
Profile 3	+	+	-	+	+	-	1
Profile 4	+	+	+	-	-	-	4
Profile 5	+	+	-	+	-	-	1
Profile 6	+	-	-	-	-	+	1
Profile 7	+	-	-	+	+	-	1
Profile 8	+	-	-	+	-	+	3
Profile 9	+	-	+	-	-	-	9
Profile 10	+	-	-	+	-	-	10

Cross-reactivity binding ELISAs reveal diverse set of profiles including human/cyno cross reactive binders

Fig 4: Epitope Binning via Arrayed SPR



Two antibodies identified via Carterra® LSA® were chosen for humanization

Fig 5: Identification of Potent Hu-Anti-LILRB2 mAbs

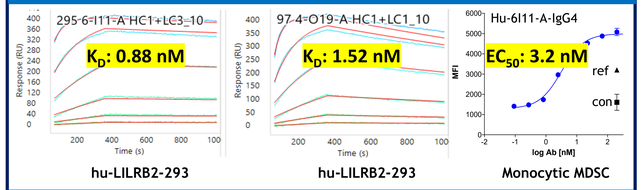
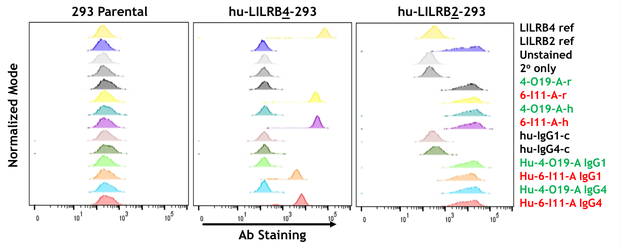


Fig 6: 6-I11-A mAb Binds to LILRB2 and LILRB4



Specificity of anti-LILRB2 mAbs via cell surface staining

References

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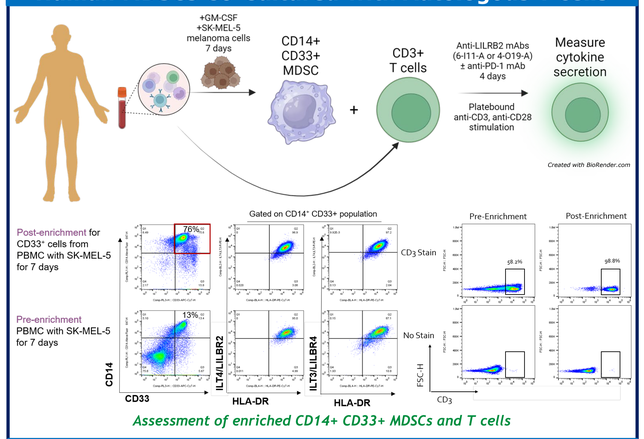
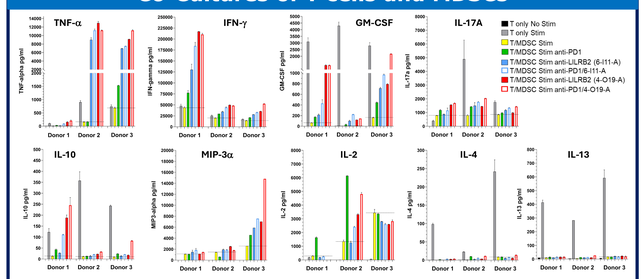
7. Redondo-Garcia, et al. *Front. Immunol.* 2023;13:1

Fig 7: In Vitro Model of Tumor Cell Line-Educated Human MDSCs Co-Cultured with Autologous T cells



Assessment of enriched CD14+ CD33+ MDSCs and T cells

Fig 8: Humanized Anti-LILRB2 mAb Treatment, Like Anti-PD-1 mAb Enhances Anti-Tumor Cytokines in Co-Cultures of T cells and MDSCs



Cytokine secretion from co-cultured cells from three human donors

Summary of the Effects of Humanized Anti-LILRB2 mAbs Treatment on Cytokine Secretion in Co-Cultures of T Cells and Tumor Adapted MDSCs ± Anti-PD-1

Treatment	TNF-α	INF-γ	GM-CSF	IL-17A	IL-10	MIP-3α	IL-2	IL-4	IL-13
Anti-PD-1	+ (3)	+ (ALL)	+ (1,3)	+ (ALL)	+ (1,3)	+ (3)	+ (1,2)	-	-
6-I11-A	+ (2,3)	+ (ALL)	+ (ALL)	+ (2,3)	+ (1,3)	+ (ALL)	-	-	-
6-I11-A + Anti-PD-1	Additive (1,2)	Additive (ALL)	Additive (1,2)	Not Additive	Additive (1)	Additive (3)	Not Additive	Not Additive	Not Additive
4-O19-A	+ (ALL)	+ (ALL)	+ (ALL)	+ (ALL)	+ (ALL)	+ (2,3)	+ (2)	-	-
4-O19-A + Anti-PD-1	Additive (3)	Additive (3)	Additive (3)	Additive (2,3)	Additive (1,3)	Additive (3)	Not Additive	Not Additive	Not Additive

Conclusions

- Using LILRB2 as an antigen, we have identified two potent mAbs, 6-I11-A and 4-O19-A.
- mAb 6-I11-A was also found to bind LILRB4 resulting in a dual anti-LILRB2/LILRB4 antagonist.
- Targeting LILRB4 is ideal since it is also expressed on a number of cancers including multiple myeloma, gastric, melanoma, colorectal, pancreatic, ovarian, hepatocellular and NSCLC, and its expression on MDSCs corelates with reduces overall survival[7].
- We believe that mAb 6-I11-A has the potential to be a novel checkpoint inhibitor to be used in a variety of immunotherapy applications for cancer.
- The potential for synergy with PD-1 blockade offers great promise for immuno-oncology.

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