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A promising cancer immunotherapy target:

Novel agonistic human antibody against the human

T-cell costimulatory receptor CD27

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A promising cancer immunotherapy target: Novel agonistic human antibodies against the human T-cell costimulatory receptor CD27

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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 effector 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.
- Agonistic stimulation of CD27 is a promising cancer immunotherapy approach to reinvigorate specific T cell driven anti-tumor responses.

Objectives

Evaluate 147 fully human monoclonal anti-CD27 antibodies (mAb)

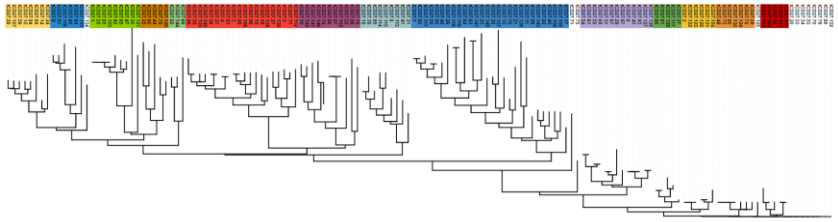
- Measure antibodies' affinity to CD27.
- Test anti-CD27 antibodies binding to human, murine and primate CD27 to evaluate species cross-reactivity.
- Perform competition assay to test if soluble CD70-ECD disrupts anti-CD27 antibody binding.
- Assess antibody agonist activity using NFkB-Luc2/CD27 Jurkat reporter assay.
- Characterize the CD27-mediated costimulatory effects of anti-CD27 antibodies on human peripheral blood T cells.

Exceptional antibody diversity in both heavy and light chains

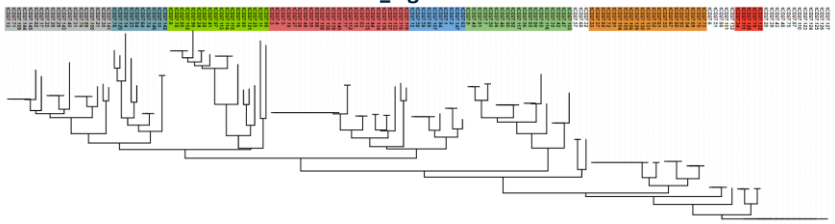
147 fully human ScFv antibodies directed against human CD27 obtained after immunization of Trianni mice and single B cell sequencing

- > 14 V_H diversity groups
- > 8 V_L diversity groups
- Highest diversity in CDR3-H

CDR3_Heavy Chains



CDR3_Light Chains



Results

Kineta's anti-CD27 mAbs bind to human-CD27 with different affinity

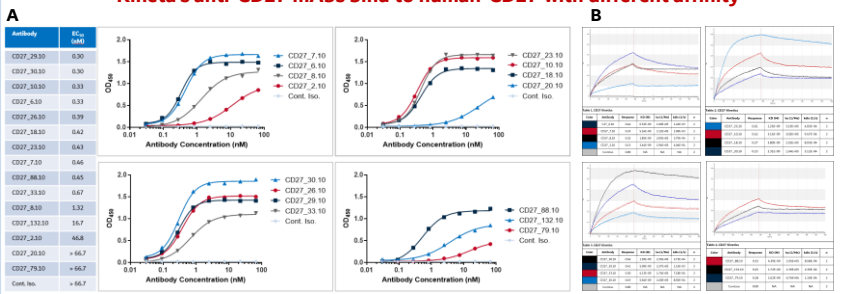


Figure 1: (A) ELISA binding studies were conducted with immobilized human CD27 (rabbit-Fc). Binding of anti-CD27 mAb was detected using biotinylated anti-human IgG (light chain) followed by streptavidin HRP. **(B)** Octet kinetics studies were performed using 1x kinetics buffer (pH 7.2) and 10 ug/ml (67 nM) of anti-CD27 mAb loaded onto an Anti-human IgG Fc Capture (AHC) biosensor for 120 seconds. The loaded biosensor was incubated with 50nM of human CD27-ECD (His-Tag) for 240 seconds for the association step and then transferred into the kinetic buffer for 360 seconds to measure the dissociation. 1:1 curve fitting analysis was performed to determine ka, kdis, and KD.

Anti-CD27 mAbs cross-react with NHP-CD27 but not with mouse-CD27

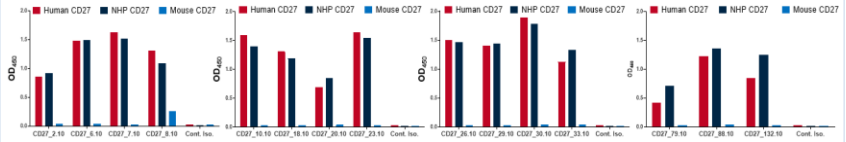


Figure 2. Binding of anti-CD27 mAb to immobilized human CD27 (rabbit-Fc), non-human primate (NHP) CD27 (human-Fc), or mouse CD27 (mouse-Fc). Bound anti-CD27 mAbs were detected using biotinylated anti-human IgG (light chain) followed by streptavidin HRP.

Anti-CD27 mAbs prevent CD70 binding

Antibody	EC_{50} (nM)	% Blockage
CD27_30.10	2.0	75%
CD27_10.10	2.7	91%
CD27_23.10	3.3	69%
CD27_18.10	4.2	92%
CD27_7.10	9.7	64%
CD27_8.10	10.3	84%
CD27_26.10	14.5	52%
CD27_29.10	66.4	42%
CD27_6.10	>66.7	42%
CD27_88.10	>66.7	31%
CD27_33.10	>66.7	28%
CD27_132.10	>66.7	20%
CD27_2.10	>66.7	17%
CD27_20.10	>66.7	15%
CD27_79.10	>66.7	7%
Cont. Iso.	>66.7	0%

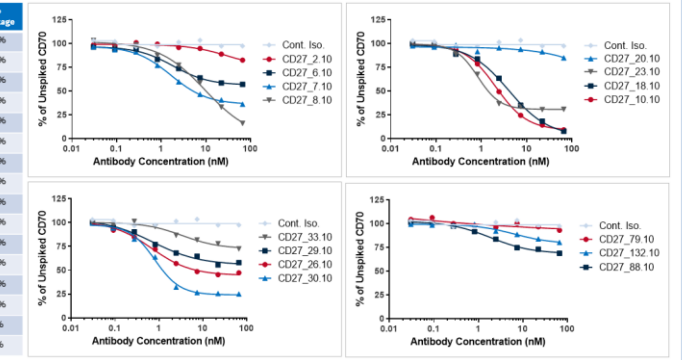


Figure 3. Disruption of soluble human CD70-ECD (mouse Fc) binding to immobilized human CD27 (rabbit-Fc) by anti-CD27 mAb in a competition ELISA. CD70-ECD (mouse-Fc) at 0.11nM was spiked with anti-CD27 mAb (0.03-66.67nM) and incubated on the plate coated with CD27 for 1 hour. Bound CD70 was detected using peroxidase anti-mouse IgG (H+L). EC_{50} and % Blockage of CD27/CD70 binding was measured.

Selected anti-CD27 mAbs induce strong agonistic activity

Antibody	EC_{50} (nM)
CD27_8.10	3.4
CD27_88.10	3.4
CD27_2.10	7.0
CD27_132.10	15.6
CD27_20.10	21.8
CD27_33.10	22.9
CD27_79.10	33.7
CD27_29.10	55.2
CD27_30.10	> 104
CD27_23.10	> 104
CD27_7.10	> 104
CD27_6.10	> 104
CD27_26.10	> 208
CD27_18.10	> 208
CD27_10.10	> 208
Cont. Iso.	> 208
CD70-ECD	0.4

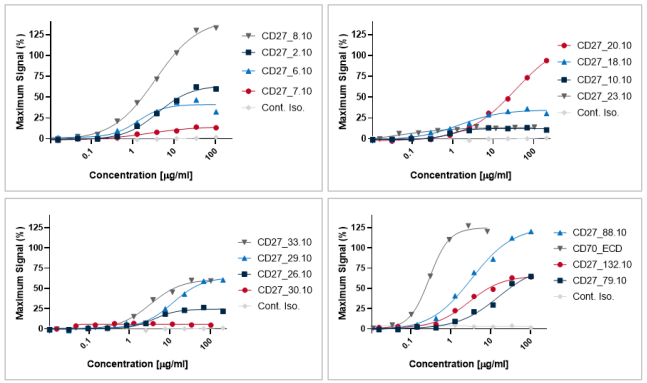


Figure 4: Anti-CD27 agonist assay was carried out with NFkB-Luc2/CD27 Jurkat cells seeded at 2e5 cells/well in X-VIVO-15 medium in the 96-well plates pre-coated with 5 µg/mL anti-CD3 mAbs. Cells were then incubated with anti-CD27 antibodies or CD27 ligand (CD70-ECD) and incubated for 4 hrs at 37°C, 5% CO_2 humidified incubator. NFkB reporter induction was measured by luminescence in the presence of Bio-Glo reagent.

Selected anti-CD27 mAbs demonstrate robust T cell activation

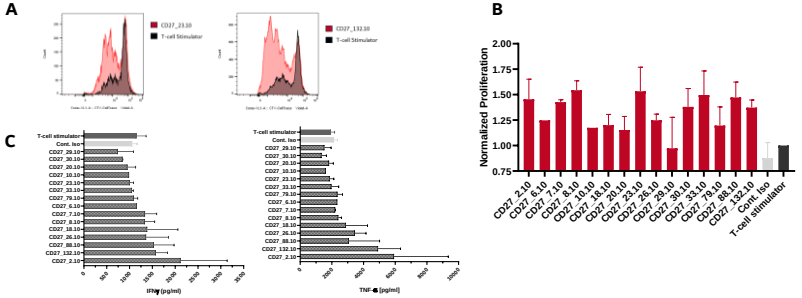


Figure 5: T-cells from two healthy donors were stained with cell trace violet (CTV) and stimulated with 10 µg/mL of anti-CD27 antibodies and T-cell activator (2 µg/mL anti-CD3 and 5 µg/mL anti-CD28). The frequency of CTV labelled cells and cytokine release were examined after 4 days using flow cytometry and Luminex assay, respectively. **(A)** Representative histogram plots comparing T-cell proliferation for 2 selected anti-CD27 mAbs to T-cell stimulator without anti-CD27 antibody. **(B)** Proliferation data were normalized against the results obtained with T-cell activator without anti-CD27 antibody for each donor. **(C)** Cytokine data are represented as pg/mL.

Conclusions

Identified lead candidates for future pre-clinical development

- 147 fully human anti-CD27 monoclonal antibodies with unique sequences were generated.
- Anti-CD27 agonist assay showed strong agonist activity for 8 pre-selected anti-CD27 antibodies.
- Human T cell activation assay showed increased proliferation and cytokine secretion for only 4 mAbs.
- Further *in vitro* and *in vivo* developments are on-going to select our lead anti-CD27 agonist antibody.