

CD27 Background

- Member of the TNF-receptor superfamily
- Constitutively expressed on the majority of mature T cells, memory B cells, and a portion of NK cells
- T cell co-stimulatory molecule, required for generation and long-term maintenance of T cell immunity
- Role in clonal B cell expansion and germinal center formation, immunoglobulin synthesis
- Role in NK cell cytolytic activity

Rationale for Targeting CD27 in Cancer

- Anti-mouse CD27 mAb previously shown to have anti-tumor activity in several tumor models
- In vivo CD27 stimulation with its ligand (CD70) promotes strong primary and secondary CD8+ cytotoxic T cell responses
- Expression of CD70 on dendritic cells improves immunity of dendritic cell vaccines
- Agonist CD27 mAb may bypass the requirement for CD40 activation in immune activation
- CD27 is expressed by some lymphomas and leukemias

Characterization of CD27 HuMAbs Binding and Ligand Blocking

Clone	KD(M) (Biacore)	sCD70 blocking (ELISA)
1G5	4.02E-10	No
1H8	1.58E-10	yes
3H12	3.58E-10	yes
3H8	5.56E-11	No
2G9	1.53E-12	No
1F5	1.86E-10	yes
3A10	2.02E-10	No
2C2	8.41E-11	No

- A panel of hybridomas were generated from splenocytes of human immunoglobulin transgenic mice after immunization with recombinant human CD27.
- Hybridoma mAbs were characterized by ELISA, Flow Cytometry and Biacore for binding and CD70 blocking.
- 8 selected antibodies were cloned, sequenced and expressed in CHO cells.

Development of huCD27 transgenic mice

- A murine model was established to test the activity of anti-human CD27 HuMAbs
- BAC clone containing the CD27 gene was used for microinjection of mice embryos
- Transgenic lines were established for evaluation of human CD27 expression

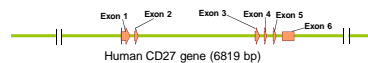


Fig. 1. CD27 Expression in Tg Mice by Flow Cytometry

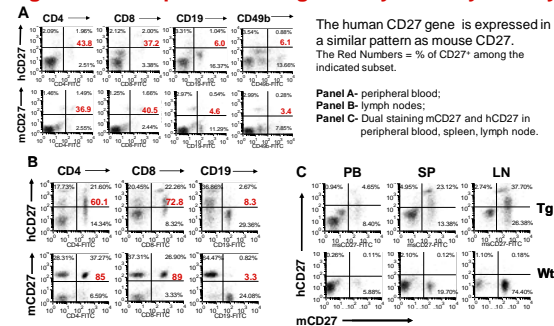
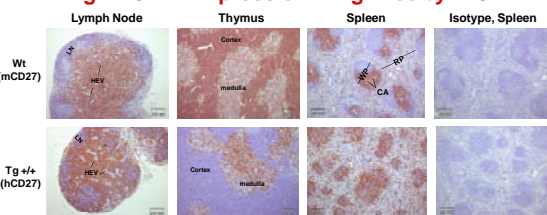
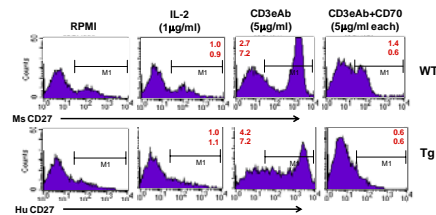


Fig. 2. CD27 Expression in Tg Mice by IHC



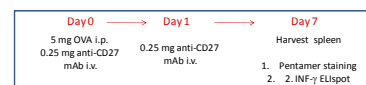
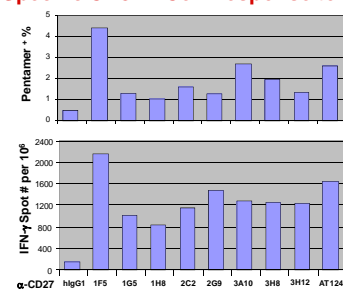
Immunostaining with anti-human (0.1 µg/ml) or mouse (0.5 µg/ml) CD27-FITC and isotype on acetone fixed frozen tissue sections. Counterstained with Mayer's hematoxylin. LN, lymph node; HEV, high endothelial venule; RP, red pulp; WP, white Pulp; CA, central artery.

Fig. 3. Regulation of CD27 Expression



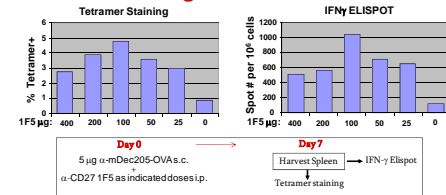
Splenocytes from huCD27 Tg mice or WT mice were activated IL-2 (does not up-regulate CD27) CD3e Ab (strong up-regulator of CD27) or CD3e Ab + CD70 (CD27 down-regulator) for 72 hrs. Histogram of CD4⁺CD8⁺ gated cells are shown. Relative fold-increases are shown in red, Top number - fold increase in % positive; Bottom number - fold increase in MFI

Fig. 4. CD27 HuMAbs Enhance Antigen-Specific CD8⁺ T Cell Response to Vaccine



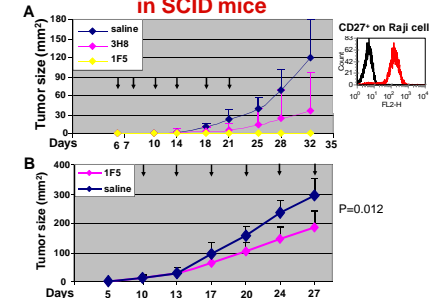
HuCD27 Tg mice were immunized with OVA and anti-CD27 HuMAbs. One week later splenocytes were analyzed for CD8⁺ T cell reactivity to the OVA SINFELK peptide (OVA peptide 257-264) by pentamer staining and IFN-γ ELISPOT. All human anti-CD27 HuMAbs enhanced T cell responses over controls.

Fig. 5. CD27 HuMAb enhances Dendritic Cell Targeted Vaccine



HuCD27 Tg mice were immunized with anti-mouse DEC-205 IgG-OVA and anti-CD27 HuMAb 1F5 at various doses. One week later splenocytes were analyzed for CD8⁺ T cell reactivity to the OVA SINFELK peptide (OVA peptide 257-264) by tetramer staining (% tetramer positive of all CD8⁺ shown) and IFN-γ ELISPOT.

Fig. 6. Anti-tumor Activity of CD27 HuMAb in SCID mice



- 1x10⁶ Raji cells were inoculated s.c. on day 0; 0.5 mg of Abs were administered i.p. on day 6, 8, 11, 14, 17 and 20. Tumor were measured twice a week.
- 3x10⁶ Raji cells were inoculated s.c. on day 0; when tumor was palpable on day 10, Ab was administered b.i.w x 3w, i.e., on day 10, 13, 17, 20, 24 & 27.

Summary

- ✓ Fully human antibodies specific for human CD27 were selected for characterization;
- ✓ A Human CD27-Tg mouse model was developed for in vivo testing of CD27 HuMAbs;
- ✓ CD27 HuMAbs enhance antigen-specific CD8⁺ T cells proliferation and activation;
- ✓ CD27 HuMAb enhances T cell responses to a dendritic cell-targeted vaccine;
- ✓ CD27 HuMAbs inhibit CD27⁺ human lymphoma growth in xenograft model.