AK132, an asymmetric Claudin18.2/CD47 bispecific antibody for cancer immunotherapy

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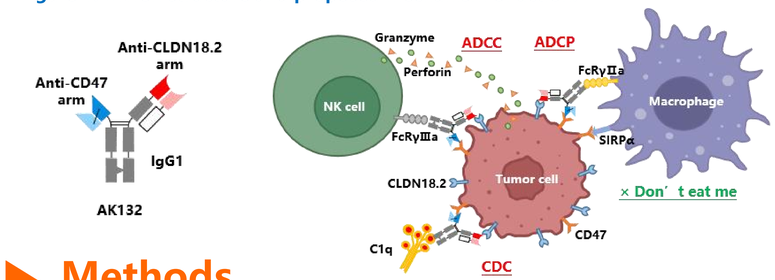
Introduction

CD47 is overexpressed on many types of cancer cells, and it can interact with its ligand SIPRα on innate immune cells and inhibit tumor phagocytosis. Although CD47 is considered as a promising target for cancer immunotherapy, the therapeutic utility of CD47 monoclonal antibodies is largely compromised due to significant red blood cell (RBCs) toxicities, as CD47 is also expressed on peripheral blood cells. To overcome these limitations and further improve therapeutic efficacy, we designed AK132, an asymmetric monovalent bispecific antibody, Claudin18.2 (CLDN18.2)/CD47, with a wild type IgG1 Fc. Claudin18.2 is a tight junction protein that has been identified as a valuable target in gastric and pancreatic cancers. AK132 aims to promote the phagocytosis of CLDN18.2+ tumor cells by attenuating CD47-SIPRα interaction, and further to enhance the tumor cell killing through Fc effector functions.

Figure 1. AK132 structure and proposed mechanism of action.

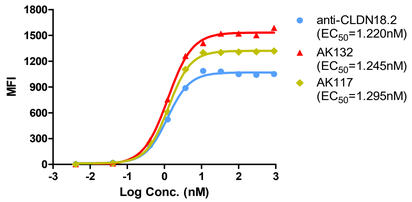


Methods

Binding activity of AK132 to CD47 or CLDN18.2 on tumor cells and transfected cells were assessed by flow cytometry. Effector functions of AK132 on tumor cells were measured in ADCC (antibody-dependent cell cytotoxicity), ADCP (antibody-dependent cell phagocytosis) and CDC (complement-dependent cytotoxicity) assays. To evaluate RBCs toxicities, we measured the binding activity, ADCC, ADCP effects of AK132 on RBCs, as well as RBCs hemagglutination. The anti-tumor activity of AK132 was investigated in C57BL/6 mice implanted with MC38-CD47-CLDN18.2 cells (a mouse colon carcinoma cell line that is engineered to express human CD47 and CLDN18.2). Mice were treated with Isotype control antibody, AK117 (a bivalent CD47 antibody with IgG4 backbone; 20 mg/kg), anti-CLDN8.2 (a bivalent CLDN18.2 antibody with IgG1 backbone; 20 mg/kg) and AK132 (1.9 mg/kg; 5.7 mg/kg; 17 mg/kg) via i.p. injection. The tumor volume and body weight were measured.

Results

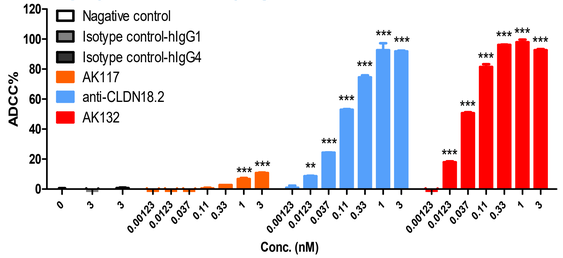
Figure 1. AK132 binds to CHO-K1-CLDN18.2/CD47 cells.



The binding activity of AK132, anti-CLDN18.2 and AK117 to CHO-K1 cells which transfected with CLDN18.2 and CD47 were detected by FACS. MFI, mean fluorescent intensity. EC_{50} were indicated.

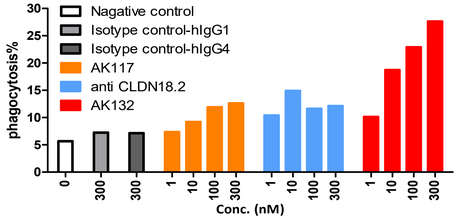
Results

Figure 2. AK132 displays ADCC activity against CHO-K1-CLDN18.2/CD47 cells.



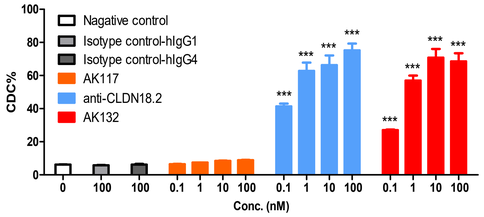
*CHO-K1-CLDN18.2/CD47 cells were served as target cells, and the human PBMCs were used as effector cells. Data shown as mean with SD for n = 2, and One-way ANOVA analysis was performed. Compared with the isotype control, ***p < 0.001, **p < 0.01.*

Figure 3. AK132 shows enhanced ADCP activity against KATOIII-CLDN18.2 tumor cells.



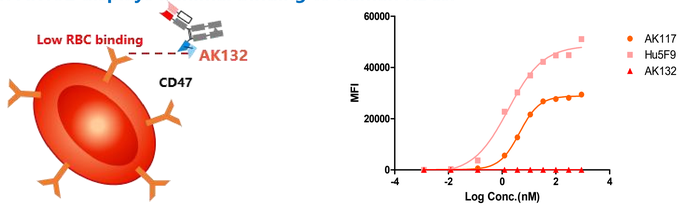
KATOIII-CLDN18.2/CD47 tumor cells were co-cultured with human monocytes-derived macrophages in the presence of indicated antibodies and the percentage of macrophages that engulfed tumor cells was measured by FACS.

Figure 4. AK132 has CDC activity against CHO-K1-CLDN18.2/CD47 cells.



*CHO-K1-CLDN18.2/CD47 cells were served as target cells, and the human serum was used as the source of complements. Data shown as mean with SD for n = 3, and One-way ANOVA analysis was performed. Compared with the isotype control, ***p < 0.001.*

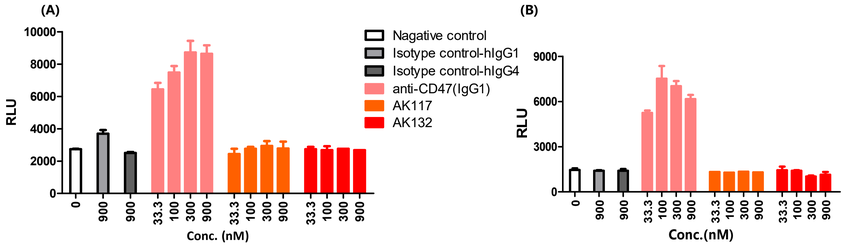
Figure 5. AK132 displays minimal binding to human RBCs.



The binding activity of AK132, Hu5F9 and AK117 to fresh human RBCS were detected by FACS. MFI, mean fluorescent intensity. RBCs, red blood cells

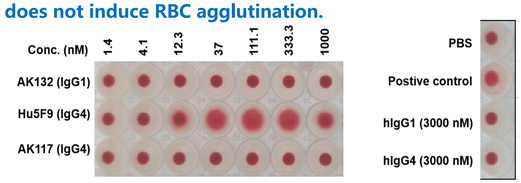
Results

Figure 6. AK132 does not promote ADCP and ADCC activity against human RBCs.



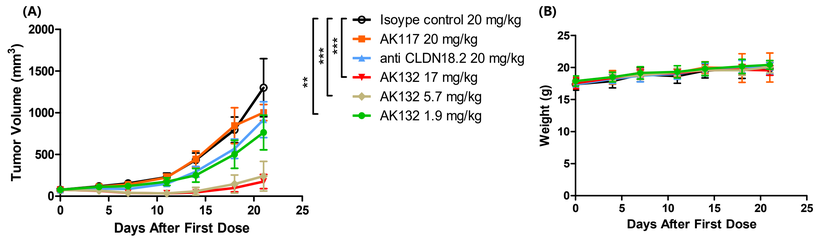
(A) ADCP of Jurkat-NFAT-CD64-CD32R cells against human RBCs in response to increasing concentrations of indicated antibodies were measured by reporter assay. (B) ADCC of Jurkat-NFAT-CD16 cells against human RBCs in response to increasing concentrations of indicated antibodies were measured by reporter assay. Anti-CD47 (IgG1) is AK117 with IgG1 backbone. Data shown as mean with SD for n = 3.

Figure 7. AK132 does not induce RBC agglutination.



50 μL RBCs suspension were mixed with an equal volume of antibodies serially diluted by threefold in normal saline in a 96-well plate and RBC aggregation was observed after incubation at room temperature for 2 h. Dextran T500 was used as positive control (right panel).

Figure 8. AK132 efficiently inhibits tumor growth in mice with subcutaneous MC38-hCD47hCLDN18.2 tumor.



*MC38-hCD47hCLDN18.2 tumor bearing mice were treated with different doses of AK132, anti-CLDN18.2, AK117 or Isotype control (anti-HEL) (n=6). The tumor volume (A) and body weight (B) of mice per group (n=6) were measured over time. Data shown as mean with SD, and One-way ANOVA analysis and Bonferron post-hoc comparisons were performed. Compared with the isotype control, ***p < 0.001, **p < 0.01.*

Conclusion

AK132, a CLDN18.2/CD47 monovalent bispecific antibody, has ADCC, ADCP and CDC effects on CLDN18.2+/CD47+ cells but not on RBCs. It shows great anti-tumor efficacy without RBC toxicities, supporting its clinical development for the treatment of human cancers.

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