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Development of a Human Anti-CD27 Antibody with Efficacy in Lymphoma and Leukemia Models by Two Distinct Mechanisms

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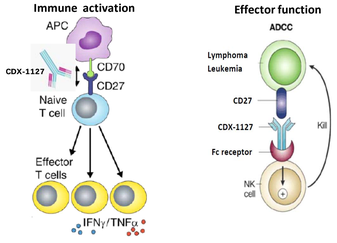
CD27 Introduction

- Member of the tumor necrosis factor (TNF) receptor superfamily
- Constitutively expressed on the surface of majority of mature T cells, memory B cells, and a portion of natural killer (NK) cells
- Also presented in B cell malignancies and adult T-cell leukemia/lymphoma
- CD70/CD27 Co-stimulatory Pathway
  - CD27 activation well-regulated by ligand CD70, which is generally only transiently expressed on activated T cells, B cells, and dendritic cells
  - On T cells: causes activation, proliferation, survival, and maturation of effector and memory cells
  - On human B cells: activates and promotes the generation of plasma cells, proliferation, and the production of immunoglobulin
  - On NK cells: induces cytolytic activity

Targeting CD27 with Antibodies

- Agonist anti-CD27 mAbs can induce potent anti-tumor immunity through T cell activation (French, RR et al. Blood 2007; Sakanish, T et al. BBRC 2010; Roberts, DJ et al. J. Immunotherapy 2010).
- CD27-targeting antibodies may also provide direct therapeutic effects against tumors with CD27 expression (see Tables below).



CD27 is a Target on lymphoid malignancies

Tab. 1, CD27 expression by IHC

Diseases	Total	CD27
Pre-B ALL	5	0/5 (0%)
MCL	18	15/15 (100%)
CLL/SLL	14	14/14 (100%)
BL	6	6/6 (100%)
FL	20	12/20 (60%)
MALT/MZL	26	23/24 (96%)
DLBCL	20	10/14 (71%)
Plasmacytoma	7	5/7 (71%)
Total	116	85/105 (82%)

Dong, HY et al Leukemia and Lymphoma, 2002

Tab. 2, CD27 expression by Flow Cytometry

Diseases	Pos./Total	MFI* (range)
Pro-B ALL	0/4	
Pre-pre-B ALL	4/17	250-320
Pre-B ALL	2/3	450/300
B-ALL	2/2	600/550
CLL	11/11	450/800
PLL	3/3	410/600
HCL	2/4	500/485
Myeloma	0/6	

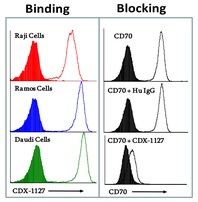
*Median Fluorescence intensity (linear scale)

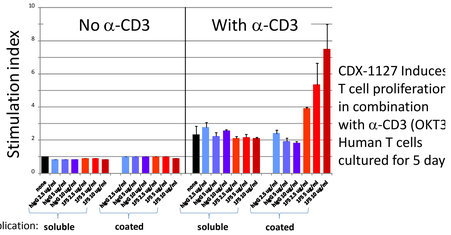
Van Oers, MHJ et al Blood 1993

CDX-1127, an agonist anti-CD27 mAb

- Fully human IgG1κ mAb
- Sub-nanomolar affinity
- Blocks CD70 binding
- Agonist in combination with α–CD3
- Cross-reacts with macaque CD27

CDX-1127 mAb binding to lymphoma cell lines and inhibition of CD70 binding

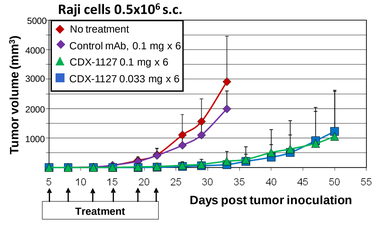


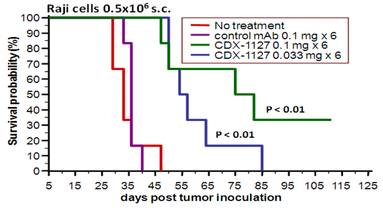


CDX-1127 Induces T cell proliferation in combination with α-CD3 (OKT3) Human T cells cultured for 5 day

CDX-1127 direct killing of CD27+ cells

Anti-tumor activity in SCID mice transplanted with human lymphoma cell lines





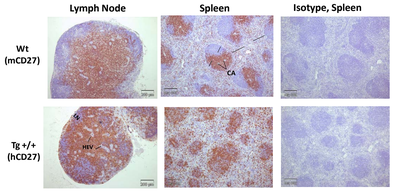
- Similar activity in models with Daudi, Namalwa, Ramos (lymphoma), and CCRF-CEM (T-ALL).

Development of huCD27 transgenic mice

Human CD27 gene (6819 bp)

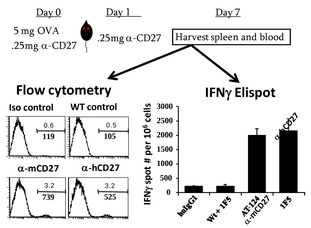
- BAC clone RP11-72G18 was used for microinjection to generate the huCD27 transgenic mice.
- 2 transgenic lines (L8 and L54) are established, and transgene expression was consistent with expected profile.

Human CD27 expression in huCD27 Tg mice



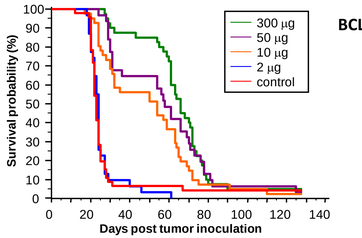
Immunostaining with LG3A10-FITC and its isotype (0.5 μg/ml) and L128-FITC and its isotype (0.1 μg/ml) on acetone fixed frozen tissue sections. Counterstained with Mayer's hematoxylin. LN, lymph nodule; HEV, high endothelial venule; RP, red pulp; WP, white Pulp; CA, central artery.

CDX-1127 stimulation of antigen specific T cells



CDX-1127 agonist activity in mouse tumor models

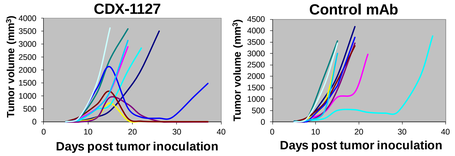
Anti-Tumor activity in huCD27 Tg mice transplanted with syngeneic lymphoma



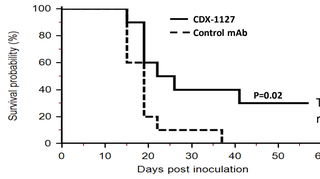
BCL1 lymphoma model

30 – 40 mice per group were inoculated with 10^7 BCL1 cells via i.v.. On day 3, 5, 7, 9 and 11, indicated amounts of CDX-1127 were i.p. injected. Control group was the merging of Tg treated with control mAb and WT treated with CDX-1127. Kaplan-Meyer survival curve was shown.

EG7 thymoma model

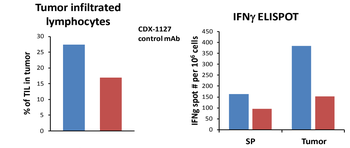


E.G7 cell 1x10^6 s.c. N=10; CDX-1127 or control mAb 50 μg on day 5, 8, 12, 15, 19, 22.



These 3 mice survived from re-challenging with same cells.

CDX-1127 enhances the number and activity of tumor infiltrating lymphocytes

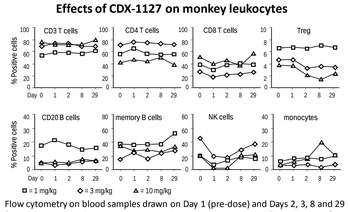


E.G7 cell 0.5x10^6 s.c. N=4; CDX-1127 or control mAb 50 μg i.p. on day 5, 8, 12, 15, 19. Mice were sacrificed when tumor volume ≈0.5 cm^3 (day 20). % of lymphocytes in tumor preparation and IFNγ-spot numbers per 10^6 cells were shown.

CDX-1127 safety and toxicology

- Well tolerated in Monkey toxicology studies (2.5-25 mg/kg X 5)
  - No significant toxicities reported
  - No significant depletion of PBL (see figure below)
  - No cytokine or temperature elevation
- No activation of human PBMC without TCR engagement
  - No cytokine secretion or proliferation
  - Using coated or soluble CDX-1127
- Did not induce proliferation of fresh human B cell lymphoma sample

Effects of CDX-1127 on monkey leukocytes



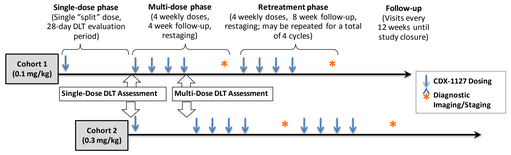
Flow cytometry on blood samples drawn on Day 1 (pre-dose) and Days 2, 3, 8 and 29 from three Cynomolgus macaques treated with a single i.v. dose of 1, 3 or 10 mg/kg CDX-1127.

CDX-1127 Phase 1 clinical trial

Open-label, dose-escalation, safety and pharmacokinetic study of CDX-1127
ARM 1: Selected solid tumors (n ~45)
ARM 2: Hematologic malignancies (n ~45)
Dose-escalation (mg/kg): 0.1⟶0.3⟶1.0⟶3.0⟶10

Refractory or Relapsed B cell hematologic malignancy known to express CD27 including but not limited to chronic lymphocytic leukemia, Burkett's lymphoma, mantle cell lymphoma, primary lymphoma of the central nervous system, and marginal zone B cell lymphoma

Dose-escalation scheme



Summary

- ✓ An anti-CD27 fully human antibody (CDX-1127) has been developed.
- ✓CDX-1127 inhibited the growth of CD27+ human lymphoma and leukemia cells in SCID mice.
- ✓CDX-1127 co-stimulatory activity and anti-tumor efficacy have been demonstrated in a human CD27 transgenic mouse model.
- ✓CDX-1127 safety was evaluated by human PBMC cytokines release assay and in monkey toxicology study.
- ✓Phase I clinical test has been initiated in both CD27+ and CD27- malignancies.

conflict of interest disclosure :
All authors are full-time employees of Celldex Therapeutics Inc.