A T cell engager (TCE), HB134, targeting LILRB4 and CD3, exhibits potent anti-tumor activity with a favorable safety profile for monocytic AML

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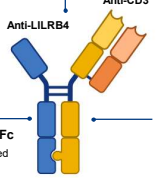
Introduction

- Monocytic leukemia, which encompasses acute myeloid leukemia (AML) subtypes M4 and M5, as well as chronic myelomonocytic leukemia (CMML), represents a heterogeneous group of malignancies characterized by a poor prognosis and limited therapeutic options[1,2].
- First-generation T cell engagers (TCEs) targeting AML-associated markers such as CD33 and CD123 lacked tumor specificity, resulting in limited clinical success[3].
- Leukocyte immunoglobulin-like receptor B4 (LILRB4), also known as the CD85K, ILT3, LIR5, and HM18, is a type I transmembrane glycoprotein belonging to the LILRB family. It features two extracellular immunoglobulin-like domains and three intracellular immunoreceptor tyrosine-based inhibition motifs (ITIMs)[4].
- LILRB4 is a monocyte-specific antigen, expressed exclusively on monocytes and monocyte-derived dendritic cells (DCs), and absent in other mature hematologic cells and non-hematologic normal tissues[5]. It is constitutively expressed during late-stage myeloid hematopoietic differentiation but absent on leukemia stem cells (LSCs) and hematopoietic stem and progenitor cells (HSPCs)[6]. This selective expression profile makes LILRB4 an attractive target for the treatment of AML and CMML.
- The anti-LILRB4 monoclonal antibody, IO-102, has demonstrated promising early responses and a favorable safety profile in CMML patients[7].
- Here, we report the development of HB134, a novel TCE consisting of one nanobody arm targeting LILRB4 and one Fab arm targeting CD3 in a 1:1 format, designed for the treatment of monocytic AML.

HB134 TCE design

Low CD3 binding activity, high LILRB4 binding activity
- Allows LILRB4-specific T cell engagement
- Avoids risk of non-specific T cell activation

Cross binding to CD3 and LILRB4 from human and cyno monkey
- Toxicity studies in cyno monkeys could predict safety risks in clinical trials



Effector-null human IgG Fc
- Avoids risk of FcγRs mediated non-specific T cell activation

Native IgG-like geometry with a LILRB4 targeting nanobody
- Highly manufacturable
- Favorable PK profile

Fig. 1. Schematic representation of HB134 molecule and its key design features.

HB134 binds specifically to LILRB4, but not to other members of the LILR family

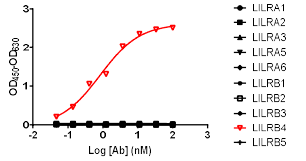
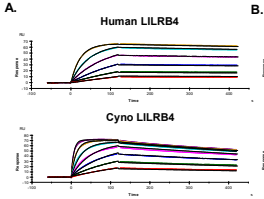


Fig. 2. Binding activity analysis of HB134 to LILR family members using ELISA. 2 μg/mL antigens were coated on a 96-well plate, three-fold serially diluted HB134 (0.14–100.00 nM) was analyzed.

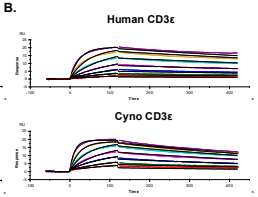
Binding profiles of HB134 to LILRB4 and CD3

HB134 cross-binds to LILRB4 and CD3 from human and cyno monkey

A.



B.



	ka (1/Ms)	kd (1/s)	KD (M)
Human LILRB4	4.16E+05	2.00E-04	4.81E-10
Cyno LILRB4	1.53E+05	9.38E-04	6.14E-09

	ka (1/Ms)	kd (1/s)	KD (M)
Human CD3ε	2.02E+05	8.74E-04	4.33E-09
Cyno CD3ε	4.02E+05	1.53E-03	3.80E-09

HB134 demonstrates high LILRB4 binding activity and low CD3 binding activity

C.

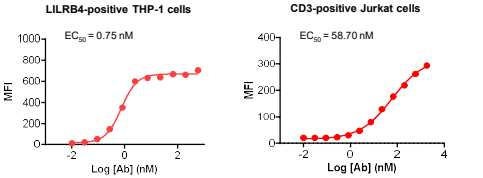
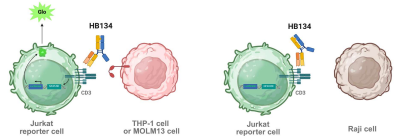


Fig. 3. Binding profiles of HB134. **A-B)** Affinity and kinetic characterization of HB134 binding to human LILRB4, cyno LILRB4, human CD3ε, and cyno CD3ε were assessed using a Biacore T200 instrument. **C)** Binding activity of HB134 to hLILRB4 and hCD3 was assessed by flow cytometry.

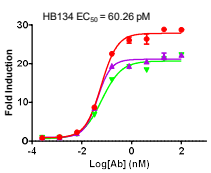
Functional analysis of HB134 using a luciferase reporter system

A.

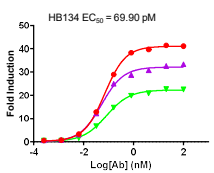


HB134 induces potent target-dependent T cell activation

B.



C.



HB134 Reference 1 Reference 2

HB134 induces T cell activation in a LILRB4-engagement-dependent manner

D.

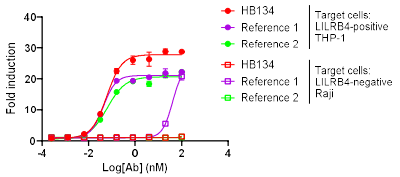
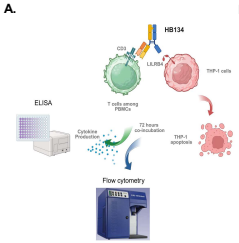


Fig. 4. Target-dependent T cell activation analysis using a luciferase reporter system. **A)** Schematic representation of the luciferase reporter system. **B–D)** T cell activation induced by HB134 in the presence of LILRB4-positive human AML cell lines THP-1 (A,C), MOLM13 (B), or in the presence of LILRB4-negative human B lymphoblastoid cell line Raji (C). The T cell activation activities are shown as fold induction of luciferase activity over PBS as blank control. Reference 1 and Reference 2 are TCEs targeting LILRB4 and CD3 in 2:1 and 1:1 formats, respectively, with their sequences sourced from patent CN115551894A.

HB134 induces potent cytotoxicity against LILRB4-positive THP-1 cells

A.



B. **Cytotoxicity and cytokines release**

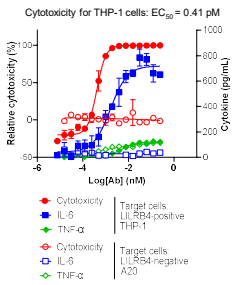


Fig. 5. Cytotoxicity and cytokines release analysis using a primary T cell-dependent cellular cytotoxicity (TDCC) assay. **A)** Schematic representation of the TDCC assay. **B)** TDCC activity of HB134 was evaluated against LILRB4-positive THP-1 cells and LILRB4-negative mouse B cell lymphoma A20 cells. Cytotoxicity, along with secreted IL-6 and TNF-α levels, was measured in parallel. Relative cytotoxicity (%) was calculated by normalizing the viable THP-1 or A20 cell count in the treated group to that in the untreated control.

HB134 exhibits a favorable pharmacokinetics (PK) profile in mice

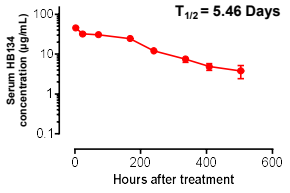


Fig. 6. Serum PK of HB134 in mice. Four BALB/c mice (6–8 weeks of age) were intravenously (IV) injected with a single dose of 5 mg/kg HB134. HB134 concentrations in serum were measured by ELISA.

HB134 demonstrates good efficacy and a favorable safety profile in mice

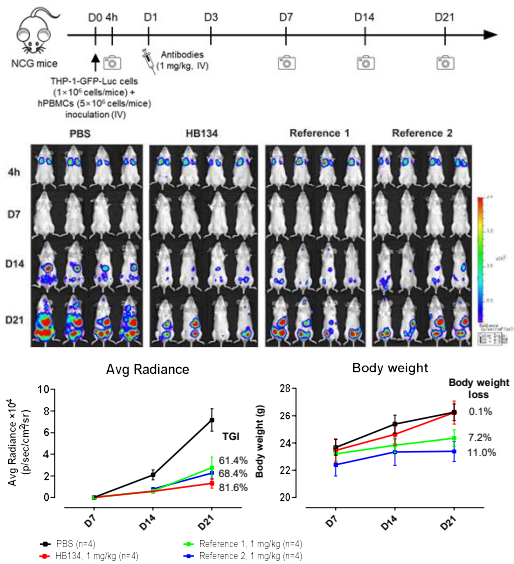


Fig. 7. Anti-tumor activity analysis of HB134 in a PBMC-engrafted mouse model. On Day 0, a mixture of 1×10^6 THP-1-Luc cells and 5×10^6 hPBMCs was IV injected into NCG mice (6–8 weeks of age). Mice were randomized into 3 groups (n=4/group) based on equivalent mean bioluminescence intensities measured 4 hours post injection using In Vivo Imaging System (PE lumina series 3). On Day 1, mice received a single IV dose of 1 mg/kg HB134, Reference 1, Reference 2 or vehicle control (PBS). Bioluminescence intensities were measured on Days 7, 14, and 21 to assess tumor growth inhibition (TGI).

Summary

- HB134 induces potent, target-dependent T cell activation and cytotoxicity against LILRB4-positive THP-1 cells.
- In a PBMC-engrafted mouse model of AML, HB134 demonstrates robust anti-tumor efficacy, achieving a TGI of 81.6% on Day 21 following a single IV dose of 1 mg/kg, while maintaining a favorable safety profile.
- HB134, a TCE designed to activate T cells via CD3 and selectively target LILRB4, represents a promising therapeutic candidate for the treatment of LILRB4-positive monocytic AML.

References

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