Functional characterization of CDX-1140, a novel CD40 antibody agonist 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 DCs promotes their conversion to APCs that are efficient for stimulation of T cell responses
- CD40 activation on macrophages promotes their ability to mediate effector function 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

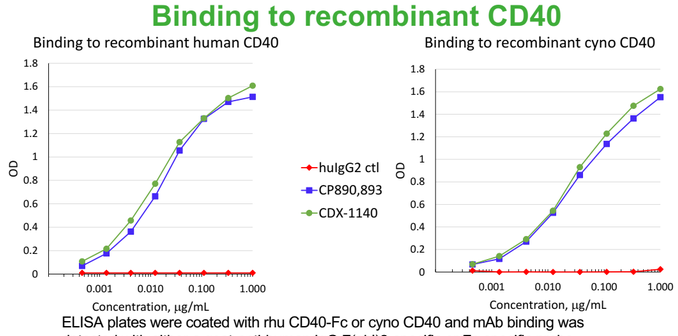
CDX-1140 represents a novel CD40 agonist antibody with unique properties

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

Making and Characterization of CDX-1140

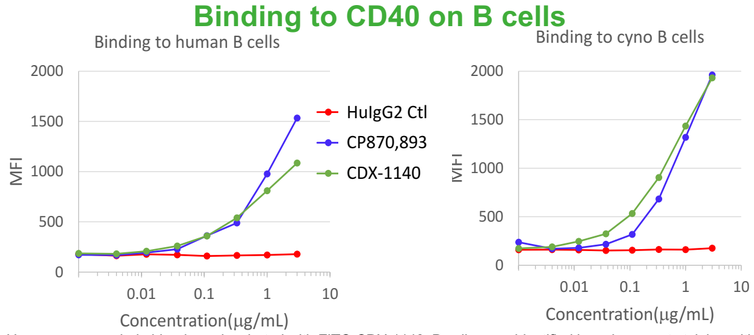
Anti-CD40 monoclonal antibodies (mAbs) were generated by immunization of human Ig transgenic mice (H2L2 strain of Harbour® transgenic mice) with recombinant and cell surface expressed human CD40. Hybridomas were selected using a reporter cell assay engineered to express CD40 and NFκB-responsive luciferase. The variable regions of lead antibodies were cloned in human IgG1 or IgG2 constant domains and expressed in CHO cells. From this panel, CDX-1140, a human IgG2 antibody, was selected for further development.

Binding to recombinant CD40



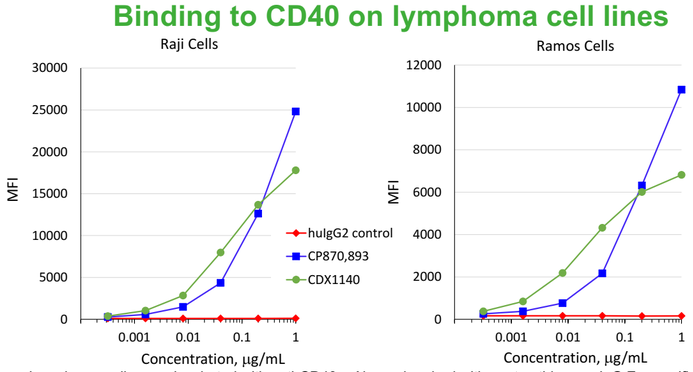
ELISA plates were coated with rhu CD40-Fc or cyno CD40 and mAb binding was detected with either a goat-anti-human IgG F(ab')2-specific or Fc-specific probe.

Binding to CD40 on B cells



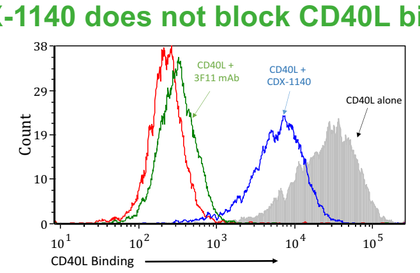
Human or cyno whole blood was incubated with FITC-CDX-1140. B cells were identified by subsequent staining with anti-CD20.

Binding to CD40 on lymphoma cell lines



Lymphoma cells were incubated with anti-CD40 mAbs and probed with goat anti-human IgG Fc-specific PE antibody

CDX-1140 does not block CD40L binding

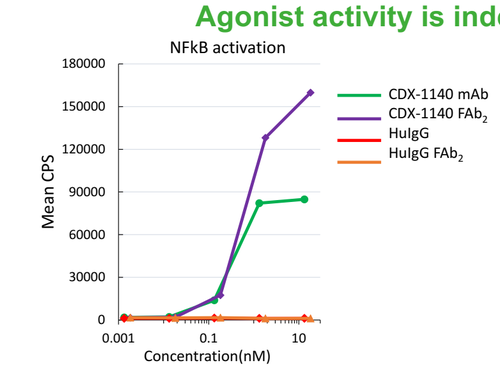


Competition with labeled CD40L for binding to CD40L on the hCD40 transfected 293 cells as measured by flow cytometry. 3F11 is an anti-CD40 mAb that binds the ligand binding domain of CD40.

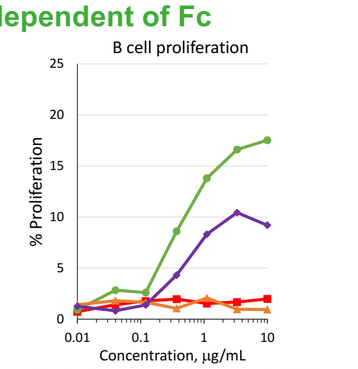
View Poster

CDX-1140: Agonist Activity

Agonist activity is independent of Fc

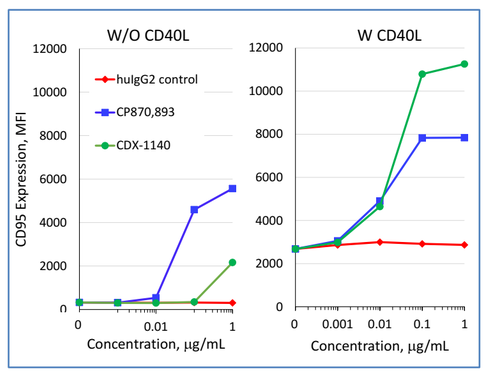


CD40-transfected NFκB-luciferase reporter cells were incubated for 6 hours with CDX-1140 or controls before determining luciferase expression.

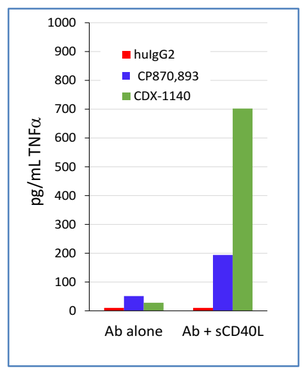


CD19 cells were isolated, stained with CFSE and incubated for 6 days with CDX-1140 or controls.

CD40L enhances CDX-1140 agonist activity

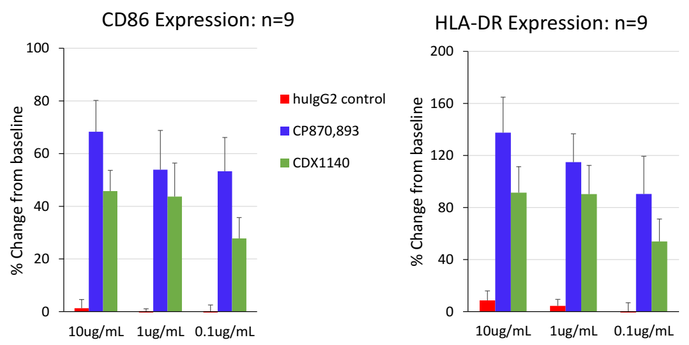


Ramos cells were incubated overnight with anti-CD40 mAbs with or without sCD40L. The cells were stained with anti-CD95-PE antibody and analyzed by flow cytometry.



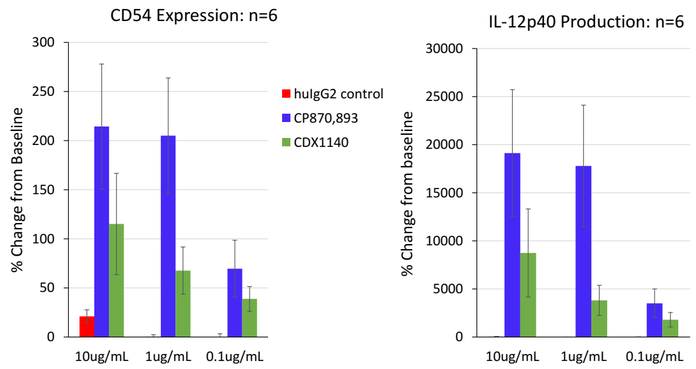
PBMC's were incubated for 6 days with anti-CD40 mAbs +/- sCD40L. Supernatant was analyzed for TNF-α by ELISA

Activation of B cells in whole blood



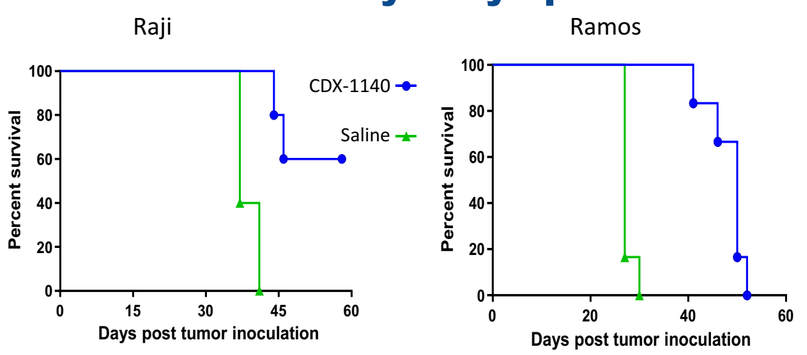
Whole blood was incubated overnight with anti-CD40 mAbs and then stained for CD19 and activation markers before analysis by flow cytometry.

DC activation



Adherent PBMC were cultured for 7 days in media containing 10ng/mL IL-4 and 100ng/mL GM-CSF. DCs were then incubated in the presence of anti-CD40 mAbs for 48 hours. The cells were collected for flow cytometry and the supernatant was analyzed for IL12p40 by ELISA.

CDX-1140: Activity in Lymphoma Model

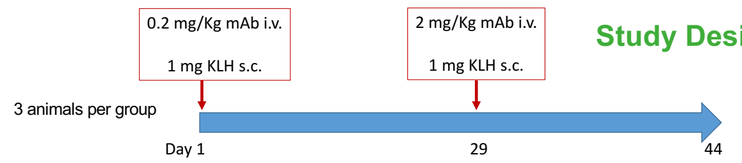


Raji cells 1x10⁶ were s.c. inoculated on day 0; N=5
CDX-1140 was dosed at 0.3 mg i.p. on day 1, 6 and 13.

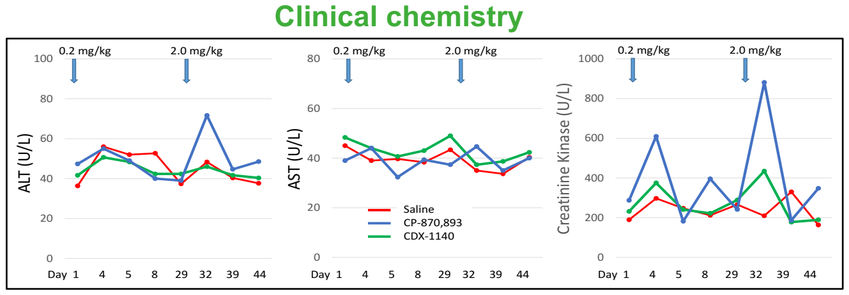
Ramos cells 0.5 x10⁶ were s.c. inoculated on day 0; N=6
CDX-1140 was dosed at 0.3 mg i.p. on day 1, 6 and 13.

CDX-1140: Non-human Primate Pilot Study

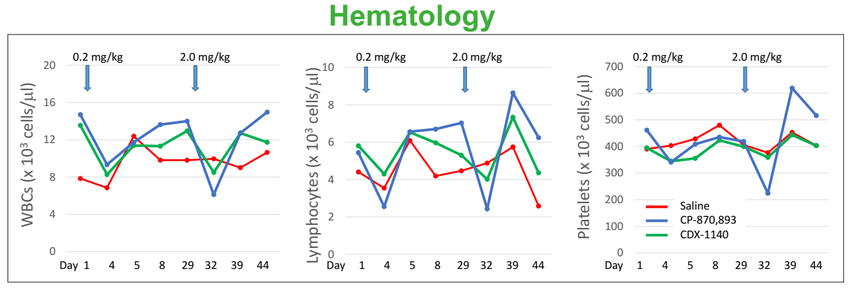
Study Design



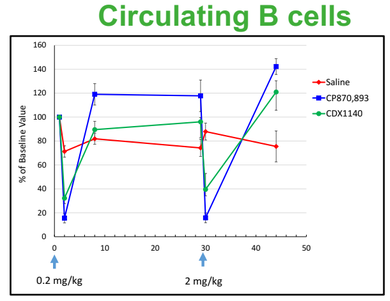
Clinical chemistry



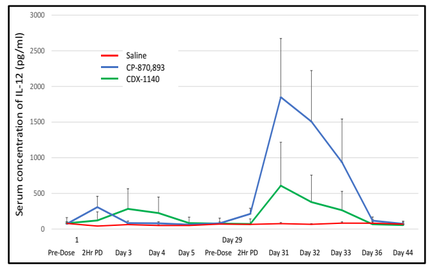
Hematology



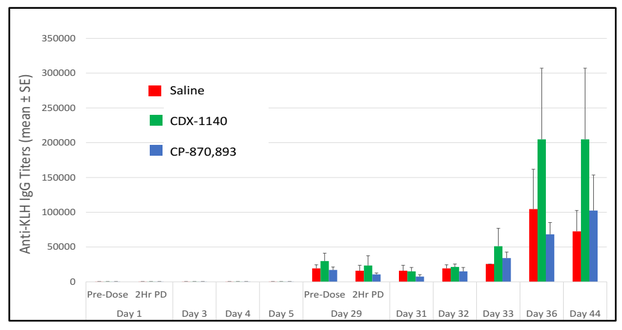
Circulating B cells



Serum IL-12



Anti-KLH titers



Administration of CDX-1140 in this study was well tolerated in cynomolgus monkeys without any toxicity parameter being significantly outside of control levels. Pharmacologic decreases in white blood cells, lymphocytes and neutrophils were seen in CDX-1140 dosed animals, with most significantly a transient decrease in B cells. Increase in KLH-specific IgG was observed with CDX-1140, but not significant due to few animals and significant variability.

Conclusions and Next Steps

- CDX-1140 represents a novel CD40 agonist antibody with a unique profile:
  - Potent agonist that functions independent of FcR interactions
  - Strong cooperation with CD40L for enhanced activity
  - Direct anti-lymphoma activity in vivo
  - Pharmacologic activity in monkeys minimal evidence of toxicity
- CDX-1140 manufacturing and IND-enabling studies are on-going
- 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

Celldex therapeutics