Efficacy of CDX-1140, an agonist CD40 antibody, in preclinical tumor models

#6289

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ABSTRACT

CD40 on antigen presenting cells plays a critical role in the induction of effective innate and adaptive immune responses. In contrast, CD40 signaling on certain malignant cells, particularly B cell lymphomas, inhibits proliferation or triggers apoptotic cell death. Thus, two independent mechanisms provide opportunities for the use of agonist anti-CD40 monoclonal antibodies in cancer therapy: enhancement of anti-tumor immunity, and direct inhibition of tumor growth.

CDX-1140 is a human IgG2 antibody selected from a panel of fully human mAbs specific for CD40 generated by hybridoma technology from human Ig transgenic mice. We previously demonstrated the potent immune enhancing effects of CDX-1140 using *in vitro* models and in non-human primates. CDX-1140 was shown to activate dendritic cells and B cells in an Fc receptor independent manner. CDX-1140 does not bind to the CD40L binding site, and synergizes with CD40L in stimulation of the CD40 receptor and subsequent functional activities.

Here we further characterized the anti-tumor activity of CDX-1140 on CD40 positive tumors using xenograft models in immunodeficient mice. Using the Ramos and Raji human lymphoblastoma cell lines, CDX-1140 was shown to attenuate tumor growth and prolong survival. Addition of CDX-1140 and human PBMC was highly effective at promoting complete rejection of both Ramos and Raji tumors. Importantly, the epithelial EJ138 bladder carcinoma cell line was also highly sensitive to CDX-1140 treatment. For example, in a study where mice received EJ138 cells subcutaneously, all animals that were treated with 300 µg of CDX-1140 on days 1, 8, and 15, showed suppression of tumor growth through day 60, in comparison to saline-treated animals which developed significant tumors in 7 of 8 animals.

These data support the potential of CDX-1140 for direct anti-tumor effects on CD40-positive tumors (including epithelial tumors) that may supplement its activity as an immune activating agent. CDX-1140 is currently in a phase 1 dose-escalation study in patients with advanced solid tumors.

INTRODUCTION

CD40 represents a unique target for immunotherapy due to its powerful effect on multiple relevant cell types:

- CD40 activation on dendritic cells (DCs) promotes their conversion to antigen presenting cells (APCs) that are efficient for the stimulation of T cell responses,
- CD40 activation on macrophages promotes their ability to mediate effector functions such as phagocytosis,
- CD40 activation on B cells promotes proliferation and antigen presentation,
- CD40 activation on malignant B cells leads to tumor growth inhibition and rejection in xenograft models.

Functional aspects of CD40 agonist antibodies will substantially influence its activity profile:

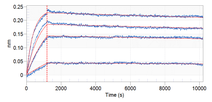
- Block/not block natural ligand (CD40L) interaction,
- Promote/lack Fc receptor interaction,
- Require/not require FcR binding for agonistic function,
- Potency of agonistic activity.

CDX-1140 represents a novel fully human CD40 agonist antibody with unique properties.

BINDING PROPERTIES OF CDX-1140

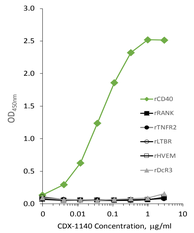
CDX-1140 Binding Kinetics to Human CD40

KD (pM)	Kon (1/Ms)	Kdis (1/s)
10.7	8.13 x 10^5	8.67 x 10^{-6}



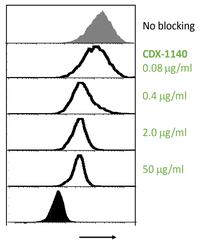
The binding rates and affinity of CDX-1140 to human CD40-Ig were determined using bio-layer interferometry.

Specificity of CDX-1140 for CD40



ELISA plates were coated with rhuCD40-Fc or 5 other TNFRSF members that share the closest homology to CD40. CDX-1140 binding was detected with a goat-anti-human IgG F(ab')$_2$-specific probe.

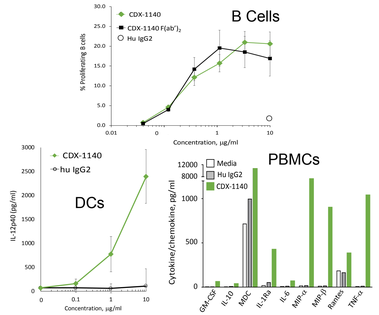
CDX-1140 Reduces but Does Not Block CD40L Binding



Competition of FITC-labeled CD40L by CDX-1140 for binding to CD40 on the rhuCD40 transfected 293 cells as measured by flow cytometry. Unlabeled CD40L was added to show maximum inhibition.

CDX-1140: AGONIST ACTIVITY

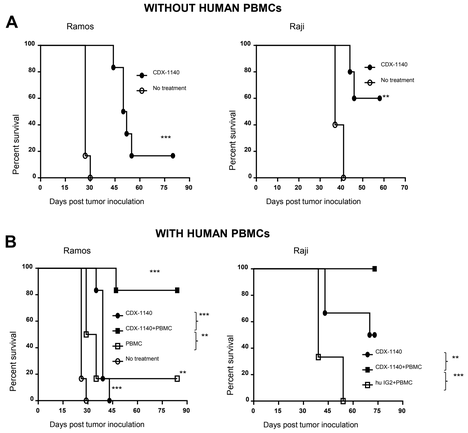
CDX-1140 has Dose-dependent and Fc-independent Agonist Activity



Concentration, µg/ml

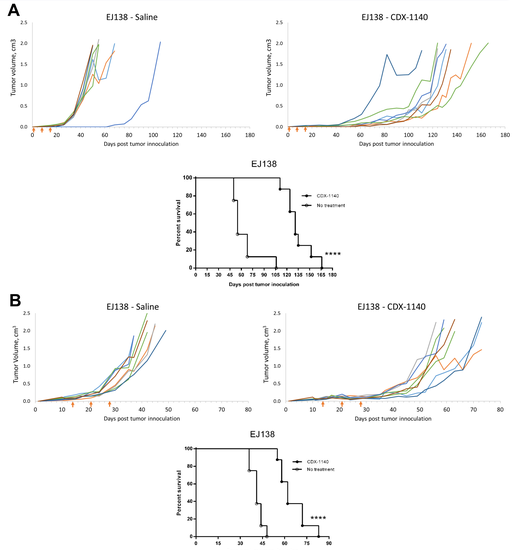
B Cells: CFSE labeled B cells were incubated for 6 days with whole antibody or F(ab')$_2$. The percent of proliferating cells was measured by flow cytometry. DCs: Monocyte-derived DCs were incubated with antibody for 48 hrs. Supernatant was analyzed for IL-12p40 production by ELISA. PBMCs: Peripheral blood mono-nuclear cells (PBMCs) were incubated with the antibodies for 6 days. Supernatants were harvested and analyzed for cytokine production by multiplex analysis.

CDX-1140 ACTIVITY IN XENOGRAFT BURKITT'S LYMPHOMA MODELS



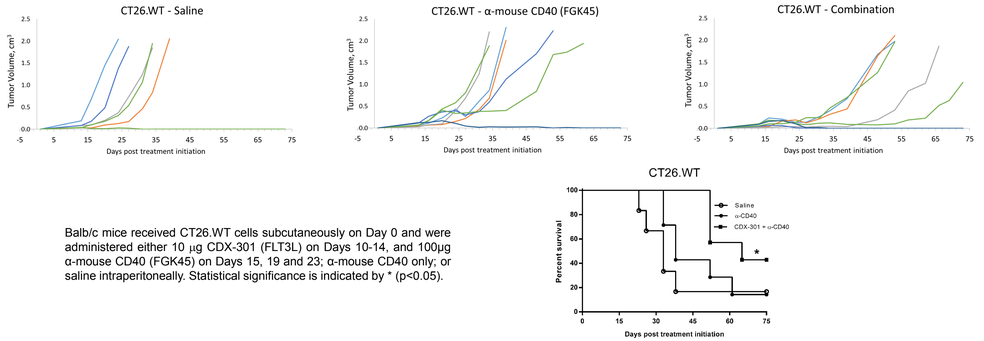
A. Ramos tumor cells (0.5 x 10^6) or Raji tumor cells (1 x 10^6 cells) were injected subcutaneously in the flanks of SCID mice. Mice were split into groups of 5 (Raji) or 6 (Ramos), and treated mice received CDX-1140 (0.3 mg) by intraperitoneal injection on days 1, 6 and 13 after tumor cell inoculation. **B.** Ramos and Raji cells were mixed with 3 x 10^6 human PBMCs and implanted into SCID mice as above and treated with CDX-1140 (0.3 mg) or controls on days 1, 8, and 15 after tumor inoculation (n=6 per group). Data are representative of at least 2 separate studies including different donors for the PBMC experiments. Statistical significance is indicated by ** (p<0.01) or *** (p<0.001).

CDX-1140 ACTIVITY IN A XENOGRAFT BLADDER CARCINOMA MODEL



SCID mice received EJ138 cells subcutaneously and were administered CDX-1140 (0.3 mg) or saline intraperitoneally, once a week for three weeks beginning either the day after tumor implantation (Panel A) or once the mean tumor volume reached approximately 0.1 to 0.2 cm^3 (Panel B). Statistical significance is indicated by **** (p<0.0001).

COMBINATION OF CD40 AGONIST mAb WITH CDX-301 (FLT3L) IN A SYNGENEIC TUMOR MODEL



Balb/c mice received CT26.WT cells subcutaneously on Day 0 and were administered either 10 µg CDX-301 (FLT3L) on Days 10-14, and 100µg α-mouse CD40 (FGK45) on Days 15, 19 and 23; α-mouse CD40 only; or saline intraperitoneally. Statistical significance is indicated by * (p<0.05).

CDX-1140 PHASE 1 CLINICAL TRIAL

A Phase 1 Study of CDX-1140, a Fully Human Agonist anti-CD40 Monoclonal Antibody, in Patients with Advanced Solid Tumors (n~105)

Study CDX1140-01 Dosing Cohorts

Study Portion	Cohort	CDX-1140 Dose Level (mg/kg)	Initial Patients (n)[1]	Additional Patients (n)[2]
Phase 1 Dose-Escalation	1	0.01	1-6	
	2	0.03	1-6	
	3	0.09	3-6	6-9 (up to 12 total)
	4	0.18	3-6	6-9 (up to 12 total)
	5	0.36	3-6	6-9 (up to 12 total)
	6	0.72	3-6	6-9 (up to 12 total)
	7	1.5	3-6	6-9 (up to 12 total)
	8	3.0	3-6	6-9 (up to 12 total)
Phase 1 Expansion Cohorts[3]	9-11	Dose(s) chosen during escalation	10-15 per cohort	

1. It is anticipated that approximately 35 patients (with a maximum of 48 evaluable patients) will be treated in the dose-escalation phase of the study,
2. Upon completion of the dose-escalation phase, selected individual cohorts may be expanded up to 12 total patients per cohort to further inform dose selection for the Phase 1 expansion cohorts.
3. A maximum of 45 patients will be treated in the expansion cohorts (up to 3 cohorts of specific tumor types with up to 15 patients enrolled into each)

SUMMARY AND FUTURE DIRECTIONS

- Two distinct mechanisms provide separate opportunities for the use of agonist anti-CD40 monoclonal antibodies in cancer therapy: enhancement of anti-tumor immunity, and direct inhibition of tumor growth.
- CDX-1140 is a human IgG2 anti-CD40 antibody.
- CDX-1140 does not bind to the CD40L binding site, and synergizes with CD40L.
- CDX-1140 attenuated tumor growth and prolonged survival in xenograft models of the CD40-positive Ramos and Raji human lymphoblastoma cell lines, and the EJ138 bladder carcinoma cell line.
- An anti-CD40 surrogate, in combination with CDX-301 (FLT3L), attenuated tumor growth and prolonged survival in a syngeneic model of the CD40-negative CT26.WT murine colon carcinoma cell line.
- These data support the current Phase 1 dose-escalation study in patients with advanced solid tumors.
- Data also support adding CDX-301 to the Phase 1 CDX-1140 study in the near future.

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