A differentiated CD47 therapeutic antibody recognizing a novel epitope and sparing erythrocytes and platelets

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ABSTRACT

Tumor cells overexpress CD47 which engages signal-regulatory protein (SIRPα) on macrophages (mϕ) to deliver a "do-not-eat" signal to avoid being phagocytosed. Blocking CD47 using SIRPα-Fc or anti-CD47 antibodies (Ab) has emerged as a promising strategy to neutralize CD47 and promote tumor eradication. However, CD47 is also expressed on red blood cells (RBC) and platelets (PLT) which can act as a large Ab sink. Targeting CD47 also led to anemia and thrombocytopenia in animal studies and phase I trials, which is of serious concern. Here we report the discovery of a new CD47 Ab (TJ-C4) with a novel epitope that endows it with enhanced phagocytic and RBC-sparing properties, thus differentiating itself from current CD47-targeting therapies.

MATERIALS AND METHODS

Antibody generation. A naïve human PBMC scFv phage library was subjected to several rounds of solution panning for binding to biotinylated recombinant human CD47 extracellular domain (ECD). All binders with unique VH and VL sequences were converted to full IgG1 antibody and screened through a series of functional assays. A reference antibody 5F9 was prepared based on sequence information from Forty-seven patent and was included in all experiments described below.

Affinity determination by BIAcore. mAbs were captured on CM5 sensor chip via anti-IgG-Fc. The CD47 ECD monomeric proteins were serially titrated from 50 nM down to 1.6 nM and injected for 3 min. The dissociation was monitored for 10 min. Data were fitted to 1:1 Langmuir binding model.

***In vitro* phagocytosis assay.** Raji cells or primary AML patient cells were CFSE-labeled and incubated with PBMC-derived mϕs in the presence of anti-CD47 or control antibodies for 3 h at 37 °C. Phagocytosis was measured by flow cytometry (FACS) by gating on $CD14^+$ mϕs and then assessing the percentages of $CFSE^+$ cells.

***In vitro* human RBC binding.** Purified RBCs were incubated with anti-CD47 or control antibodies for 1 h at 4 °C followed by addition of fluorochrome-conjugated secondary antibodies. Binding of CD47 antibodies to RBCs was analyzed by FACS.

***In vivo* antibody treatment of Raji xenograft (CDX) or liver cancer patient derived xenograft (PDX) in mice.** NSG mice were engrafted with 10^6 luciferase-labelled Raji cells by i.v. injection. Treatment of CD47 antibodies started from 5 days post engraftment at a dose of 10 mg/kg every other day i.p. (n=6/group). All mice were imaged *in vivo* for tumor growth by IVIS Lumina III imaging system. For PDX model, NOD-SCID mice were transplanted s.c. with liver tumor samples. When engrafted tumors grew to 100-300 mm^3, mice were randomized to receive treatment of anti-CD47 or control antibodies at a dose of 10 mg/kg every other day i.v. (n=5/group). Tumor growth was monitored by tumor dimension measurements.

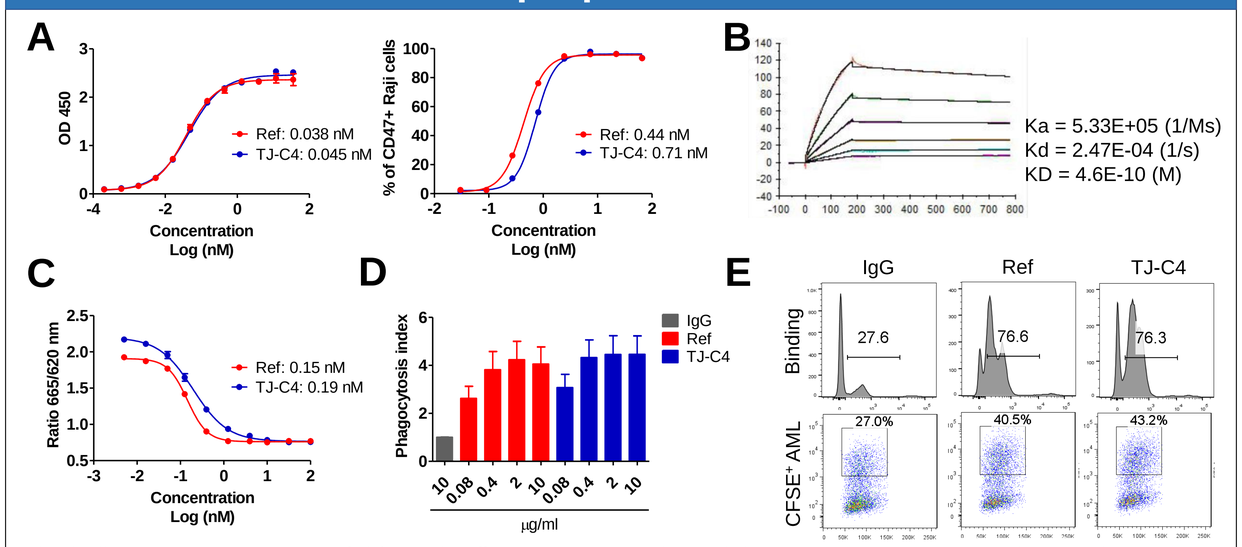
Hematology in non-human primates. Naive cynomolgus monkeys were given a single 1-hour i.v. infusion of 15 mg/kg of CD47 mAb or PBS control (3 animals/group) on Day 1. Peripheral blood was withdrawn at pre-dose Days 7 and 2, and at Days 3, 6, 10, 14 and 21 post administration. A complete CBC panel was examined.

Epitope binning of anti-CD47 antibodies. CD47 protein and first anti-CD47 mAb were pre-incubated followed by the addition of a second biotinylated anti-CD47 mAb. Antibodies were deemed to occupy the same or overlapping epitope bin if the biotinylation signal was reduced.

Crystallization and structure determination. CD47-ECD/TJ-C4 complex was co-crystallized by combining 1 μl protein solution with 1 μl precipitation solution. Crystallographic data were collected at beamline BL19U1, Shanghai Synchrotron Radiation Facility at a resolution of 2.7 Å.

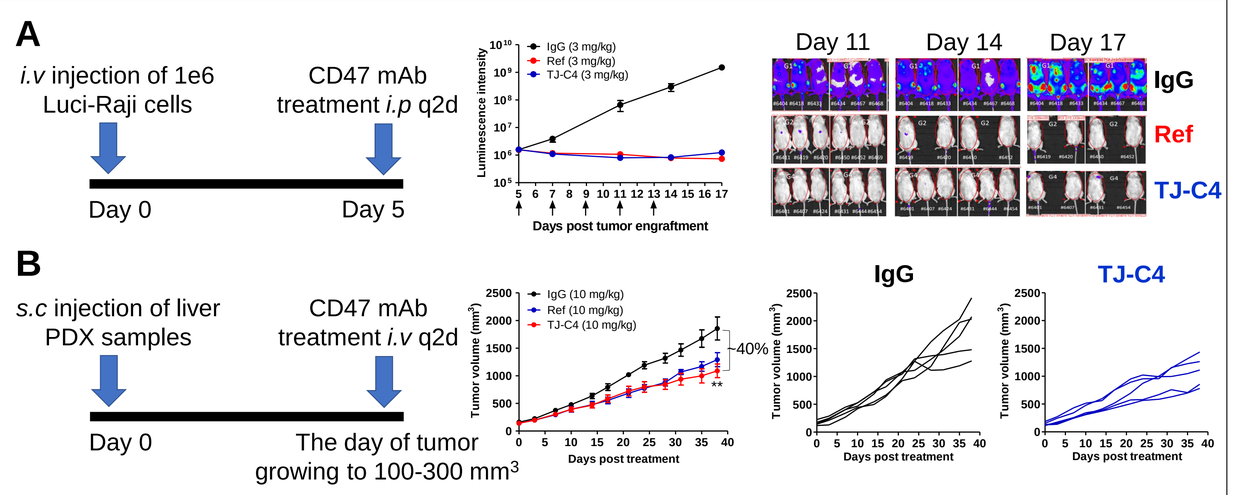
RESULTS

General properties of TJ-C4



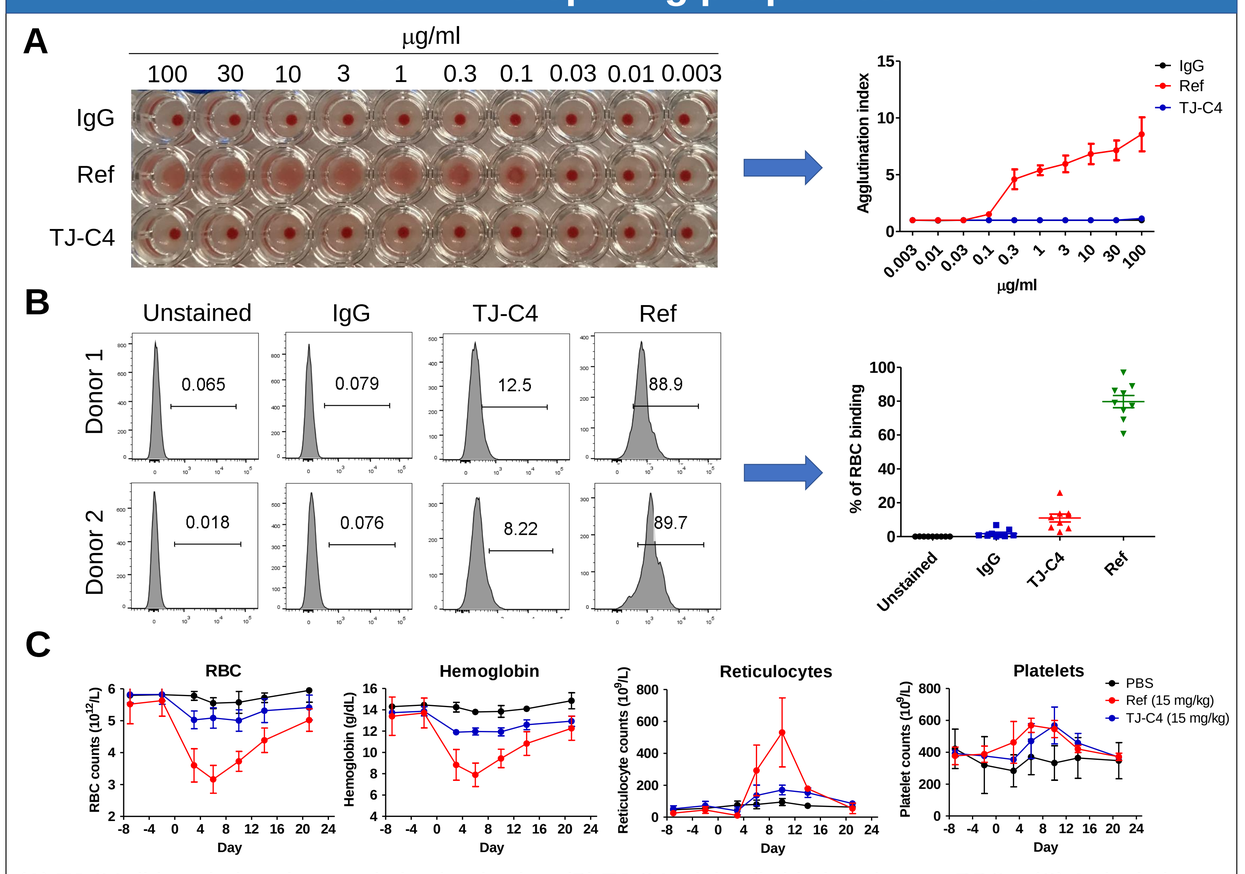
(A) Binding of CD47 mAb to soluble and cell surface CD47. (B) Affinity of TJ-C4 to CD47 by Biacore. (C) Competition of CD47 binding with SIRPα by CD47 mAb. (D) Phagocytosis of Raji cells by human MΦ in response to CD47 mAb. (E) Binding and phagocytosis of primary AML cells by CD47 mAb at 10 μg/ml.

Anti-tumor activity of TJ-C4 in Raji B cell lymphoma xenograft and liver cancer patient derived xenograft model



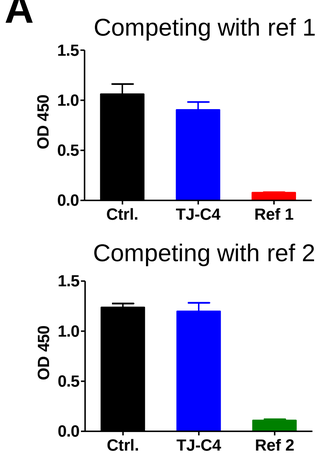
TJ-C4 anti-CD47 mAb treatment completely eliminated the tumor cells in Raji B cell lymphoma xenograft model (A). TJ-C4 anti-CD47 mAb demonstrated significant efficacy in liver PDX model (B).

Differentiated RBC sparing properties of TJ-C4



(A) TJ-C4 did not induce hemagglutination *in vitro*. (B) TJ-C4 minimally binds to human RBCs. (C) A single i.v. dose of TJ-C4 at 15 mg/kg did not alter hematological parameters throughout the study in contrast with a ref. Ab.

An unique binding epitope of TJ-C4 in complex with CD47



B

	TJ-C4	Ref 1	Description
Structure of mAb/CD47	TJ-C4 CD47	Ref CD47	The structure of TJ-C4/CD47 complex adopts straighter head to head orientation, unlike the structure of ref/CD47 complex (PDB 5iwl) presenting tilted head to head orientation.
Binding epitope on CD47	R103 L101 T26 E35 T34 Q31 L74	Hu5F9-G4 CD47 *JCI 2016.126(7):2610*	The epitope of TJ-C4 on CD47 is conformationally discontinuous and includes a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103) of CD47.

(A) Epitope binning of TJ-C4 with ref Ab 1 or 2. (B) Crystal structure and epitope regions of TJ-C4/CD47 complex.

CONCLUSIONS

- TJ-C4 is an affinity-matured, sub-nanomolar, fully human, CD47 blocking mAb that strongly competes with SIRPα binding and promotes mϕ phagocytosis.
- TJ-C4 demonstrated significant efficacy in the complete eradication of tumor cells in the Raji CDX and reduction of tumor growth in the liver PDX model.
- TJ-C4 is endowed with a differentiated RBC-sparing property with minimal RBC-binding and no agglutination *in vitro*, and no RBC-depletion *in vivo*.
- TJ-C4 binds a novel epitope on CD47 with a unique orientation distinct from all current CD47 antibodies. A series of patents have been filed around TJ-C4, its unique properties and the novel epitope.
- TJ-C4 has good drug-like properties and the molecule is at CMC/preclinical stage of development.