Abnormal 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 showed 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 site.

TJC4 is a specifically designed fully human CD47 blocking mAb with superior anti-tumor efficacy in mono- and combination xenograft models. Animals were treated with TJC4 five days per dose (n=10/group) and the survival rate of the treated group was compared with untreated controls. The survival rate of the treated group was significantly higher than that of the control group. The tumor weight of the treated group was also significantly lower than that of the control group. The survival rate of the treated group was significantly higher than that of the control group.

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 showed 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 site.

TJC4 inhibits hematological tumor growth in vivo

TJC4 showed good safety in cynomolgus monkeys without inducing anemia. 4-Week GLP toxicity study of TJC4 in cynomolgus monkeys

- OW 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
- TJC4 was well tolerated in cynomolgus monkeys following QW IV infusion of TJC4 up to 100 mg/kg for 4 weeks.

Figure 2. Cynomolgus monkeys received OW 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 decreases in RBCs and hemoglobin (not shown) was observed following 1st dosing, and reversed from 2nd to 3rd dosing; the right graph showed RBC percentage v.s. control to rule out procedure-related impact on RBCs.

Figure 3. (A) Raji lymphoma model: Treatment: TJC4 (10 mg/kg) >61 (49~>63) >57.1 b<0.0001 TJC4 (2 mg/kg) >61 (49~>63) >57.1 b<0.0001. (B) HL60 leukemia model: Treatment: TJC4 (5 mg/kg) >99 1.000 TJC4 (0.4 mg/kg) >99 1.000. (C) WSU-DLCL2 DLBCL model: Treatment: TJC4 (2 mg/kg) >99 1.000 TJC4 (0.4 mg/kg) >99 1.000. (D) Simulated concentration-time profiles for TJC4 in humans: Weekly dosing at least for the 1 mg/kg dose is justified. Weekly doses of 3 and 10 mg/kg is likely to maintain receptor occupancy over the dose 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 the number of TJC4 bound to the cells. (B) Cytotoxicity was measured using an in vitro TJC4 uptake in healthy volunteer whole blood followed by the incubation of a saturating concentration of either IgG4 (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 doses. (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 and platelets.

- TJC4 was well tolerated in cynomolgus monkeys following OW IV infusion of TJC4 up to 100 mg/kg for 4 weeks.

- The in vivo kinetic profile was consistent with an IgG4 mAb with TKD effect at lower doses.

- TJC4 has been approved by US FDA and China NMPA for further clinical development.