Development of CDX-1140, an agonist CD40 antibody for cancer immunotherapy

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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.

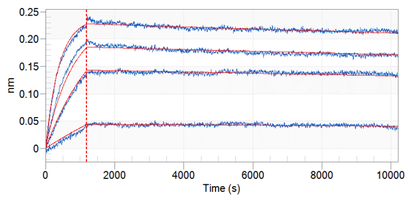
CDX-1140 represents a novel fully human CD40 agonist antibody with unique properties.

Comparisons are presented with CP-870,893, also known as clone 21.4.1 (US patent 8388971)

Binding Properties of CDX-1140

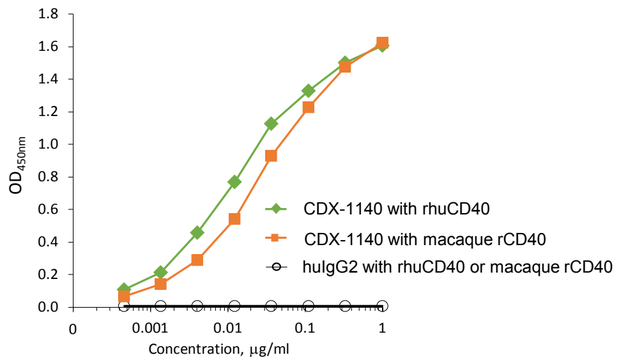
CDX-1140 Binding Kinetics to Human CD40

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



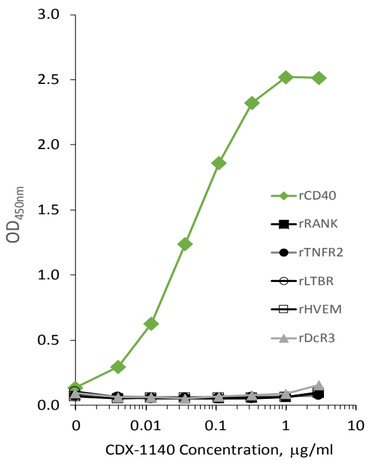
The binding rates and affinity of CDX-1140 to human CD40-Ig were determined using bio-layer interferometry.

CDX-1140 Has Similar Binding to Human and Macaque CD40



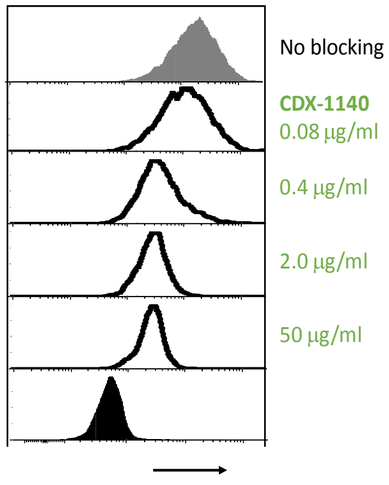
ELISA plates were coated with rhuCD40-Fc or recombinant cynomolgus macaque CD40. CDX-1140 binding was detected with a goat-anti-human IgG F(ab')$_2$-specific probe.

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

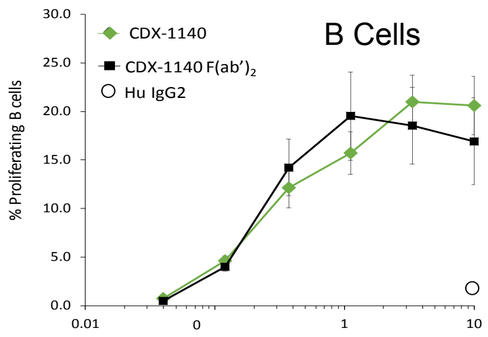


Competition of FITC-labeled CD40L by CDX-1140 for binding to CD40 on the rhuCD40 transfected 293 cells as measured by flow cytometry. Unlabeled CD40L was added to show maximum inhibition.

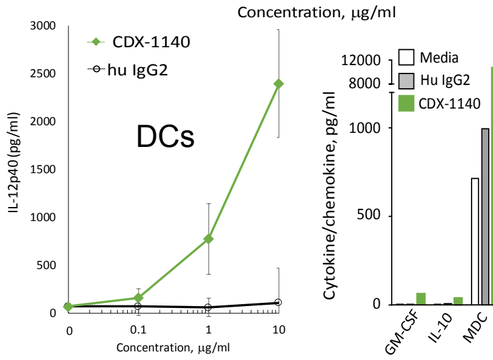
View Poster

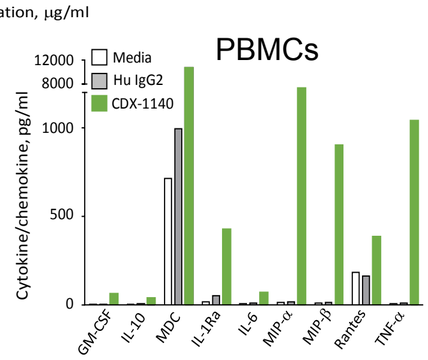
CDX-1140: Agonist Activity

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



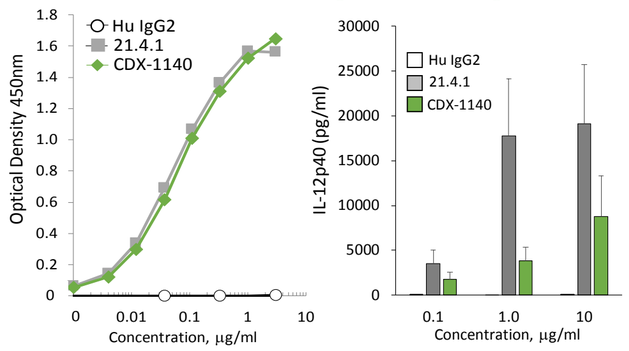
B Cells: CFSE labeled B cells were incubated for 6 days with whole antibody or F(ab')$_2$. The percent of proliferating cells was measured by flow cytometry.
DCs: Monocyte-derived DCs were incubated with antibody for 48 hrs. Supernatant was analyzed for IL-12p40 production by ELISA.
PBMCs: Peripheral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





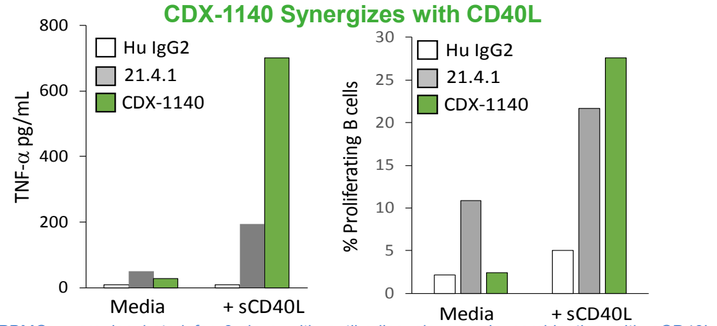
CDX-1140 comparison with 21.4.1

CDX-1140 has Similar Binding but Lower Agonist Activity



ELISA plates were coated with rhuCD40-Fc and antibody binding was detected with a goat anti-human F(ab')$_2$ specific probe. Monocyte derived DCs were incubated with the antibodies for 48 hrs. Supernatant was analyzed for IL-12p40 production by ELISA.

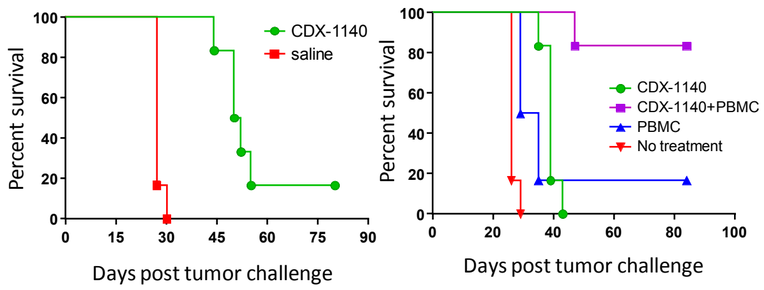
CDX-1140 Synergizes with CD40L



PBMCs were incubated for 6 days with antibodies alone or in combination with sCD40L. Supernatant was harvested and analyzed for TNF-α production by ELISA. Purified CFSE labeled B cells were incubated for 5 days with antibodies alone or in combination with sCD40L. The percent of proliferating B cells was measured by flow cytometry.

CDX-1140: Anti-lymphoma Activity

CDX-1140 has Direct and Indirect Anti-lymphoma Activity



Left Panel: Ramos cells 0.5 x 10^6 were s.c. inoculated on day 0 (n=6). CDX-1140 (0.3 mg) was injected i.p. on day 1, 6 and 13. Right Panel: Ramos cells 1.0 x 10^6 with or without 3 x 10^6 PBMC (from healthy human donor) were injected s.c. on day 0 (n=6). CDX-1140 (0.3 mg) was injected i.p. on day 1, 8 and 15.

IND-Enabling Studies

CDX-1140 *in vitro* Cytokine Response Assays

- CDX-1140 was tested for cytokine production in whole human blood assays in plate-bound and solution format. No significant increases of cytokines (TNFα, IL6, IL1β, IFNγ, IL8, IL-12p40, and IL2) were observed above isotype control.

Tissue Cross-reactivity Studies

- Membrane staining was present only in epithelium in a number of tissues and mononuclear cells in GLP-compliant tissue cross-reactivity study.
- CDX-1140 stained human and monkey tissues similarly.

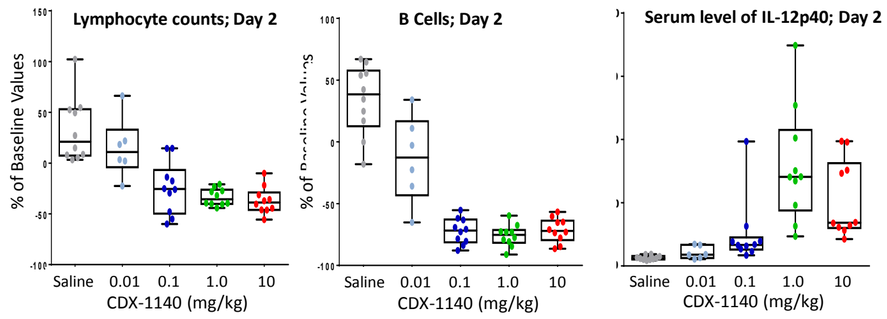
Toxicology Study

- Study Design:

Treatment Group (Dose Level)	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Number of Animals			
				Main		Recovery	
				M	F	M	F
1. Control	0	0	3.5	3	3	2	2
2. CDX-1140 (very-low dose)	0.01	0.00286		3	3	-	-
3. CDX-1140 (low dose)	0.1	0.0286		3	3	2	2
4. CDX-1140 (mid dose)	1	0.286		3	3	2	2
5. CDX-1140 (high dose)	10	2.86		3	3	2	2

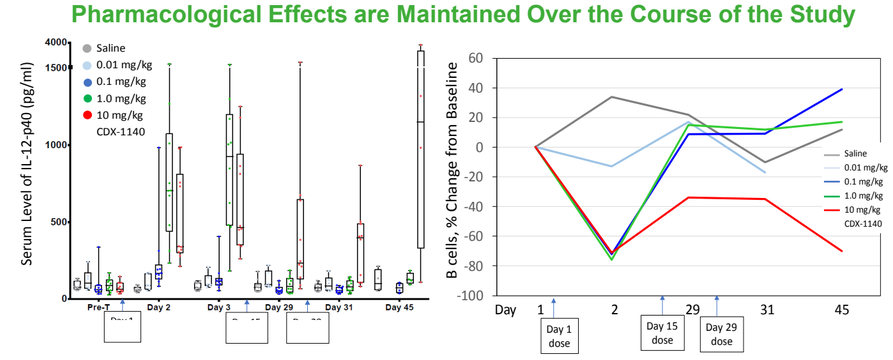
- CDX-1140 was well tolerated in cynomolgus monkeys. The No Observable Adverse Effect Level (NOAEL) was determined to be the high dose level of 10 mg/kg.

Pharmacological Effects Show Dose-dependent CD40 Activation



Analysis of samples at baseline and day 2 (24 hours after dosing) of GLP toxicology study. Lymphocytes are plotted from the absolute lymphocyte counts; B cells were enumerated by flow cytometry, and serum levels of IL-12p40 were measured by ELISA.

Pharmacological Effects are Maintained Over the Course of the Study



The serum IL-12p40 levels and B cells were determined as above. Of note dose levels lower than 10 mg/kg all had significant ADA responses that likely impact the evaluation of their potential pharmacological effects.

CDX-1140: Phase 1 Clinical Trial

Study in Patients with Advanced Solid Tumors

Study Portion	Cohort	CDX-1140 Dose Level (mg/kg)	Initial Patients (n)	Additional Patients (n)
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	9-11	Dose(s) chosen during escalation	10-15 per cohort	

Conclusions and Next Steps

- CDX-1140 represents a novel CD40 agonist antibody with a unique profile relative to other CD40 agonist antibodies:
  - Designed to achieve systemic doses with good tissue penetration,
  - Synergy with CD40L at low doses may promote local agonist activity.
- A Phase 1 Study with CDX-1140 in advanced cancer patients is planned to initiate in 2017.
- Following dose escalation of CDX-1140, combinations will be explored with immunotherapy and conventional therapies.