DUAL TARGETING OF CTLA-4 AND CD47 ON TREG CELLS REJUVENATES IMMUNITY AGAINST SOLID TUMORS

Anli Zhang*, Zhenhua Ren, Kuo-fu Tseng, Xiajuan Liu, Huiyu Li, Changzheng Lu, Yueqi Cai, John Minna, Yang-Xin Fu. 1UTSW, Dallas, TX, United States; 2Aetio Biotherapy, Dallas, TX, United States; 3Institute of Biophysics, Beijing, China

Background: Although approved by FDA, anti-CTLA-4 treatment has severe side effect that limits its clinical usage. Blockade of CD47, the “don’t eat me” signal, has limited effects in solid tumors despite its potent anti-tumor effects in hematopoietic malignancies. Targeted delivery of immune blockers into tumor tissues are desirable.

Methods: Taking advantage of the high expression of CTLA-4 on Treg cells and abundant Fc receptor+ active phagocytes inside the tumor microenvironment (TME), we design and test an anti-CTLA-4×SIRPα (CD47 ligand)-Fc heterodimer that selectively blocks CD47 on intratumoral Treg cells and increases antibody-dependent cellular phagocytosis (the “eat me” signal).

Abstract 800 Figure 1 Anti-CTLA-4×SIRPα selectively targets intratumor Treg. (A) Expression of CTLA-4 transcripts in colorectal cancer patient tissues based on single cell sequencing online database (29). (B and C) CTLA-4 expression on Treg cells from PBMC, spleen and tumor cells in MC38 tumor-bearing mice on day 14. (D) Schematic diagram of anti-CTLA-4×SIRPα heterodimer. (E) Kinetic association (ka), dissociation (kd), and calculated affinity (KD) for binding of the indicated analyte to mouse CTLA-4 or CD47 antigen were measured by surface plasmon resonance using OpenSPR instrument. (F) Binding of SIRPα, CTLA-4 and anti-CTLA-4×SIRPα to RBC from C57BL/6 mice, n=4. (G) Anti-CTLA-4×SIRPα binding on Treg of PBMC and tumor cells from MC38 tumor-bearing mice. (H) C57BL/6 mice were inoculated with 5×105 MC38 tumor cells. After 13 days, 20 mg anti-CTLA-4×SIRPα was injected intraperitoneally. Five days later, mice were perfused with PBS, and tumor and other tissues were harvested and homogenized. Anti-CTLA-4×SIRPα protein abundance was determined with ELISA, n=4.

Abstract 800 Figure 2 Anti-CTLA-4×SIRPα preferentially depletes ICOShi Treg. (A–C) C57BL/6 mice (n=4) were inoculated with 5×105 MC38 tumor cells and treated with Combo or anti-CTLA-4×SIRPα on day 13. Five days later, Treg cells from tumor, spleen and draining lymph node (dLN) were analyzed. The representative flow cytometric gating was shown in (A). Treg cells frequency from different groups was shown in (B). The ratio of CD8+ T cells to Treg was shown in (C). (D–F) Foxp3-EGFP reporter mice (n=3) were inoculated with 5×105 MC38 tumor cells and treated with Combo or anti-CTLA-4×SIRPα on day 13. Five days later, Foxp3-EGFP+ cells from tumor, spleen and dLN were analyzed. The representative flow cytometric gating was shown in (D). Foxp3-EGFP+ cell frequency from different groups was shown in (E). The ratio of CD8+ T cells to Foxp3-EGFP+ cells was shown in (F). (G–I) Same experiment scheme as (A–F). Representative ICOS expression on Treg cells was shown in (G). (H) ICOS++ frequency among Treg cells (H) and ICOS++ frequency among YFP+ cells (I) were shown.
Abstract 800 Figure 3  Anti-CTLA-4×SIRPα reduces tumor in T cell dependent way. (A) C57BL/6 mice were inoculated with $5 \times 10^5$ MC38 tumor cells and treated with equal moles of anti-CTLA-4 plus SIRPα (Combo, 12 µg + 8 µg) or anti-CTLA-4×SIRPα (20 µg) on day 14. Tumor growth was measured every 3 days, n=5. (B) BALB/C mice were inoculated with $5 \times 10^5$ CT26 tumor cells and treated with Combo (30 µg+ 20 µg) or anti-CTLA-4×SIRPα (50 µg) on day 6. Tumor growth was measured every 3 days, n=5. (C) Rag1-/- mice were inoculated with $1 \times 10^5$ MC38 tumor cells and treated with Combo (12 µg+ 8 µg) or anti-CTLA-4×SIRPα (20 µg) on day 13. Tumor growth was measured every 3 days, n=5. (D) MuMT mice were inoculated with $1.5 \times 10^6$ MC38 tumor cells and treated with anti-CTLA-4×SIRPα (20 µg) on day 14. Tumor growth was measured every 3 days, n=5. (E) C57BL/6 mice were inoculated with $5 \times 10^5$ MC38 tumor cells and treated with Combo (12 µg+ 8 µg) or anti-CTLA-4×SIRPα (20 µg) on day 14. Tumor growth was measured every 3 days, n=5. (F) The treatment was started with anti-CD4 (E) or anti-CD8 (F) before week 2. Five days later, tumor-infiltrating lymphocytes (TILs) were analyzed for total CD45 in tumor (E), Ki67 expression on CD4 and CD8 T cells from different groups (F and G) after treatment.

Abstract 800 Figure 4  Anti-CTLA-4×SIR enhances tumor-specific T cell IFN-gamma. (A) C57BL/6 mice (n=4) were inoculated with $5 \times 10^5$ MC38 tumor cells and treated with Combo or anti-CTLA-4×SIRPα on day 13. Five days later, TILs were analyzed for tumor specific T cells. (B and C) C57BL/6 mice (n=4) were inoculated with $5 \times 10^5$ MC38 tumor cells and treated with Combo or anti-CTLA-4×SIRPα on day 13. Five days later, TILs were purified with CD45+ positive selection magnetic beads. TILs (A) and dLN (B) were re-stimulated with irradiated MC38 tumor cells or irrelevant control Lewis lung cancer (LLC) cells for 48 h. IFN-γ producing cells were determined by ELISPOT assay. (D) WT or Ifng-/- C57BL/6 mice were inoculated with $5 \times 10^5$ MC38 tumor cells and treated with anti-CTLA-4×SIRPα on day 13. Tumor growth was measured every 3 days, n=4. (E) C57BL/6 mice were inoculated with $5 \times 10^5$ MC38 tumor cells and treated with anti-CTLA-4×SIRPα on day 13. 150 µg of anti-IFN-γ was administrated one day before treatment initiation and then twice a week for 2 weeks. Tumor growth was measured every 3 days, n=5. (F and G) C57BL/6 mice were inoculated with $5 \times 10^5$ MC38 tumor cells and treated with anti-CTLA-4×SIRPα on day 13. Six weeks later, anti-CTLA-4×SIRPα cured mice and control naive mice were re-challenged with $5 \times 10^6$ MC38 tumor cells on the left flank (opposite to the original injection flank) (F), and $5 \times 10^5$ LLC tumor cells were injected on the right flank (G). Tumor growth was measured every 3 days, n=5.
Abstract 800 Figure 5  CD47 expression on Treg cells is essential. (A) Cd47-/ tumor bearing C57BL/6 mice were treated with Combo or anti-CTLA-4×SIRPα on day 13. Tumor growth was measured every 3 days, n=5. (B) WT or Cd47-/ C57BL/6 mice were inoculated with 5 × 105 MC38 tumor cells and treated with anti-CTLA-4×SIRPα on day 14. Tumor growth was measured every 3 days, n=5. (C) WT or Cd47-/- T cells were purified by negative selection magnetic beads and intravenously transferred to Rag1-/- mice. 2 × 105 MC38 tumor cells were inoculated into the recipient mice the next day. Mice were treated with 40 μg anti-CTLA-4×SIRPα on day 7 and day 12. Experiment scheme was shown in upper panel and tumor growth was shown in lower panel, n=5. (D) C57BL/6 mice were inoculated with 5 × 105 MC38-cEGFR tumor cells and treated with 30 μg anti-EGFR×SIRPα or anti-CTLA-4×SIRPα on day 14. Tumor growth was measured every 3 days, n=5. (E) WT or Fcer1g-/- C57BL/6 mice were inoculated with 1 × 105 MC38 tumor cells and treated with anti-CTLA-4×SIRPα on day 13. Tumor growth was measured every 3 days, n=5. (F) WT C57BL/6 mice were inoculated with 5 × 105 MC38 tumor cells and treated with anti-CTLA-4×SIRPα or anti-CTLA-4×SIRPα with mutant Fc (Anti-CTLA-4×SIRPα-InFc) on day 14. Tumor growth was measured every 3 days, n=5. (G) Bone marrow derived macrophages from WT or Fcer1g-/-mice were mixed with CFSE labelled in vitro differentiated Treg cells from WT or Cd47-/- mice for 3 h. Phagocytosis was determined with flow cytometry. Phagocytosis index was defined as the percentage of CFSE+ macrophages among total macrophages.

Abstract 800 Figure 6  A human version anti-CTLA-4×SIRPα heterodimer depletes Treg. (A and B) CD47 and CTLA-4 expression level on Treg cells of PBMC and tumor from NSCLC patients. The representative flow cytometric gating was shown in (A), pooled data from different patients was show in (B). (C) Anti-hCTLA-4, SIRPα and anti-hCTLA-4×SIRPα binding on Jurkat and Jurkat-hCTLA-4 expressing cells. (D) Comparison of anti-hCTLA-4×SIRPα binding on Jurkat and Jurkat-hCTLA-4 cells based on (C). (E and F) PBMC-humanized NSG mice were inoculated with 2.5 × 106 A549 tumor cells and treated with anti-hCTLA-4 plus SIRPα (h-Combo, 18 μg +12 μg) or anti-hCTLA-4×SIRPα (30 μg) on day 12. Two days later, the frequency of tumor-infiltrating Treg cells (G) and ICOS expression level on Treg cells (F) were analyzed, n=4. (G) PBMC-humanized NSG mice were treated with 200 μg anti-hCTLA-4 or anti-hCTLA-4×SIRPα twice a week for 4 times. Mouse body weight was monitored every 3 days, n=5. Data are shown as mean ± SEM from two independent experiments. P value was determined by paired t test (B), unpaired t test (E and F) and two-way ANOVA with Geisser-Greenhouse’s correction (G). The normality of data was confirmed by Shapiro-Wilk test.

Results  Anti-CTLA-4×SIRPα preferentially depletes ICOShigh immunosuppressive Treg cells in the TME (figure 1–3) and enhances immunity against solid tumors. Mechanistically, we discovered that CD47 expression on Treg cells limits anti-CTLA-4 mediated depletion while Fc on the heterodimer enhances the depletion. Furthermore, anti-human CTLA-4×SIRPα depletes tumor Treg cells (figure 4–6) and exhibits less toxicity than anti-human CTLA-4 in a humanized mouse model.

Conclusions  Collectively, these results highlight coordinatively modulating “eat me” and “don’t eat me” signals for depleting Treg cells inside the TME as a unique strategy for solid tumor treatment.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.800