Supplemental Methods

Binding activity to CD47

Antibody-antigen binding activity was measured by ELISA and FACS. For ELISA binding activity detection, gradient diluted antibodies were added into 96-well plates precoated with CD47-IgV-TEV-His for 30min incubation at 37℃. Plates were washed 3 rounds with PBST (PBS with 0.05% Tween 20) and incubated with HRP conjugated Goat anti Human IgG (H+L) secondary antibody at 37℃ for 30 min. Plates were washed 3 rounds and incubated with TMB substrate at 25℃ for 10min, followed by adding stop buffer (2M H_2SO_4). OD values were read at 450nM with Molecular Devices and data were calculated using SoftMax Pro 6.2.1.

For FACS binding activity detection, gradient diluted antibodies were incubated with Jurkat or Raji cells for 40min at 4℃. Cells were washed with precool (4℃) PBSA (1% BSA in PBS) 3 times, followed incubation with APC labeled mouse antiHuman IgG Fc (Biolegend) for 30min at 4℃. Cells were subsequently washed three times and analyzed using FACSCalibur. Data were calculated using Flowjo and analyzed using Prism Graphpad.

Blockade of SIRPα to CD47

Blocking activity of AK117 to CD47/SIRPα was determined by competitive ELISA and FACS.

Competitive ELISA were carried out as follow: AK117 incubated with CD47 precoated on 96-well plates. Plates were washed, incubated with 0.469nM SIRPα ECD-hFc-bio, washed and incubated with HRP conjugated streptavidin, washed and incubated with TMB substrate, followed by adding stop buffer (2M H_2SO_4). OD values were read at 450nM with Molecular Devices and data were calculated with SoftMax Pro 6.2.1.

Competitive FACS binding were carried out as follow: AK117 incubated with Jurkat

or Raji tumor cells. Cells were washed and incubated with 30nM SIRPα ECD-hFc-bio, washed and stained with PE labeled streptavidin, washed and analyzed using FACSCalibur. Data were calculated using Flowjo and analyzed using Prism Graphpad.

Modeling and docking

Based on the sequence of AK117, the Fab AK117 was constructed, the FR and the CDRs residues were identified by IgFold[1] and the whole sequences were renumbered by ANARCI after uploading the sequences of AK117 light chain and heavy chain. IgFold predicted AK117 structure by deep learning and returned a predicted RMSD to evaluate the modeled structure. The structural data of Hu5F9-G4 Fv diabody in complex with CD47-ECD (PDB ID: 5IWL) were used and one Fv with antigen binding was separated by PyMOL.[2] The Fab Hu5F9-G4 was completed by grafting the missing Fab residues into Hu5F9-G4 Fv and modeling with IgFold, and the modeled Fv part coincided with PDB file.

The protein-protein docking script, "light docking", was used to analyze the potential epitopes on CD47 using PyMOL.[3] CD47-ECD (PDB ID: 5IWL) was set as the antigen, the binding site of AK117 antibody was restrained in CDRs residues and 100,000 times docking was performed. All docking results were collected, and the interacting residues of CD47-ECD were recorded. The frequency of each residues combination was statistically counted and the stability of antigen-antibody complex they formed was calculated. The residues combination with highest frequency and acceptable stability was defined as the binding epitopes of AK117 on CD47-ECD. The docking system was tested by simulating Hu5F9-G4 to recognize the CD47-ECD, and the top residues combination of L39/E97/T99/L101/T102/E104 was selected, which was consistent with the PDB structure file description.

CD47-Transmembrane domain (TMD) modeling

The simulated structural data of AK117 and Fab Hu5F9-G4 in complex with

CD47-ECD were extracted by PyMOL. The PDB file 7MYZ was chosen as CD47 structure framework, which was the whole-length CD47 in complex with an antibody Fab. The CD47-TMD structure was built for the antibody-antigen complex files. In Gustavo's research, this whole-length CD47 was determined by Lipidic cubic phase to simulate the conformation of CD47 anchored on the cytomembrane. There was a 80 degrees angle between CD47 ECD and TMD. It was reported that Gustavo also used molecular dynamics to simulate the flexibility of the relative position of the ECD and TMD domains, suggesting that the CD47 has different conformational states when anchored on cytomembrane.[4] The crystalline structure was used as the antigen modeling framework in this study.

Conformational comparison of antibody-antigen complexes

For the construction of whole-length IgG4 antibody, the structure file of PDB:5DK3 was chosen as the frame structure of the hinge and Fc region. The conserved parts (CL and CH1) in AK117 and Hu5F9-G4 Fab-antigen structure were superposed to the corresponding parts in IgG4 structure, the original Fab of IgG4 was deleted, and AK117 and Hu5F9-G4 were linked with the hinge and Fc regions of IgG4.

The antigen part in Fab-antigen simulated structure was also superposed to IgG4 antibody to present the conformation of the whole antibody binding multiple antigens. CD47 was roughly defined as a bar-shaped protein linking N and C terminals, and two Fab and Fc regions of IgG4 formed a plane. The angle and the distance between two bound CD47 proteins were determined. The angle between two antigens was defined as the angle between N-C terminals lines on the IgG4 antibody plane, and the distance was the spacing between two C15 residues in CD47-ECD.

Supplemental References:

1. Ruffolo JA, Gray JJ. Fast, accurate antibody structure prediction from deep learning on massive set of natural antibodies. *Biophys J* 2022;121(Suppl 1):P155a-156a.

2. Schrödinger L & DeLano W, 2020. PyMOL, Version 4.6.0, Available at: http://www.pymol.org/pymol. Accessed in August 2022.

3. Jiménez-García B, Roel-Touris J, Romero-Durana M, et al. LightDock: a new multi-scale approach to protein-protein docking. *Bioinformatics* 2018;34:49-55.

4. Fenalti G, Villanueva N, Griffith M, et al. Structure of the human marker of self 5-transmembrane receptor CD47. *Nat Commun* 2021;12:5218.