Targeting the Immune Inhibitory Receptor LILRB4 to Treat Acute Myeloid Leukemia (AML)

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Abstract

Immune checkpoint blockade therapy has been successful in treating certain types of cancers but has not shown clinical benefits for treating leukemia. This observation suggests that leukemia exploits unique escape mechanisms. Certain immune inhibitory receptors that are expressed by normal immune cells are also present on leukemia cells. Whether these receptors can initiate immune-related primary signaling in tumor cells remains unknown. Here we use mouse models and human cells to show that LILRB4, an ITIM-containing receptor and a marker of monocytic leukemia, supports tumor cell infiltration into tissues and suppresses T cell activity via a signaling pathway that involves APOE, LILRB4, SHP-2, uPAR and ARG1 in acute myeloid leukemia (AML) cells.

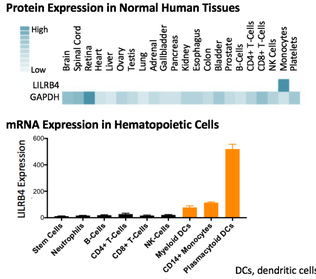
We will present data to show that deletion of LILRB4 or the use of antibodies to block LILRB4 signaling impeded AML development. Additionally, mechanistic studies in vitro and in vivo revealed three major modes of action for the anti-AML activity mediated by our antibodies: 1) reversal of T cell suppression; 2) inhibition of monocytic AML cell tissue infiltration and induction of AML cell mobilization; 3) targeted cell killing via ADCC and ADCP. Furthermore, a novel anti-LILRB4 CAR-T cell was engineered to specifically target monocytic AML cells with no toxicity to normal hematopoietic progenitors, as demonstrated in vitro and in xenografted mice.

In summary, LILRB4 orchestrates tumor invasion pathways in monocytic leukemia cells by creating an immunosuppressive microenvironment. Targeting LILRB4 with antibodies or cell therapies represents new therapeutic strategies for treating monocytic AML.

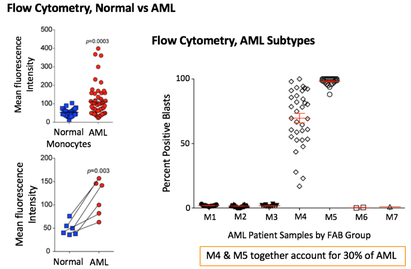
Results

LILRB4 Expression is Restricted in Normal Cells and Upregulated in AML

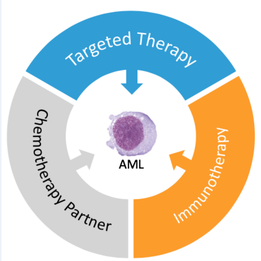
Expression in Normal Tissues is Largely Restricted to Monocytes and Dendritic Cells



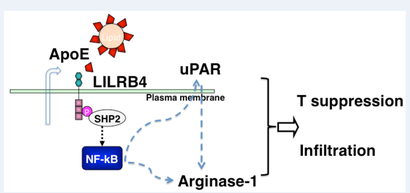
LILRB4 Expression is Upregulated on Monocytic AML Blasts Target Patient Population Readily Identified



Anti-LILRB4 Antibody has Three Potential Modes of Action in AML

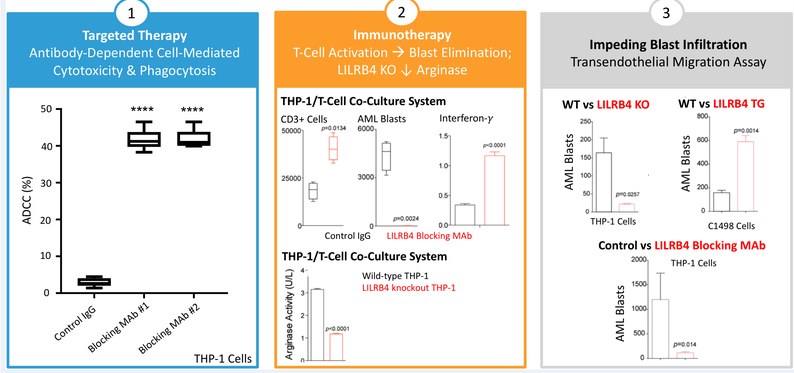


1. Target expression is highly restricted and elevated on AML blasts compared to normal monocytes, facilitating the clearance of blasts through ADCC and ADCP
2. Activates effector T-cells *in vivo* by inhibiting arginase-1 activity in the tumor microenvironment
3. Impedes blast infiltration to the bone-marrow and extra-medullary sites, mobilizing leukemia cells and increasing sensitivity to chemotherapy



Schematic for the mechanisms by which LILRB4 suppresses T cells and promotes leukemia infiltration.

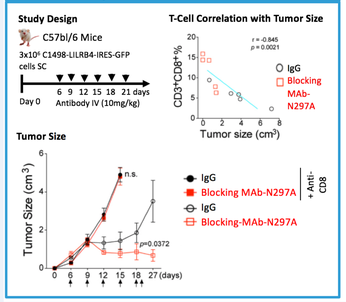
LILRB4 Blocking Antibody Shows Activity in In Vitro Models



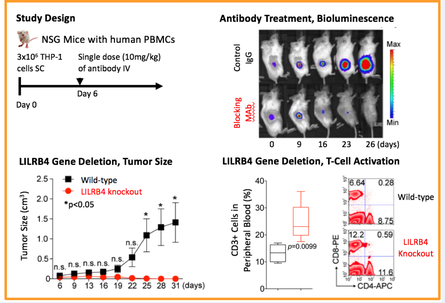
Three potential mechanisms of actions demonstrated in vitro.

LILRB4 Blocking Antibody Causes Regression of Established AML Tumors and Increases T-Cell Activation In Vivo

Allograft Tumors Expressing LILRB4 in Immunocompetent Mice



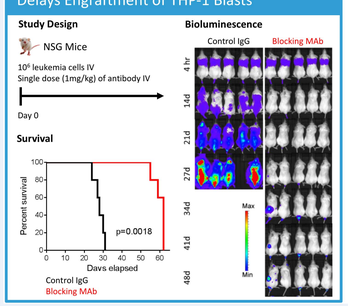
Xenograft Tumors in Human PBMC-reconstituted NSG Mice (Functional Human T-Cells)



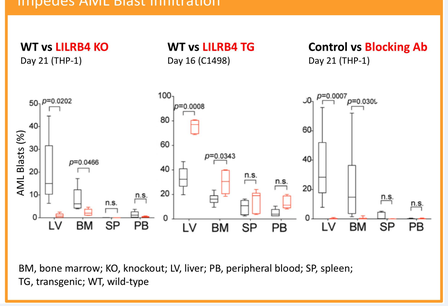
Subcutaneous-introduced AML cells led to reduced tumor size upon blockade of LILRB4, either by blocking antibodies or through gene knock-out, in immune-competent models (syngeneic mice or human PBMC reconstituted mice), and accompanied by T-cell activation.

LILRB4 Blocking Antibody Impedes Engraftment of AML Blasts In Vivo

LILRB4 Blocking Antibody Prolongs Survival and Delays Engraftment of THP-1 Blasts



Genetic and Therapeutic Evidence Show that Inhibiting LILRB4 Impedes AML Blast Infiltration

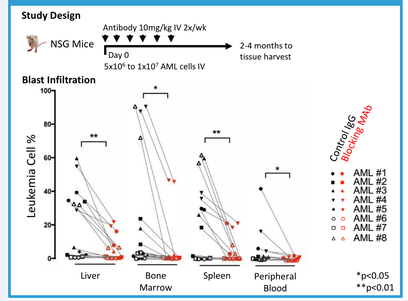


BM, bone marrow; KO, knockout; LV, liver; PB, peripheral blood; SP, spleen; TG, transgenic; WT, wild-type

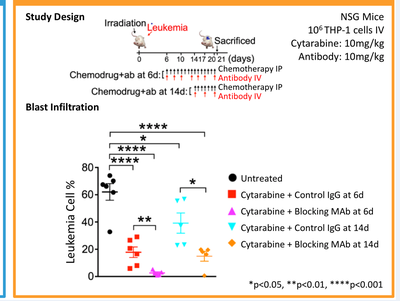
Blockade of LILRB4 (with blocking antibody or gene knockout) led to reduction of leukemia engraftment in vivo. Conversely, LILRB4 expression led to increased leukemia infiltration to tissues.

LILRB4 Blocking Antibody Shows Activity in AML Patient-Derived Xenografts (PDX) and Additive Activity with Chemotherapy In Vivo

Activity in Eight AML Patient-Derived Xenografts



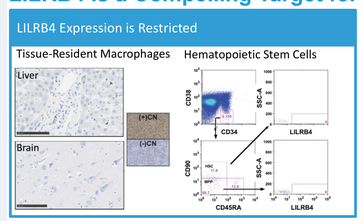
Combination Activity with Chemotherapy



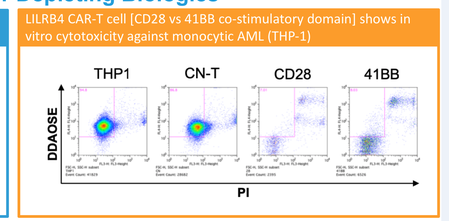
Anti-LILRB4 antibody reduced leukemia burden in AML patient-derived xenograft (PDX) model and showed additive activity with chemo-combo.

LILRB4 is a Compelling Target for Depleting Biologics

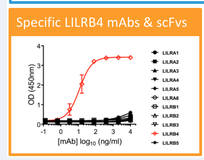
LILRB4 Expression is Restricted



LILRB4 CAR-T cell [CD28 vs 41BB co-stimulatory domain] shows in vitro cytotoxicity against monocytic AML (THP-1)

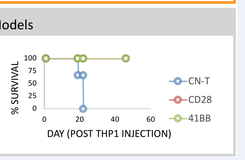


Specific LILRB4 mAbs & scFvs



LILRB4 CAR-T Constructs Show *In Vivo* Efficacy in AML Models

- NSG mice
- Day -4: Irradiation
- Day -4: THP-1 IV (1x10⁶/mouse)
- Day 0: T-cell IV (1x10⁶ Viable CAR-T/mouse)



Proof-of-concept data of LILRB4 CAR-T in vitro and in vivo.

Summary

- LILRB4 is an immune-inhibitory receptor expressed on monocytes, dendritic cells and macrophages
- LILRB4 is upregulated on some monocytic AML blasts
- When expressed on AML cells, LILRB4 suppresses T cell activity and also supports tumor infiltration into tissues and organs. Mechanistically, a signaling pathway involving APOE, LILRB4, SHP-2, NF-kB, uPAR, and ARG1 in AML cells is required for both functions of LILRB4. (see Schematic on the left).
- Targeting LILRB4 with genetic knockout or blocking antibodies in preclinical systems showed:
  - Fc effector function-mediated blast depletion
  - Anti-leukemic T-cell activation
  - Reduced blast infiltration/engraftment
- LILRB4 shows additive activity with cytotoxic chemotherapy in preclinical AML models
- A novel anti-LILRB4 CAR-T cell was engineered to specifically target monocytic AML cells with no toxicity to normal hematopoietic progenitors, as demonstrated in vitro and in xenografted mice.
- LILRB4 may act as an immune checkpoint in solid tumors. LILRB4 blocking antibodies may modulate myeloid-derived suppressor cell activity and facilitate immune-mediated tumor regression

Acknowledgements

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