# A Novel Bispecific LILRB4 x CD3 Antibody with Potent killing of Monocytic Acute Myeloid Leukemia Cells

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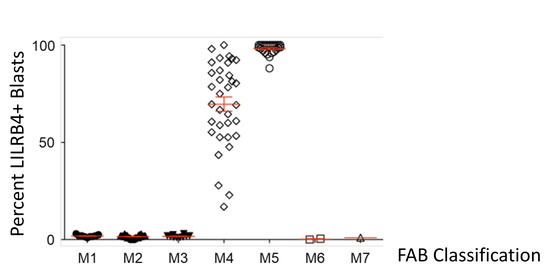
## Abstract

**Background**:
Acute myeloid leukemia (AML) remains one of the highest unmet needs among human cancers. LILRB4 is specifically expressed on M4 myelomonocytic and M5 monocytic AML cells with a lack of expression on normal hematopoietic stem cells and progenitors, making LILRB4 an attractive target for a T-cell redirecting bispecific antibody to treat AML. Using a proprietary bispecific antibody platform, we engineered and optimized a LILRB4 x CD3 bispecific antibody demonstrating potent and specific killing of monocytic AML cells in vitro and in vivo. The strong chemistry, manufacturing and control (CMC) attributes and cross-reactivity to non-human primate LILRB4 and CD3 favor this bispecific for rapid advancement to the clinic.

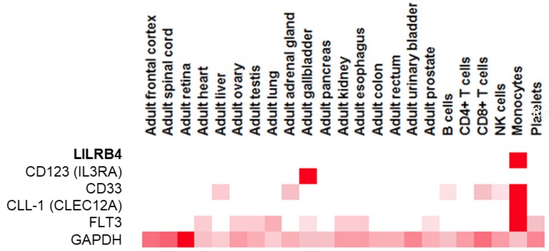
**Results**:
Two top candidates were selected from multiple different CD3-bispecific configurations using biophysical criteria: 1) A monovalent anti-LILRB4 arm together with a monovalent anti-CD3 (1+1); 2) A bivalent anti-LILRB4 arm together with a monovalent anti-CD3 (2+1). The binding EC50 values of the 2+1 or the 1+1 variants to LILRB4 in the THP-1 cells were 0.7 nM and 1.3 nM, respectively. ELISA showed the anti-LILRB4 arm to be highly selective for LILRB4. Both 2+1 and 1+1 variants demonstrated potent killing of the THP-1 cells with EC50 values of 0.5 pM and 4.3 pM, respectively. In the PBMC assay, both variants induced killing of monocytes while sparing B cells, supporting the specificity in inducing T-cell directed killing of the LILRB4-expressing primary cells. The anti-CD3 arm was designed to have a low affinity for CD3 to mitigate the risk of cytokine release while ensuring sufficient T cell activation. Both variants showed potent tumor-growth inhibition at doses as low as 0.2 mg/kg in the NSG mice with the 2+1 molecule showing superiority in vivo. The PK profile of the 2+1 bispecific in human FcRn-transgenic mice was similar to what is expected of human IgG1 with linear clearance.

## LILRB4 expressed in M4 and M5 AML

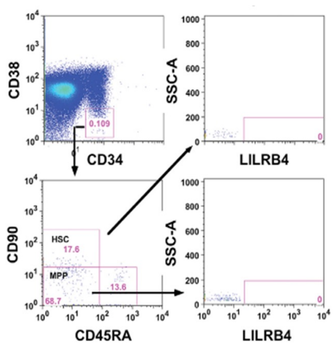



## Restricted Tissue Expression of LILRB4

Human Proteome Map
http://www.humanproteomemap.org/query.php



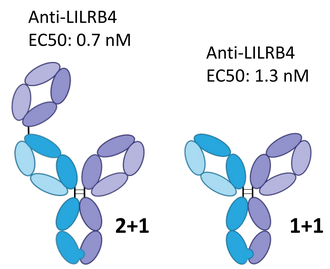

## No LILRB4 Expression in Normal Blood Stem Cell



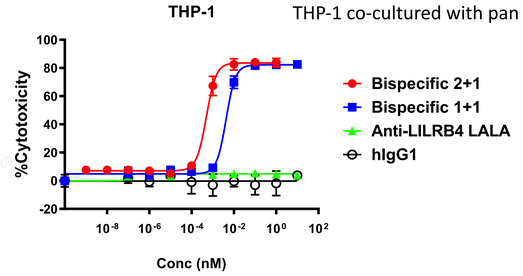

Flow cytometry analysis of LILRB4 expression on human hematopoietic stem cells (HSCs) and multi-potent progenitors (MPPs) obtained from normal healthy adult bone marrow

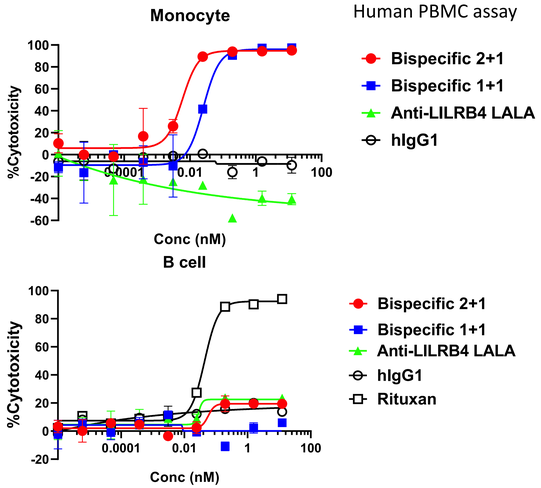
## Novel CD3 Bispecific Ab Targeting LILRB4

- Anti-LILRB4 arm (purple color) with high affinity to LILRB4 and demonstrated clinical activity
- Monovalent and low affinity for CD3 with EC50 > 100 nM
- IgG-like (effector-less Fc) for stability and long t1/2
- Cyno cross-reactive to LILRB4 and CD3



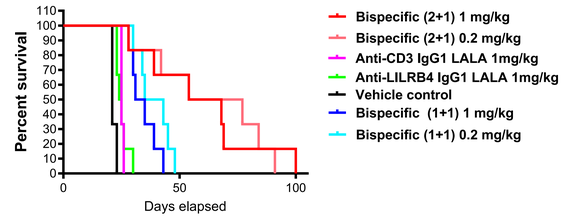

## Potent in vitro Killing of LILRB4-expressing Cells



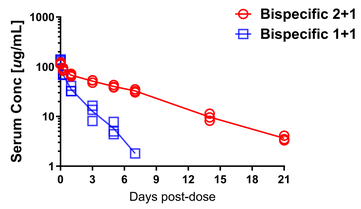




## Bispecific 2+1 is More Efficacious Than 1+1

THP-1-GFP-Luc and expanded T cells co-implanted to NSG mice treated with single dose of the antibodies at day 5

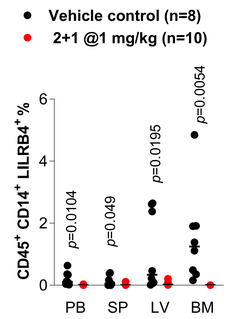



### 2+1 Has Acceptable PK Profiles in human FcRn Transgenic Mice




- 2+1 has exposure as expected in human FcRn transgenic mice for a typical hIgG1 with linear CL
- 2+1 has CL of 8.5 mL/day/kg and half-life of ~4.66 days in FcRn transgenic mice

## Bispecific 2+1 Inhibited AML Engraftment




NSG mice inoculated with PBMC from a M5 monocytic AML patient and treated with the Bispecific 2+1 or vehicle control starting at day 7 for a total of three doses. The mice were sacrificed at day 40

### Conclusions:

A novel bispecific LILRB4 x CD3 antibody with potent and specific killing of monocytic AML cells in vitro and in vivo has been identified. The desirable PK profile, strong CMC attributes, and cross-reactivity to non-human primate LILRB4 and CD3 favor this bispecific for rapid advancement to the clinic.

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**References**

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2. Samuel John, Heyu Chen, Mi Deng, Xun Gui, Guojin Wu, Weina Chen, Zunling Li, Ningyan Zhang, Zhiqiang An, Cheng Cheng Zhang. A Novel Anti-LILRB4 CAR-T Cell for the Treatment of Monocytic AML. 2018, Molecular Therapy, 26(10):2487-2495