#4560 Anti-tumor Activity of a Fully Human anti-CD27 Monoclonal Antibody in a Transgenic Mouse Model

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CD27 AS A TARGET FOR IMMUNOTHERAPY

Antibodies that recognize immune cell surface molecules can be used to enhance or target immune responses against tumors. These include antibodies that activate antigen presenting cells (*e.g.* anti-CD40), antibodies that block immune checkpoints (*e.g.* anti-CTLA-4, anti-PD-1), and T cell co-stimulatory antibodies (*e.g.* anti-4-1BB). The costimulatory molecule CD27 is a member of the tumor necrosis factor (TNF) receptor superfamily, and is constitutively expressed on the majority of mature T cells, memory B cells, and a portion of natural killer (NK) cells. The interaction of CD27 with its ligand CD70 plays key roles in the following processes:

- Costimulation through CD27 on T cells causes activation, proliferation, survival, and maturation of effector capacity and memory;
- Costimulation through CD27 on human B cells activates and promotes the generation of plasma cells, proliferation, and the production of immunoglobulin;
- Costimulation through CD27 on NK cells induces cytolytic activity.

In addition to the immune enhancing properties of agonist anti-CD27 mAbs, CD27-targeting antibodies may also provide direct therapeutic effects against tumors with CD27 expression. CD27 expression is well documented on a variety of lymphomas and leukemias and we have previously demonstrated significant anti-tumor activity in xenograph models using human lymphoblastoid cell lines.

Finally, agonist anti-mouse CD27 mAbs have shown significant anti-tumor efficacy in murine tumor models (French, RR *et al.* (2007) *Blood,* 109, 4810-4815; Sakanishi, T *et al.* (2010) *Biochem Biophys Res Commun*, 393, 829-835; Roberts, DJ, *et al.* (2010) *J Immunotherapy* 33:769–779).

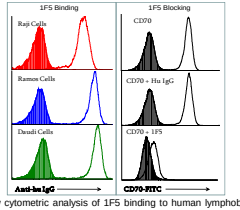
HUMAN ANTI-HUMAN CD27 ANTIBODIES

- Hybridomas expressing human anti-human CD27 mAbs were generated using human Ig Tg-mice.
- Eight selected antibodies have been cloned, sequenced and expressed in CHO cells, as below.

Clone	KD(M) (Biacore)	sCD70 blocking (Flow and ELISA)
1G5	4.02E-10	Partial
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

Biacore - Binding affinity was performed by surface plasmon resonance analysis using a CD27coated-CM5 sensor chip. Blocking studies were performed by flow cytometry (below) or ELISA with labeled sCD70.

Human mAb 1F5 is lead development candidate



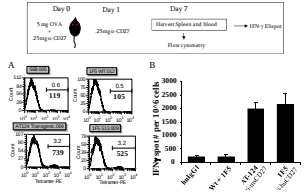
Flow cytometric analysis of 1F5 binding to human lymphoblastoid cell lines, and blocking of sCD70 binding.

1F5 ACTIVATES T CELLS FROM HUMAN CD27 TRANSGENIC MICE

Human CD27 Transgenic mice

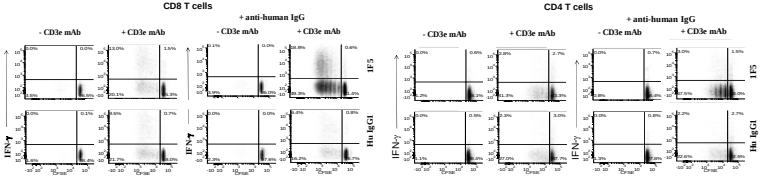
- A murine model was established to test the activity of anti-human CD27 HuMAbs.
- BAC clone, containing the CD27 gene, was used for microinjection of mouse embryos.
- A huCD27-expressing strain was established that showed appropriate expression and regulation of human CD27.
- The huCD27 Tg mice have been backcrossed to C57BL/6, BALB/c, and C3H mouse strains.
- These mice retain functional mouse CD27.

1F5 Enhances Antigen Specific T cell Response in vivo



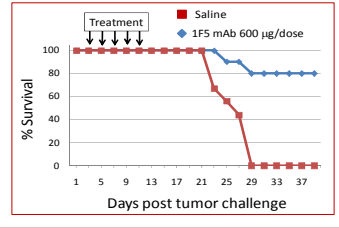
HuCD27 Tg mice were injected i.v. with 5 mg of ovalbumin on day 0 and 0.25 mg of 1F5 or anti-Mouse CD27 mAb AT-124 on day 0 and 1. On day 7, spleen was harvested and analyzed for CD8+ T cell reactivity to the OVA SIINFEKL peptide (OVA peptide 257–264) by tetramer (A) and IFNγ ELISPOT(B).

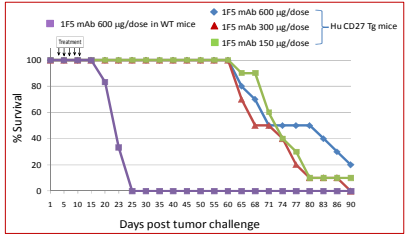
1F5 Combined with TCR Activation Induces Proliferation and Cytokine Production from T cells in vitro



T cells were purified from spleen of hCD27-Tg mice by negative selection with beads. Cells were labeled with CFSE and incubated with antibodies for 3 days. The cross-linking anti-human IgG was passed through an endotoxin removal column before use. The concentration of 1F5 was 0.2 ug/ml . TNFα-ICS showed the same pattern as IFNγ.

EFFICACY OF 1F5 IN BCL$_1$ SYNGENEIC TUMOR MODEL



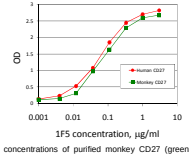


Groups of 9-10 hCD27 Tg mice (Balb/c background) were challenged with 10^7 BCL$_1$ B-lymphoma cells administered intravenously On Day 0. Animals were then treated with 5 doses of anti-human CD27 mAb 1F5 as indicated. Animals were euthanized upon reaching defined clinical criteria as approved by Celldex IACUC.

1F5 BINDING TO MACAQUE CD27

Cynomolgus macaques were established as a relevant model for testing 1F5.

- Similar binding to recombinant CD27 from human and macaque.
- Similar distribution of 1F5 binding to peripheral blood cells.
- Similar tissue cross-reactivity by immunohistochemistry (not shown).



Various concentrations of purified monkey CD27 (green line) or human CD27 (red line) were captured to ELISA plates with anti-Flag antibody, followed by incubation with 1F5 mAb. A goat anti-human IgG Fc-HRP antibody and substrate Super Blue TMB were used for detection.

Analysis	CD4+ T cells		CD8+T cells		B cells (CD20+)		NK cells	
	human	monkey	human	monkey	human	monkey	human	monkey
% CD27+[b]	84 ± 5	81 ± 1	70 ± 12	90 ± 1	37 ± 4	15 ± 1	11 ± 4	88 ± 6
MFI[c]	1517± 123	416± 14	1415± 153	519±11	893± 101	491± 113	667± 28	1050± 42

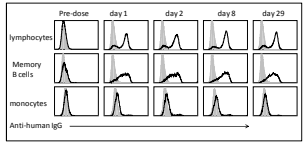
PBMCs were isolated from whole blood of 3 human and 3 cynomolgus macaques. The cells were stained with 1F5 mAb together with markers to delineate the major T cell and B cell populations that express CD27. The table summarizes the mean ± standard error of results for human and monkey cells with respect to the percent of cells expressing CD27 and the intensity of expression (MFI).

IF5 IS WELL TOLERATED AND NON-DEPLETING IN PILOT NON-HUMAN PRIMATE STUDY

3 cynomolgus monkeys were treated with one i.v. dose of 1, 3 or 10 mg/kg of anti-human CD27 mAb 1F5.

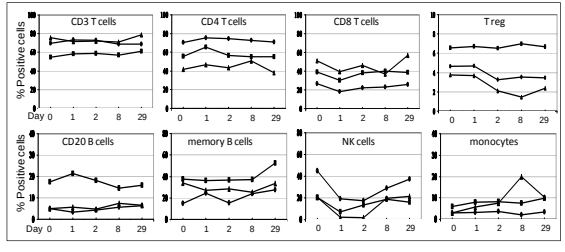
- Animals were followed for 29 days.
- No clinical symptoms.
- No significant changes in clin/path parameters.
- No elevation in body temperatures and no detectable levels of TNF-α, IL-6, or IL-1β.
- Flow was performed on blood drawn at indicated times.

1F5 on Circulating Lymphocytes after a Single 1 mg/kg Dose



Total lymphocytes (based on side and forward scatter size), memory B cells (CD20+ and CD95 bright), and monocytes (based on side and forward scatter size) were stained with anti-human IgG antibody (bold line) and compared to unstained controls (shaded histogram).

1F5 does not Significantly Deplete Circulating Lymphocytes after a Single 1 - 10 mg/kg Dose



Lymphocytes were stained with subset markers and the % positive cells plotted vs time for each animal treated at the different doses.
■ = 1 mg/kg ; ● = 3 mg/kg; ▲ = 10 mg/kg.

SUMMARY AND FUTURE DIRECTIONS

- CD27 is a critical molecule in regulating immunity and tolerance, and is expressed at high level by hematologic malignancies thus representing a new opportunity as an immunotherapy target.
- We have developed and characterized a fully human monoclonal antibody specific for CD27:
  - mAb 1F5 has potent anti-tumor activity in a syngeneic tumor challenge model of the BCL$_1$ B Cell lymphoma, and
  - mAb 1F5 was well tolerated at 1-10 mg/kg i.v. without signs of inflammation or significant lymphocyte depletion.
- Taken together the data support the therapeutic potential of mAb 1F5 (CDX-1127) in cancer therapy.
- A phase 1 clinical trial in hematologic and solid cancers is planned to initiate in 2011.

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