TJC4, A Differentiated RBC Sparing Anti-CD47 Antibody for Anti-Cancer Therapy

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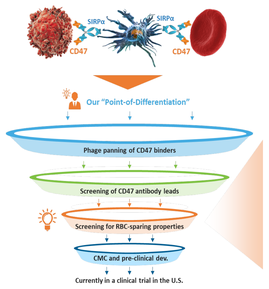
ASH 2019 #4063

ABSTRACT

Blocking CD47 using anti-CD47 antibodies (Ab) to block the “do not eat” signal expressed by tumor cells has emerged as a promising strategy. However, significant anemia and thrombocytopenia were observed in pre-clinical studies and early clinical trials, as CD47 is also expressed on normal red blood cells (RBCs) and platelets.

Here we reported the development of a novel anti-CD47 antibody TJC4, endowed with an RBC sparing property and unique binding epitope through special selection process. TJC4 had minimal binding to RBCs without inducing hemagglutination, and showed minimal and transient impact on RBCs following single or repeated administrations in monkeys. Crystal structural analysis revealed the unique binding epitope of TJC4 which is hypothesized to be shielded by a nearby N-linked glycosylation on RBCs to prevent TJC4 binding. In brief, our data demonstrated that TJC4 is differentiated from other CD47 targeting agents that is devoid of the hematological liabilities while maintaining anti-tumor properties.

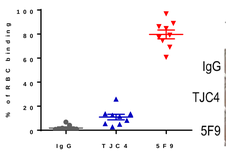
TJC4: A differentiated anti-CD47 antibody



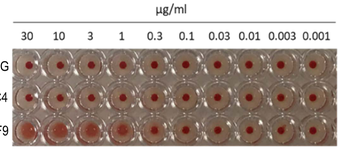
- ❖Fully human IgG4 monoclonal antibody;
- ❖Blocks CD47 and SIRPα interaction to enhance tumor phagocytosis;
- ❖Anti-tumor efficacy in tumor xenograft model as mono- or combination therapy;
- ❖Minimal binding to normal RBCs, and no RBC agglutination;
- ❖Well tolerated in cynomolgus monkeys following repeated administration up to 100 mg/kg;
- ❖Unique binding epitope leading to the RBC sparing property.

TJC4 is endowed with a unique binding epitope and RBC sparing property

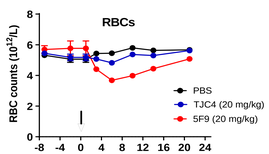
A. In vitro RBC binding



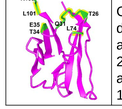
B. RBC agglutination



C. In vivo RBC accounts



D. TJC4 epitope

TJC4	Description
	The epitope of TJC4 on CD47 is conformationally discontinuous and includes a TNMEAQ loop (residues 26-31), T34, E35, L74, and an LTR hinge (residues 101-103).

E. Binding of TJC4 with deglycosylated RBCs

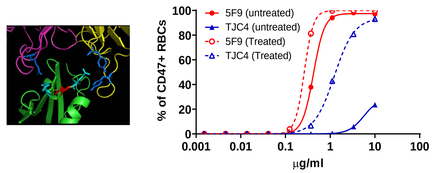


Figure 1. (A) Minimal binding of TJC4 to human RBCs as compared with the reference antibody 5F9**. (B)** *In vitro* RBC agglutination assay showing that TJC4 did not induce hemagglutination**. (C)** Single injection of TJC4 or 5F9 at 20 mg/kg was administered to cynomolgus monkeys. As compared with apparent reduction of RBCs induced by 5F9,TJC4 showed no apparent impact on RBCs. **(D)** Identification of TJC4 epitope by crystal structural analysis. **(E)** Crystal structural analysis demonstrated a N-linked glycosylation site located nearby the epitope of TJC4. Deglycosylation of RBCs by PNGase treatment can increase the binding of TJC4**.**

TJC4 showed good safety in cynomolgus monkeys without inducing anemia

4-Week GLP toxicity study of TJC4 in cynomolgus monkeys

- QW IV infusion of TJC4 for 4 weeks (5 doses) at 10, 30 and 100 mg/kg; NOAEL was 100 mg/kg
- No noteworthy toxicity; the only finding was transient and mild local effect at the injection sites
- No anemia or impact on thrombocytes; RBC decrease was mild and reversible over treatment

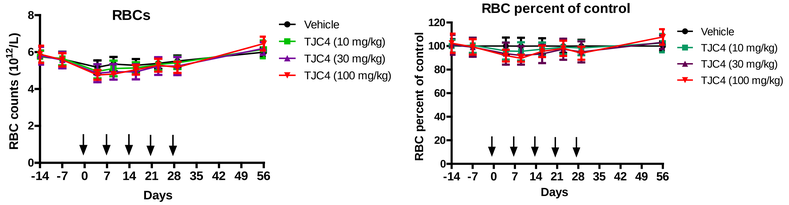
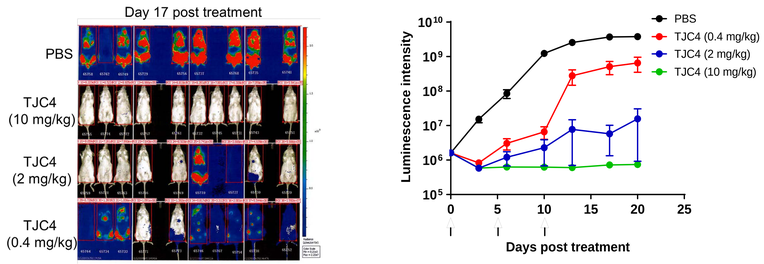


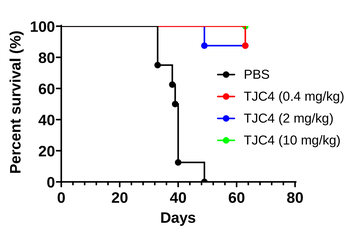
Figure 2. Cynomolgus monkeys received QW IV infusion of TJC4 at 10, 30, and 100 mg/kg for 4 weeks, The blood was collected on Day 4 after 1st dosing, the next day following 2nd, 3rd, 4th and 5th dosing, and one day before the recovery necropsy, respectively, for hematology analysis. Minimal decrease in RBCs(left) and hemoglobin (not shown) was observed following 1st dosing, and reversed from 2nd or 3rd dosing; the right graph showed RBC percentage v.s. control to rule out procedure-related impact on RBCs.

TJC4 inhibits hematological tumor growth *in vivo*

A. Raji lymphoma model



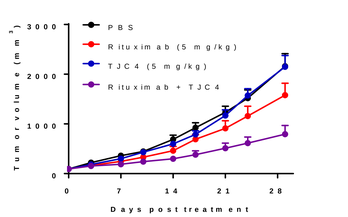
B. HL60 leukemia model



Group	Treatment	MST (days)	ILS (%)	p value
1	Vehicle control	39 a(33~49)	–	–
2	TJC4 (0.4 mg/kg)	>63 (63~>63)	>61.5	b<0.0001
3	TJC4 (2 mg/kg)	>61 (49~>63)	>57.1	b<0.0001
4	TJC4 (10 mg/kg)	>63	>61.5	b<0.0001

MST: Median survival time; ILS: in life-span
a: The range of survival times;
Statistics: Kaplan-Meier method for MST

C. WSU-DLCL2 DLBCL model



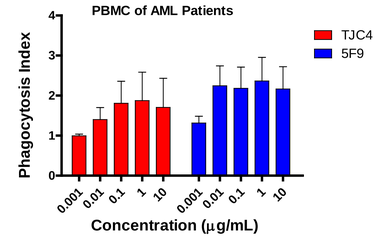
Group	Treatment	Tumor Size (mm3)a on day43	TGI (%)	T/C (%)	P value
1	Vehicle	2161.32±252.88	-	-	-
2	Rituximab 5mg/kg	1578.37±241.53	27	73	0.376
3	TJC4 5mg/kg	2148.89±227.87	1	99	1.000
4	Rituximab+ TJC4 5mg/kg+5mg/kg	794.9±174.05	63	37	0.004

Statistics: One-way ANOVA analysis for tumor volume

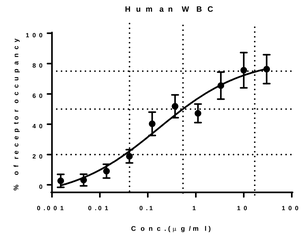
Figure 3. (A) NSG mice transplanted intravenously with Raji-luciferase cells were treated with different dosages of TJC4 on days 0, 5 and 10 (n=10/group). Luciferase imaging of the mice at day 17 post treatment were shown (left panel) and the bioluminescence values throughout the study were analyzed (right panel). **(B)** NSG mice transplanted intravenously with HL60 cells were treated with TJC4 five days per dose (n=10/group). The survival rate of the treated mice were analyzed. **(C)** NOD/SCID mice were subcutaneously injected with WSU-DLCL2 human diffuse large B cell lymphoma cells and treated with TJC4 as a single agent and in combination with Rituximab twice a week.

PK/PD modeling of TJC4 for FIH dose estimation

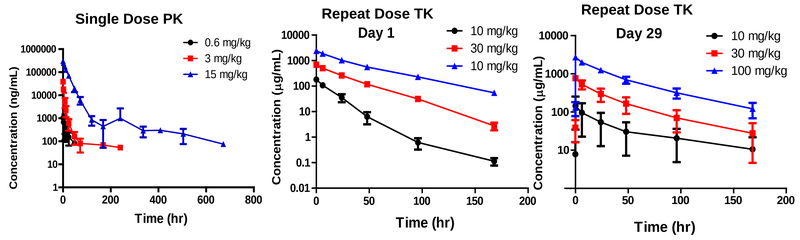
A. Phagocytosis of AML PBMCs by TJC4



B. Receptor occupancy in human PBMCs

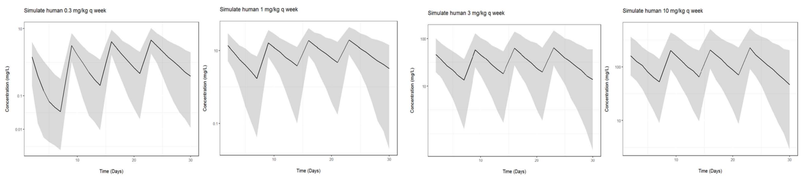


C. PK and TK profiles in cynomolgus monkeys



D. Simulated concentration-time profiles for TJC4 in humans

- o Weekly dosing at least for the 1 mg/kg dose is justified.
- o Weekly doses of 3 and 10 mg/kg is likely to maintain receptor occupancy over the dose interval.



Note: solid black line is median simulated concentration, shaded gray area is the 95% confidence interval.

Figure 4. **(A)** Macrophage-mediated phagocytosis assay was conducted by labeling PBMCs from AML patients with CSFE, added into MDMs and incubated for 3 hours. Phagocytosis was measured by gating on CD14+ cells and then assessing the percentage of CFSE+ cells. **(B)** Receptor occupancy was measured using an *in vitro* TJC4 spike-in into healthy volunteer whole blood followed by the incubation of a saturating concentration of either huIgG4 (isotype control) or TJC4. The occupied and total receptors were detected by the addition of AF633-conjugated mouse anti-human IgG4 antibody. **(C)** Single dose PK (up) and TK (bottom) profiles of TJC4 in cynomolgus monkeys following single or repeated IV administration at multiple dose levels. **(D)** Predicted human PK profile cross 0.3 to 10 mg/kg using a PPK model, and 1 mg/kg was the FIH dose.

SUMMARY

- ❖ TJC4 is a specifically designed fully human CD47 blocking mAb with superior anti-tumor efficacy in mono- and combination xenograft models.
- ❖ TJC4 has a unique binding epitope, potentially shielding it from binding to the RBCs attributed to a potential N-linked glycosylation site, thus endowed with a differentiated RBC sparing property *in vitro* and *in vivo*.
- ❖ TJC4 was well tolerated in cynomolgus monkeys following QW IV infusion of TJC4 up to 100 mg/kg for 4 weeks. The *in vivo* kinetic profile was consistent with an IgG mAb with TMDD effect at lower doses.
- ❖ TJC4 has been approved by US FDA and China NMPA for further clinical development.