ATG-031, A FIRST-IN-CLASS ANTI-CD24 ANTIBODY, SHOWED POTENT PRECLINICAL ANTI-TUMOR EFFICACY BY BLOCKING "DON'T-EAT-ME" SIGNAL

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Background By overexpressing anti-phagocytic surface proteins, often known as "don't eat me" signals, cancer cells can evade macrophage-mediated elimination. Therapeutic antibodies targeting "don't eat me" protein, such as CD47, demonstrates promising anti-tumor efficacy in preclinical models and in clinic. However, the clinical development of anti-CD47 mAbs that retain substantial FcR activating capacity (e.g. human IgG1) has been hampered by the on-target-off-tumor toxicity, such as erythrocyte depletion.1 CD24, a GPI-anchored, highly glycosylated surface protein interacting with Siglec-10 on innate immune cells, was reported to be a novel "don’t eat me" protein. CD24 is over-expressed in multiple tumor types. Knock-out of CD24 or blockade of CD24/Siglec-10 interaction enhances macrophage-mediated phagocytosis of tumor cells.2

In this study, we developed a first-in-class, humanized anti-CD24 antibody, ATG-031. The in vitro and in vivo anti-tumor activity of ATG-031 was evaluated in preclinical models.

Methods The affinity of ATG-031 was measured using Surface Plasmon Resonance (SPR). Cell-based binding to HEK293, HEK293-CD24, human red blood cell (hRBC) and a panel of tumor cells was evaluated by flow cytometry. The ability of ATG-031 in enhancing macrophage-mediated phagocytosis of tumor cells was evaluated using human monocyte-derived M2 macrophage. in vivo efficacy of ATG-031 was test in mouse bearing MC38 murine syngeneic colorectal cancer cell stable-expressing human CD24 (MC38-hCD24). Expression profile of CD24 in human tumor and normal tissues were analyzed using TCGA/GTEx database or using an in-house developed companion diagnostic antibody on tissue microarray (TMA) by IHC staining.

Results CD24 is overexpressed in multiple types of solid tumors and hematological malignancies. ATG-031 specifically binds to human CD24 with a single-digit nM affinity. ATG-031 potently binds to CD24-positive tumor cells, while showed no binding with parental HEK293 cells or hRBC. ATG-031 blocks the interaction between CD24 and Siglec-10 and induced potent macrophage-dependent tumor cell phagocytosis (figure 1A, B). Upon phagocytosis, M2 macrophages start to release M1-like cytokines suggesting a repolarization from M2 to M1. ATG-031 significantly inhibited in vivo tumor growth in MC38-hCD24 mouse model. A dose of 3mg/kg ATG-031 administered biweekly induced tumor regression (figure 1C).

Conclusions Blocking CD24 by ATG-031 enhances macrophage-mediated phagocytosis of cancer cells, and polarized M2 macrophage towards anti-tumor M1 subtype (figure 2). It demonstrates potent in vitro anti-tumor tumor efficacy, suggesting promising therapeutic strategies against a broad range of solid tumor or hematological malignancies, which warrants further clinical investigation. A clinical study to investigate the safety and efficacy of ATG-031 in cancer patients is being developed.

REFERENCES

Ethics Approval This study was performed in strict accordance with institutional guidelines and approved by the Institutional Animal Care and Use Committee of the Shanghai Model Organisms Center, Inc. and the IACUC permit number was 2019-0011.

Abstract 482 Figure 1 ATG-031 showed potent anti-tumor efficacy Immunofluorescence imaging of macrophage (green) mediated tumor cell (Nalm-6, red) induced by ATG-031 or IgG control. (B) Phagocytic index of macrophage. Monocyte-derived M2 macrophages were co-cultured with NALM-6 cells and phagocytosis was induced by ATG-031 or anti-CD47 (5F9). (C) Average tumor growth curves of mouse bearing MC38-hCD24 tumor cell. The mice were treated with 1mg/kg or 3mg/kg ATG-031. 3mg/kg of ATG-031 induced complete remission of tumor in 4 out of 8 mice. n=8 per group. CR, complete remission

Abstract 482 Figure 2 Schematic cartoon of the mechanism of action for ATG-031. Tumor associated macrophages were repolarized to pro-inflammation status when CD24/SigLec-10 interaction was blocked by ATG-031