Novel fully human agonist antibodies against the T-cell costimulatory receptor CD27 shape adaptive anti-tumor immunity. #133P

DISCLOSURE

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Novel fully human agonist antibodies against the T-cell costimulatory receptor CD27 shape adaptive anti-tumor immunity. #133P

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Background

- CD27 is a member of the TNF-Receptor superfamily and plays a critical role in T-cell activation by providing a costimulatory signal after binding to its ligand CD70 .
- CD27 signaling enhances T-cell proliferation, activation and differentiation of naive and memory T cells and therefore promotes cytotoxic T cell (CTL)-based anti-tumor immunity.
- CD27 is expressed on CD4+ and CD8+ T cells, B cells and NK cells.
- Agonist stimulation of CD27 is a promising cancer immunotherapy approach to reinvigorate specific T cell driven anti-tumor responses.
- CD27 is expressed on tumor infiltrating lymphocytes (TIL) to provide the necessary co-stimulatory signal to T cells, especially CD8+ T cells. Stimulation of TILs through CD27 can lower the threshold of activation to low affinity antigens and provide a broader repertoire of Ag-reactive T cells within the tumor.

CD27 Staining of Human Peripheral Blood populations

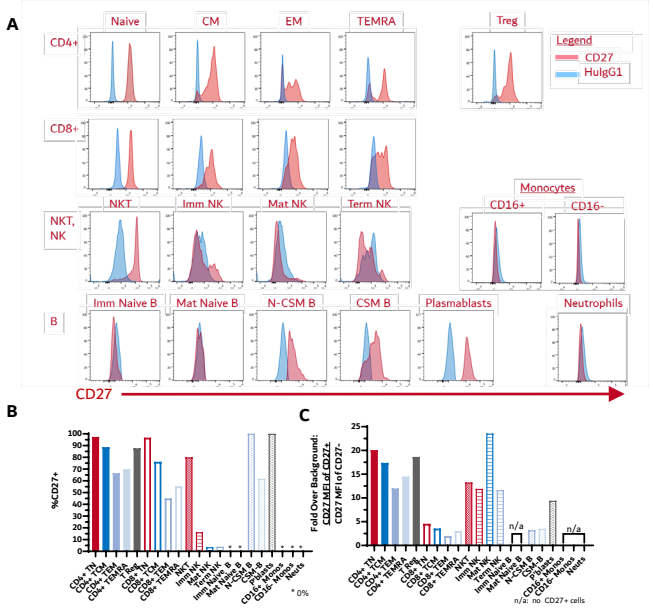

Figure 1: Anti-CD27 staining of human peripheral blood populations with Kineta clone CD27.30.10 conjugated to Alexa Fluor 647. Whole peripheral blood from two donors was stained with four immunophenotyping panels and Kineta's anti-CD27 clone CD27_30.10 or HuIgG1 conjugated to Alexa Fluor 647. Representative flow cytometry data from one donor shown. (A) Histogram overlays of CD27 (red) and HuIgG1 (blue) stained samples. CD27 was strongly stained in CD4+T, NKT and memory B cell populations; (B) % of CD27 positive cells of each population is shown. (C) Fold-Over-Background staining intensity of CD27 positive cells was measured as (CD27 positive cells Median Fluorescence Intensity)/(CD27 negative cells Median Fluorescence Intensity)).

Results

Selected anti-CD27 mAbs demonstrate robust T cell activation and cytokine induction

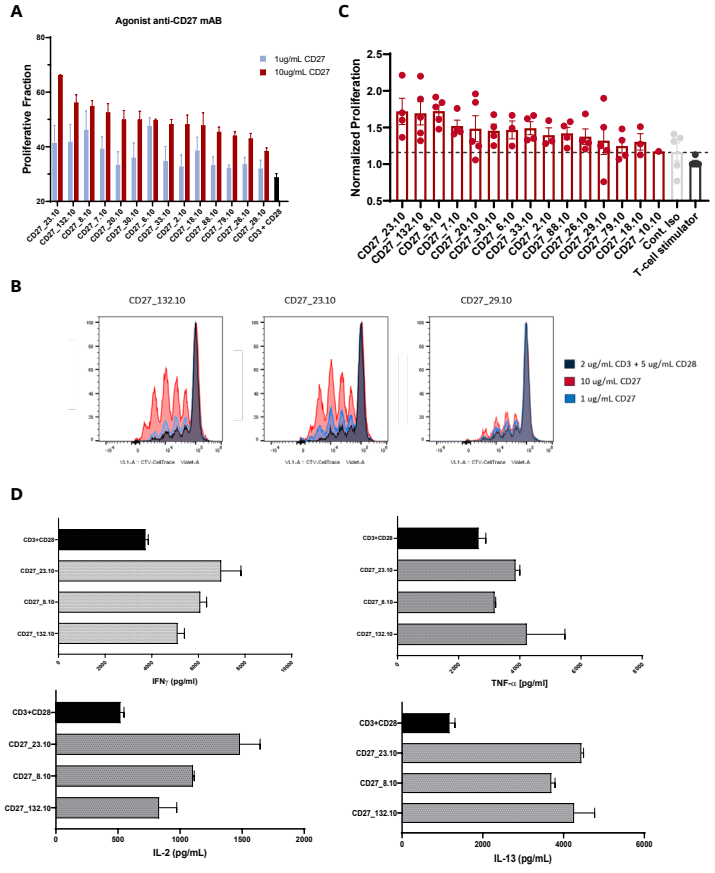

Figure 2: T cell proliferation assay. T-cells from healthy donors were stained with cell trace violet (CTV) and stimulated with 10 mg/mL of anti-CD27 antibodies and T-cell activator (2 mg/mL anti-CD3 and 5 mg/mL anti-CD28). The Frequency of CTV labelled cells and cytokine release into the supernatant were examined after 4 days using flow cytometry and Luminex assay, respectively. (A) Proliferative fraction for each antibody for a single donor is represented. (B) Histogram plots comparing T-cell proliferation for selected anti-CD27 at 10ug/mL and 1ug/mL to T-cell stimulator without anti-CD27 antibody (C) Proliferation data from 5 different donors, normalized to T-cell activators but without anti-CD27 antibodies in each donor (C) Cytokine data from lead antibodies is represented as pg/mL. (Error bars represent SEM).

Anti-mCD27 agonist mAb induce anti-tumor response as a single agent

- Proof of concept at low concentration -

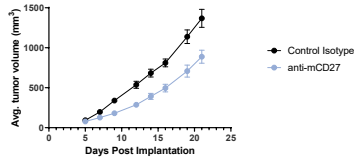

Figure 3: Inhibitory effect of anti-mCD27 agonist of tumor growth. Plot of mean tumor volumes vs. time following subcutaneous implantation of 5e5 MB49 WT cells/mouse in C57BL/6J mice. Final tumor volumes were carried forward for animals that met euthanasia endpoints prior to termination of the entire group. Animals received control isotype or anti-mCD27 (BioXCell BE0348) 5mg/kg 2x/week beginning on Day 5 through Day 21.

Characterization of CD27 surface expression in huCD27 transgenic mice

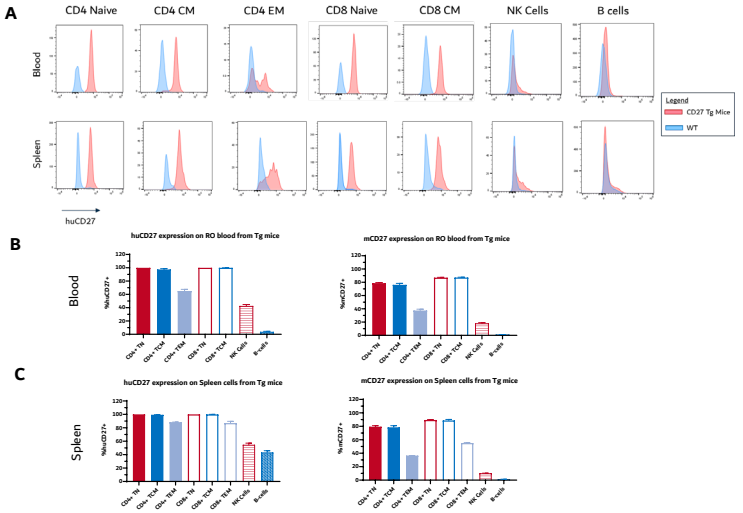

Figure 4: huCD27 transgenic mouse model characterization. RO bleeds and spleen were harvested from 11 huCD27 Transgenic C57BL/6 mice and dissociated to single cell suspension for staining and flow cytometry. Representative plots are graphed: (A) Histogram comparison of huCD27 expression in huCD27 transgenic mice and WT C57BL/6 mice (B) Frequency of human and mouse CD27 on cells from RO blood, (C) Frequency of human and mouse CD27 on cells from Spleen.

Conclusions

3 lead candidates for future pre-clinical development

- We focused on evaluating 15 leads out of 147 fully human anti-CD27 monoclonal antibodies with unique sequences
- Human T cell activation assay showed strong increased in proliferation and cytokine secretion for 3 mAbs
- Surrogate *in vivo* model with anti- mouse CD27 agonist showed significant anti-tumor response
- huCD27 transgenic mice will serve as our model for further in vivo studies to select our lead anti-CD27 agonist antibody by Q1 2022.