1391 ANTIBODY TARGETING A SPECIFIC EPITOPE OF LILRB4 INDUCES POTENT ADCC/ADCP EFFECT AGAINST LEUKEMIA CELLS

Ao Sun, Enlin Zheng, Mengshi Gao, Suya Bai, Peng Chen, Jay Mei, Bo Shan, Bing Hou*.
Antengene Corporation Limited, Shanghai, China

Background Acute myeloid leukemia (AML) is the most common acute leukemia in adults and the treatment of AML, especially monocytic AML subtypes still has a poor outcome. Therapies targeting CD33 and CD123 for the treatment of AML demonstrated hematological toxicity due to the distribution of the targets on hematopoietic stem cells (HSCs). Leukocyte immunoglobulin-like receptor B4 (LILRB4), an inhibitory receptor belonging to the LILR family, is highly expressed on monocytic M4/M5 AML and leukemic stem cells (LSCs), but not on normal HSCs.[1] Fc-mediated ADCC/ADCP activity is one of the driving mechanisms of action for therapeutic monoclonal antibodies (mAbs) targeting tumor-associated or specific antigen against malignancies. However, owing to the epitope and/or IgG4 Fc subtype, the ADCC/ADCP effect of current therapeutic mAbs targeting LILRB4 antigen on AML in the clinic is relatively weak.[2] Here we report a novel humanized LILRB4 antagonist antibody, ATG-034-S3, binding to a unique epitope that is distinct from the epitopes of other clinical benchmark LILRB4 antibodies, induces strong ADCC/ADCP effects, resulting in a potent anti-tumor efficacy *in vitro* and *in vivo*.

Methods The protein-based and cell-based binding affinity of ATG-034-S3 was measured using SPR, ELISA and FACS analysis. Competitive ELISA assay was used to evaluate the ability of ATG-034-S3 to block the interaction of LILRB4 with its ligand ApoE. ADCC assay was PBMC-mediated cytotoxicity and measured by FACS analysis. ADCP assay was M2 macrophage-mediated phagocytosis and measured using FACS analysis. T cell killing assay was $CD8^+$ T cell-mediated cytotoxicity measured by FACS analysis. The *in vivo* anti-tumor efficacy of ATG-034-S3 was evaluated in the EL4-LILRB4 syngeneic model.

Results Compared with clinical benchmark antibodies, ATG-034-S3 bound to a unique epitope on the human LILRB4 protein (figure 1A). It blocked the interaction between LILRB4 and ApoE and reversed THP-1 (AML)-mediated T cell suppression. ATG-034-S3 triggered strong ADCC and ADCP activity with EC50s of 1.13 nM and 0.22 nM, respectively (figure 1B,C,G), while clinical benchmark antibody demonstrated limited ADCC/ADCP effect. ATG-034-S3 potentiated $CD8^+$ T cell-mediated cytotoxicity (figure 1D,G), and significantly inhibited tumor growth in the EL4-LILRB4 syngeneic model *in vivo*. 3 mg/kg ATG-034 showed a TGI of 77%.

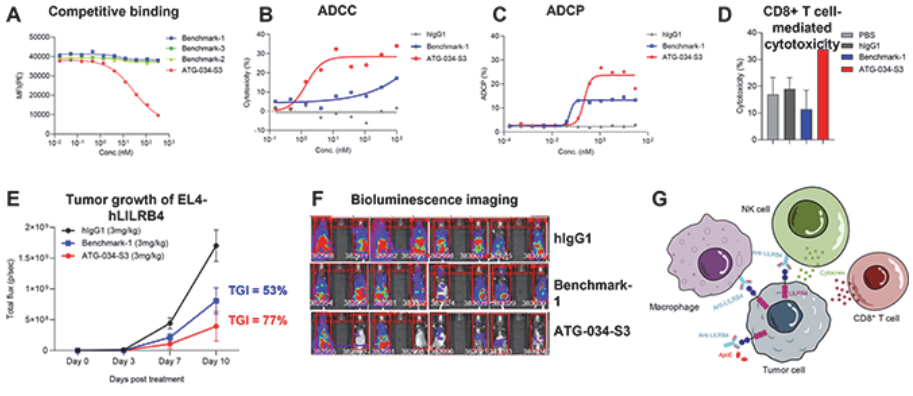
Conclusions Our data showed that ATG-034-S3 potentiates NK-mediated ADCC, macrophage-mediated ADCP, $CD8^+$ T cell-cytotoxicity and reverses AML-mediated T cell suppress, enhancing anti-tumor immunity (figure 1G), and demonstrates potent *in vivo* anti-tumor efficacy. ATG-034-S3 may be a promising strategy for the treatment of monocytic M4/M5 AML and other LILRB4 positive hematologic malignancies.

REFERENCES

1. Deng M, Gui X, Kim J, Xie L, Chen W, Li Z, He L, Chen Y, Chen H, Luo W, *et al*. LILRB4 signalling in leukaemia cells mediates T cell suppression and tumour infiltration. *Nature*. 2018;**562**:605–09.
2. Gui X, Deng M, Song H, Chen Y, Xie J, Li Z, He L, Huang F, Xu Y, Anami Y, *et al*. Disrupting LILRB4/APOE interaction by an efficacious humanized antibody reverses T-cell suppression and blocks AML development. *Cancer Immunol Res*. 2019;**7**(8):1244–1257.

Ethics Approval The protocol and any amendment(s) or procedures involving the care and use of animals in this study were reviewed and approved by the IACUC of CrownBio. All studies were conducted following an approved IACUC protocol. AUP NO.:2004–12-1465, 2004–12-1000; IACUC approval number: IACUC-2021-M-003

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1391



Abstract 1391 Figure 1 ATG-034-S3 shows significant antitumor effects in vitro and in vivo. (A) Competitive binding of labeled ATG-034-S3 to human LILRB4-expressing HEK293F cells in the presence of titrated unlabeled benchmark-1, benchmark-2 and benchmark-3 that were obtained from WO2020056077A1, WO2021127200A1 and US2019153093A1 patents, respectively. (B) CFSE labeled THP-1 cells as target cells were seeded to 96 wells Flat-bottom sterile plate, then freshly isolated human PBMCs were added as effector cell. Antibody concentration is 0.1 to 1000 nM with three-fold serial dilution. The E:T ratio is 6:1. (C) ADCP was performed with human PBMC- derived M2 macrophage. Macrophages were stained with Far-Red and target MV-4-11 cells were stained CFSE. Antibody concentration is 0.0002 to 30 nM with three-fold serial dilution. The E:T ratio is 1:1. (D) ATG-034-S3 potentiates CD8+ T cell-mediated cytotoxicity. CFSE-labeled THP-1 and CD8+ T cells were cocultured at a 20:1 ratio with 20 Âµg/mL ATG-034-S3 for 18 hours. THP-1 cells were counted as CFSE+ cells, and T cell-mediated cytotoxicity was calculated as the decrease of THP-1 cell number. (E) ATG-034-S3 inhibits tumor growth in the EL4-LILRB4 models. The tumor measurement data are presented as mean ± SEM (n = 8). EL4-LILRB4 tumor-bearing human LILRB4 transgenic mice were treated with 3 mg/kg antibodies, BIW. (F) Bioluminescence imaging on day 10 in E. (G) Mechanism of action of LILRB4 antibody ATG-034-S3. ATG-034-S3 potentiates NK-mediated ADCC, macrophage-mediated ADCP, CD8+ T cell-cytotoxicity and reverses AML-mediated T cell suppress, enhancing anti-tumor immunity