

# # 1848 CDX-1140, A Novel Agonist CD40 Antibody with Potent Anti-lymphoma Activity

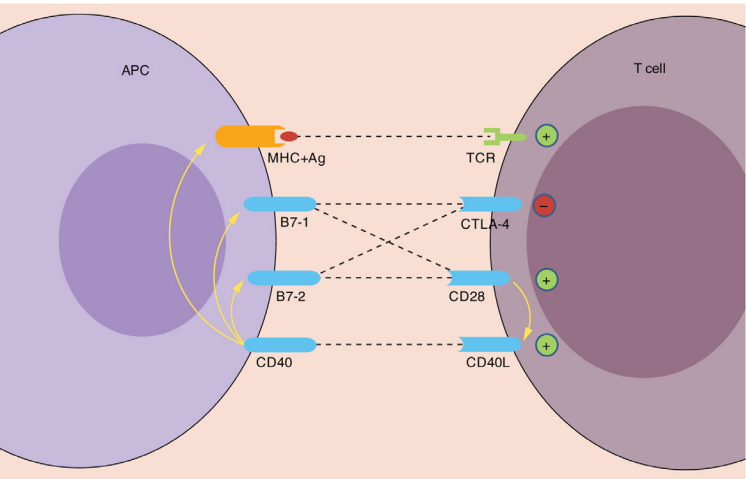
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## CD40 Introduction

- CD40 is a cell surface glycoprotein in the TNF receptor (TNFR) family.
- CD40 is expressed constitutively and upregulated upon activation on antigen-presenting cells (APCs), including B cells, dendritic cells (DCs) and macrophages.
- CD40 interacted with its ligand (CD40L, aka CD154), which is rapidly induced on T cells following TCR activation, plays essential roles in the modulation of adaptive immunity.
  - Humoral responses:
    - B cell proliferation,
    - immunoglobulin (Ig) production
    - isotype switching
    - memory B-cell generation
  - T-cell-mediated licensing of APCs for antigen-presenting function:
    - increase surface expression of MHC molecules
    - upregulate costimulatory molecules to provide “second signals”
    - release pro-inflammatory cytokines and chemokines to facilitate the polarization of Th1-type immune responses and cytotoxic T lymphocyte priming
  - Enhancement of macrophage effector functions, such as phagocytosis
- CD40 is expressed in a variety of malignances, especially B cell lymphoma, being a target for antibody therapy.

## Role of CD40/CD40L in T Cell Activation



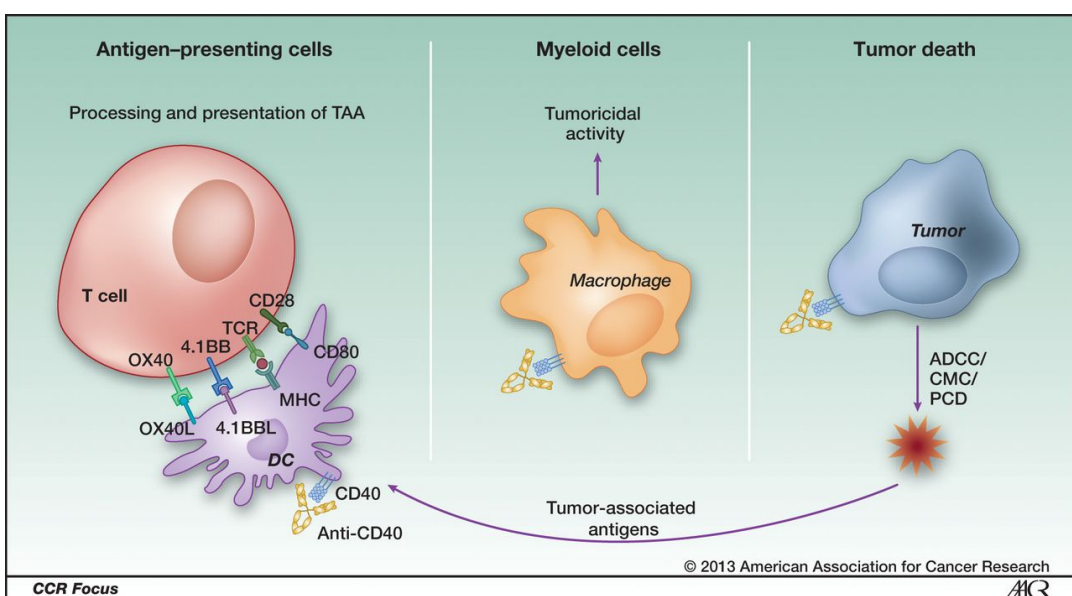
Tianshu Zhang, Richard N Pierson III Agnes M Azimzadeh  
*Immunotherapy*. 2015 August ; 7(8): 899–911.

## CD40 Antibody for Lymphoma Therapy

- Stimulating CD40 signaling on B cell lymphoma can inhibit proliferation and promote apoptosis.
- Ligation of CD40 on APCs can substitute for stimulation normally provided by helper T cells via CD40L to activate and promote antitumor T-cell responses.
- Triggering CD40 on myeloid cells, especially macrophages, can activate them with the potential to control malignancies in a T cell-independent manner.
- Targeting CD40 on B cell lymphoma with a depleting mAb can induce killing by ADCC, CMC and ADCP.
- CD40 mAb in combination can sensitize other anti-cancer agents, including vaccine, cytotoxic drugs, etc.
- Due to the broad expression profile of CD40 and the potency of this signaling pathway, toxicity is a critical challenging in exploiting CD40 target as antitumor therapeutics.

We set out to develop a panel of human CD40 mAbs with different levels of agonist activity. A lead candidate, named as CDX-1140, for systemic use is identified based on unique properties in agonist activity, anti-lymphoma activity in xenograft models and safety profile in non-human primate. Here we focus on the anti-lymphoma efficacy both in single agent and in combination with anti-CD27 mAb or with human PBMC.

## Potential Mechanisms of Action of Agonistic CD40 mAb on Various Immune Effectors



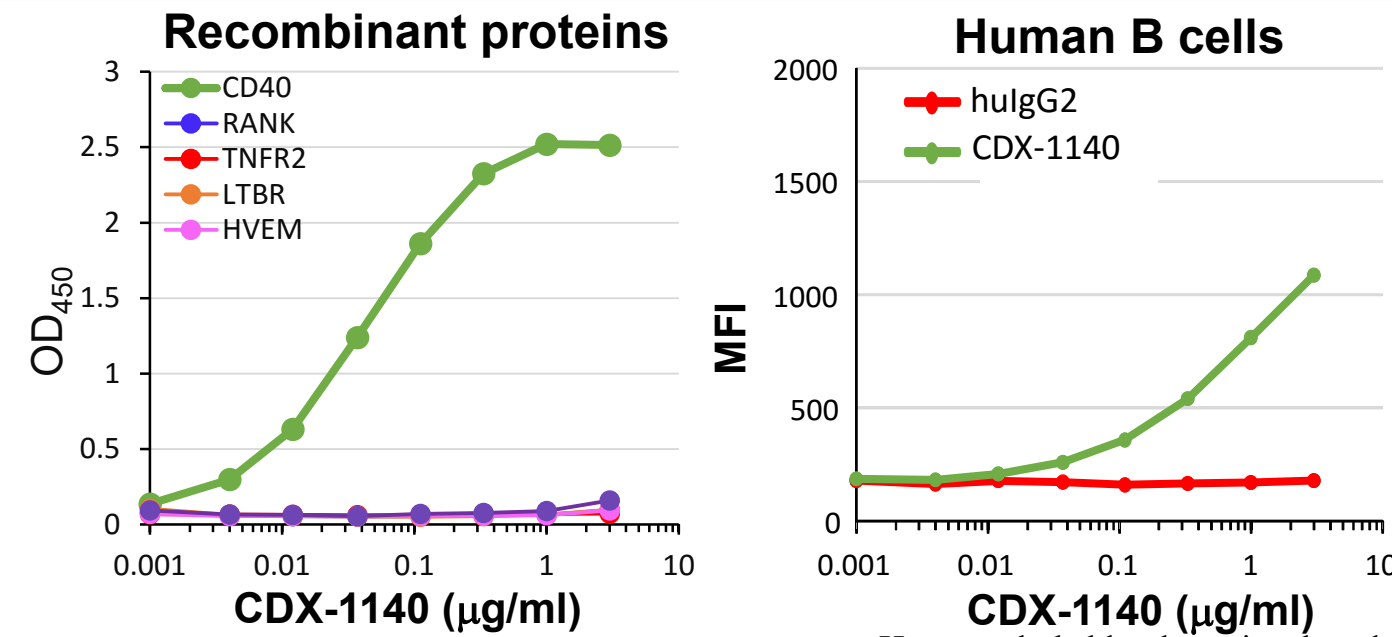
Robert H. Vonderheide, and Martin J. Glennie  
*Clin Cancer Res* 2013;19:1035-1043  
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Clinical  
Cancer Research

## Development and Characterization of CDX-1140

- A panel of 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.
- VL and VH sequences were obtained from hybridoma and expressed as huIgG1 and huIgG2a by transient transfection.
- CDX-1140 (human IgG2) was identified as a lead candidate after in vitro and in vivo characterization and non-human primate pilot study.

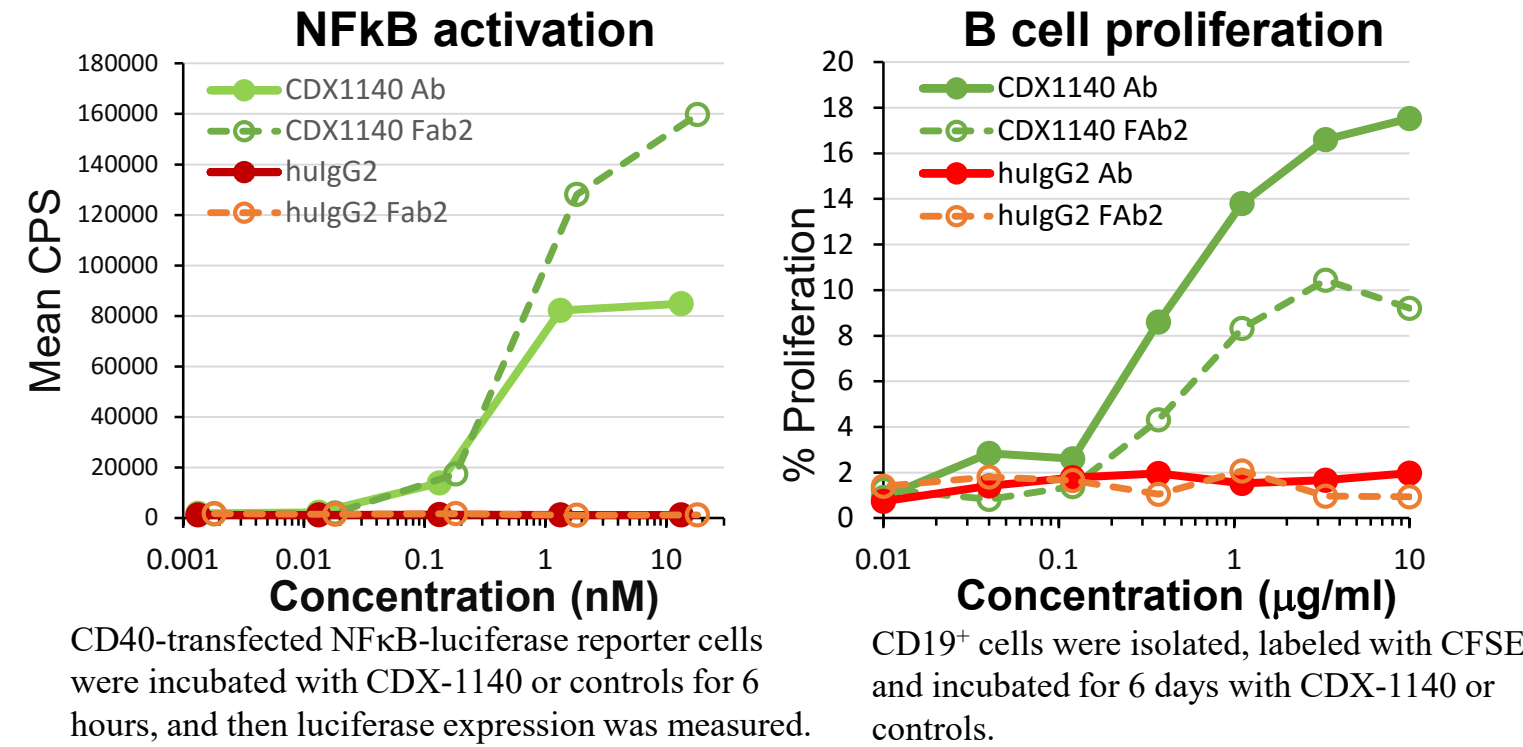
## CDX-1140 Binding to CD40 by ELISA and Flow Cytometry



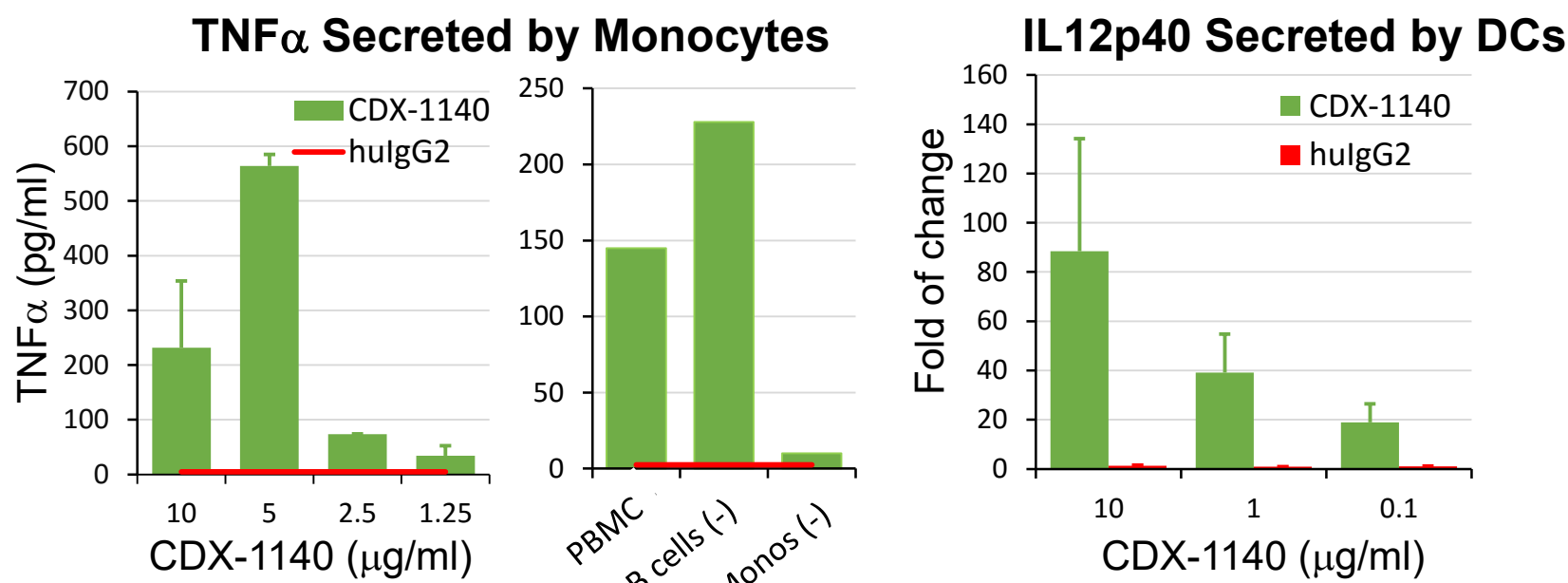
The TNFR antigens were coated on a plate at 2 μg/ml. CDX-1140 were added and the binding to each antigen was detected with an HRP labeled goat anti-human IgG Ab.

Human whole blood was incubated with FITC-CDX-1140. B cells were identified by anti-CD20 mAb.

## CDX-1140 Agonist Activity Is Fc-independent



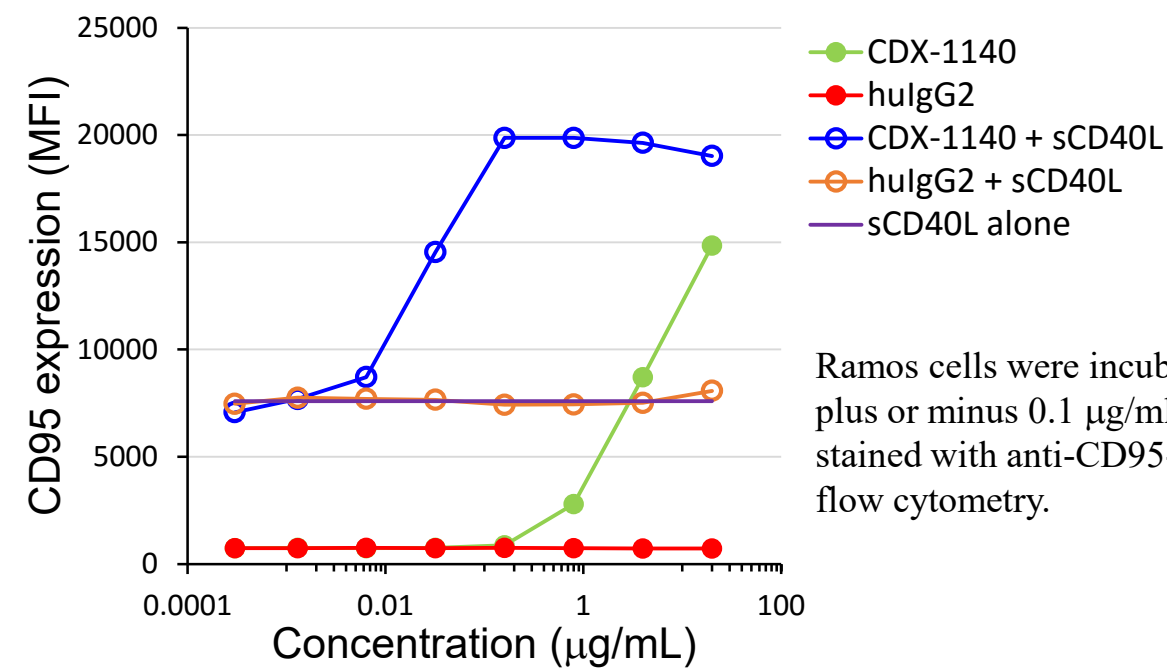
## CDX-1140 Induces Cytokines Production



Cells were cultured for 6 days with CDX-1140. Supernatants were harvested and tested for TNFα production by ELISA. (-) B cells = B cell depleted PBMC (Miltenyi CD19 kit) (-) monos = Monocyte depleted PBMC (Miltenyi CD14 kit)

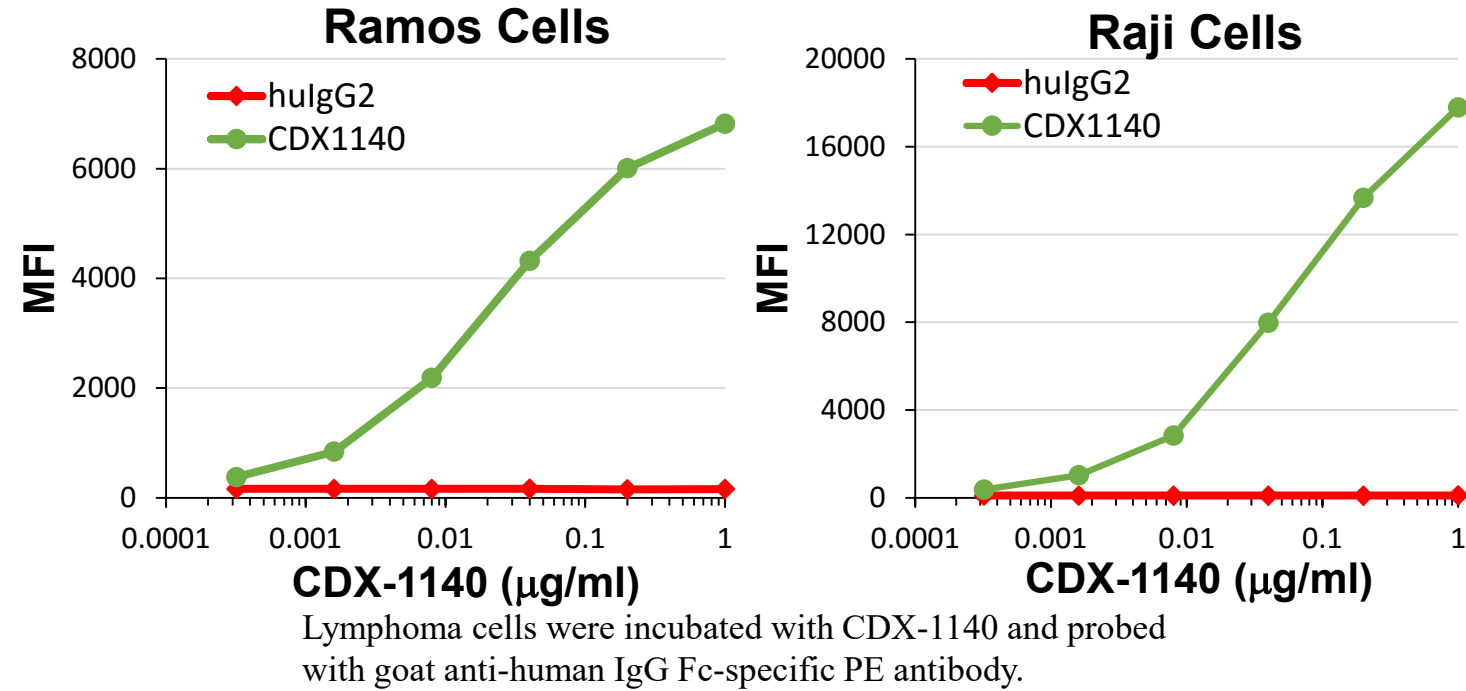
DCs were generated by culturing adherent PBMC for 7 days in the presence of 10ng/ml IL4 and 100ng/ml GM-CSF (N=6), and then incubated in the presence of CDX-1140 for 48 hours. The supernatant was analyzed for IL12p40 by ELISA.

## CDX-1140 Synergizes with sCD40L

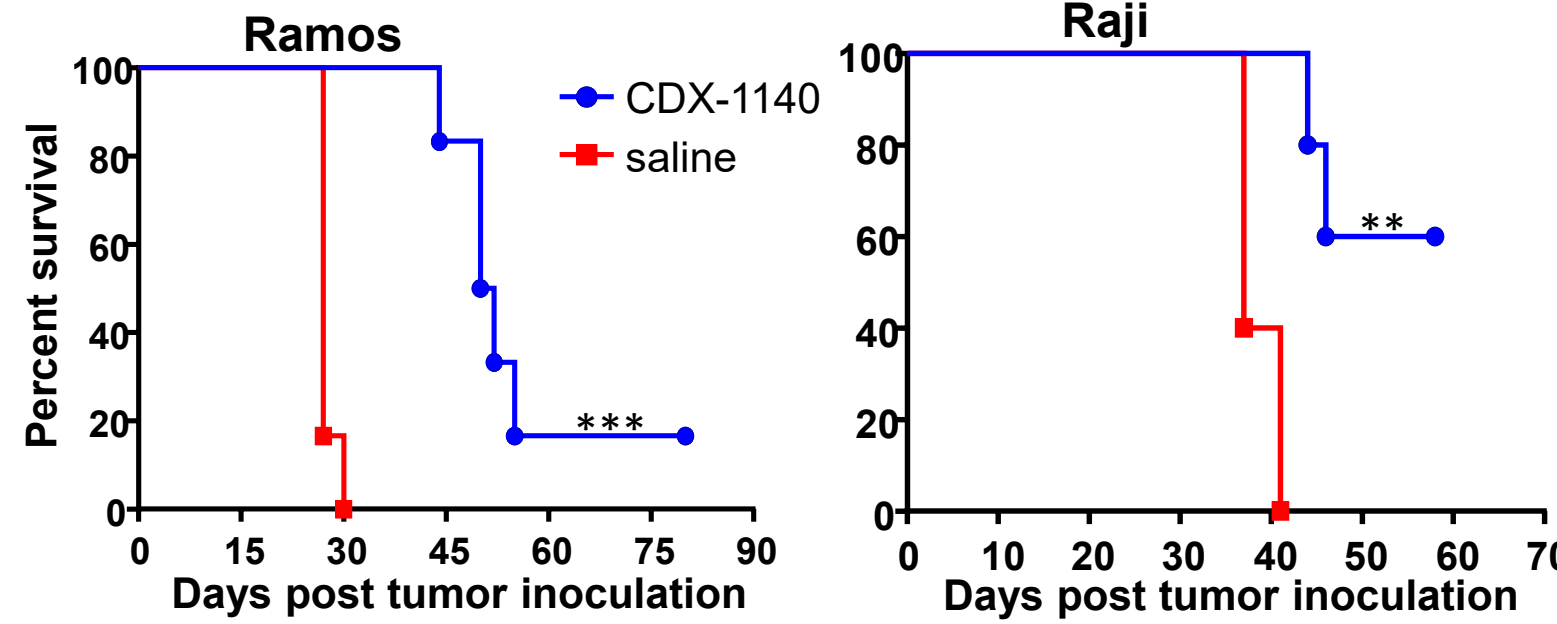


Ramos cells were incubated overnight with CDX-1140 plus or minus 0.1 μg/ml sCD40L. The cells were then stained with anti-CD95-PE antibody and analyzed by flow cytometry.

## CDX-1140 Anti-lymphoma Activity in Xenograph Models

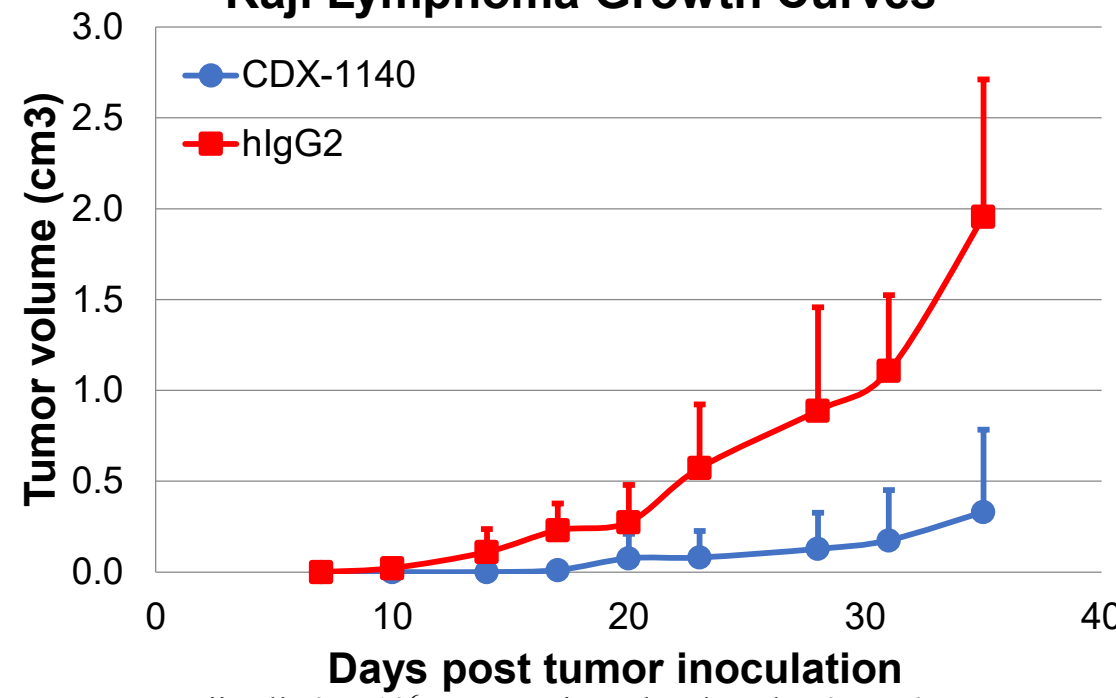


## CDX-1140 Direct Anti-lymphoma Activity



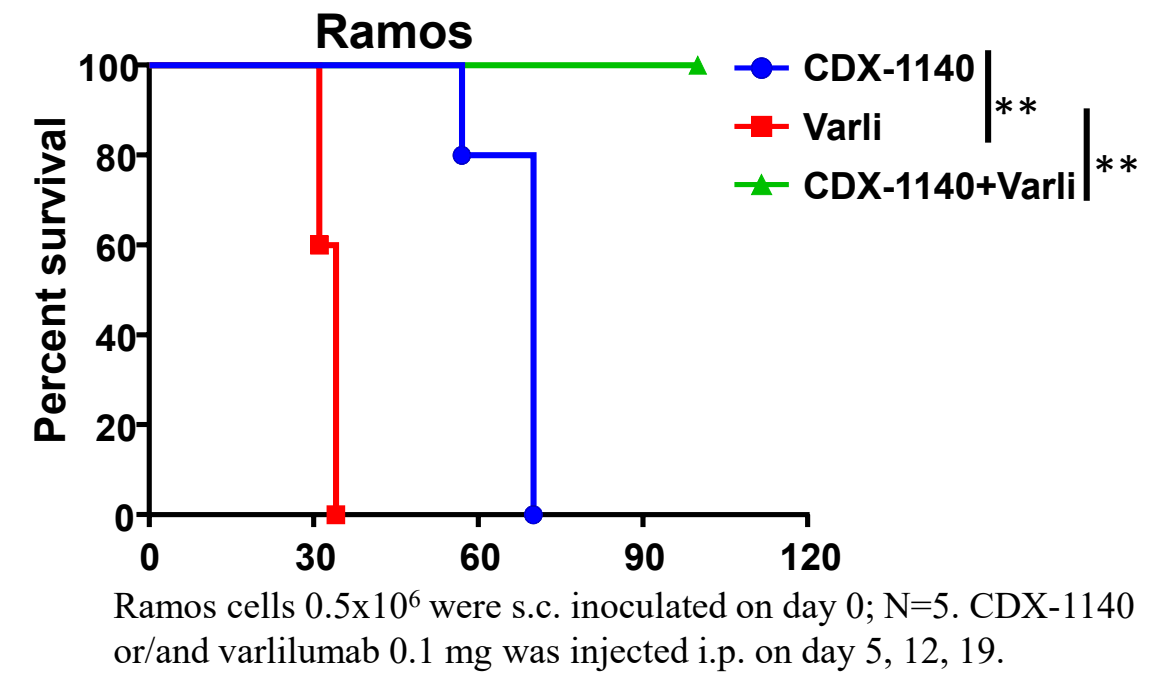
Ramos cells 0.5x10<sup>6</sup> were s.c. inoculated on day 0; Raji cells 1.0x10<sup>6</sup> were s.c. inoculated on day 0; N=6. CDX-1140 0.3 mg i.p. on day 1, 6, 13. N=5. CDX-1140 0.3 mg i.p. on day 1, 6, 13.

## Raji Lymphoma Growth Curves

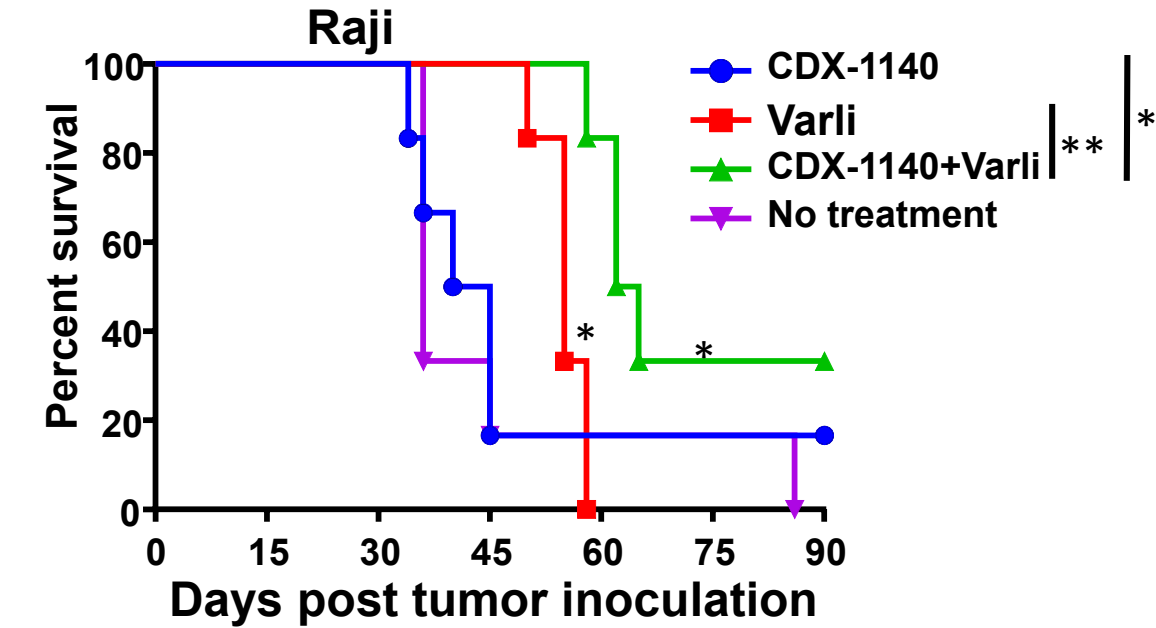


Raji cells 0.5x10<sup>6</sup> were s.c. inoculated on day 0; N=6. CDX-1140 or huIgG2 0.3 mg was injected i.p. on day 1, 8, 15.

## Synergy of CDX-1140 & Varlilumab

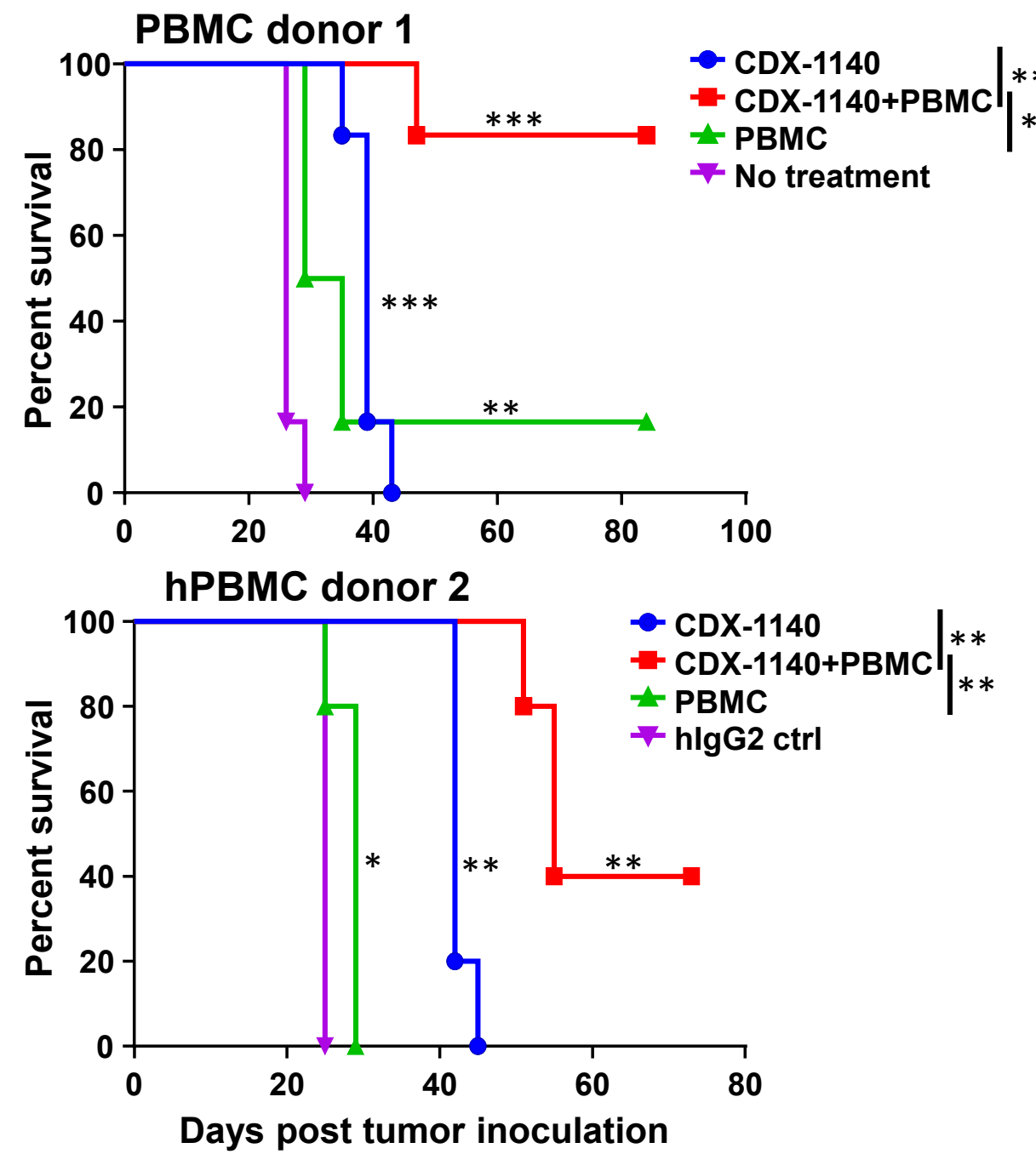


Ramos cells 0.5x10<sup>6</sup> were s.c. inoculated on day 0; N=5. CDX-1140 or/and varlilumab 0.1 mg was injected i.p. on day 5, 12, 19.

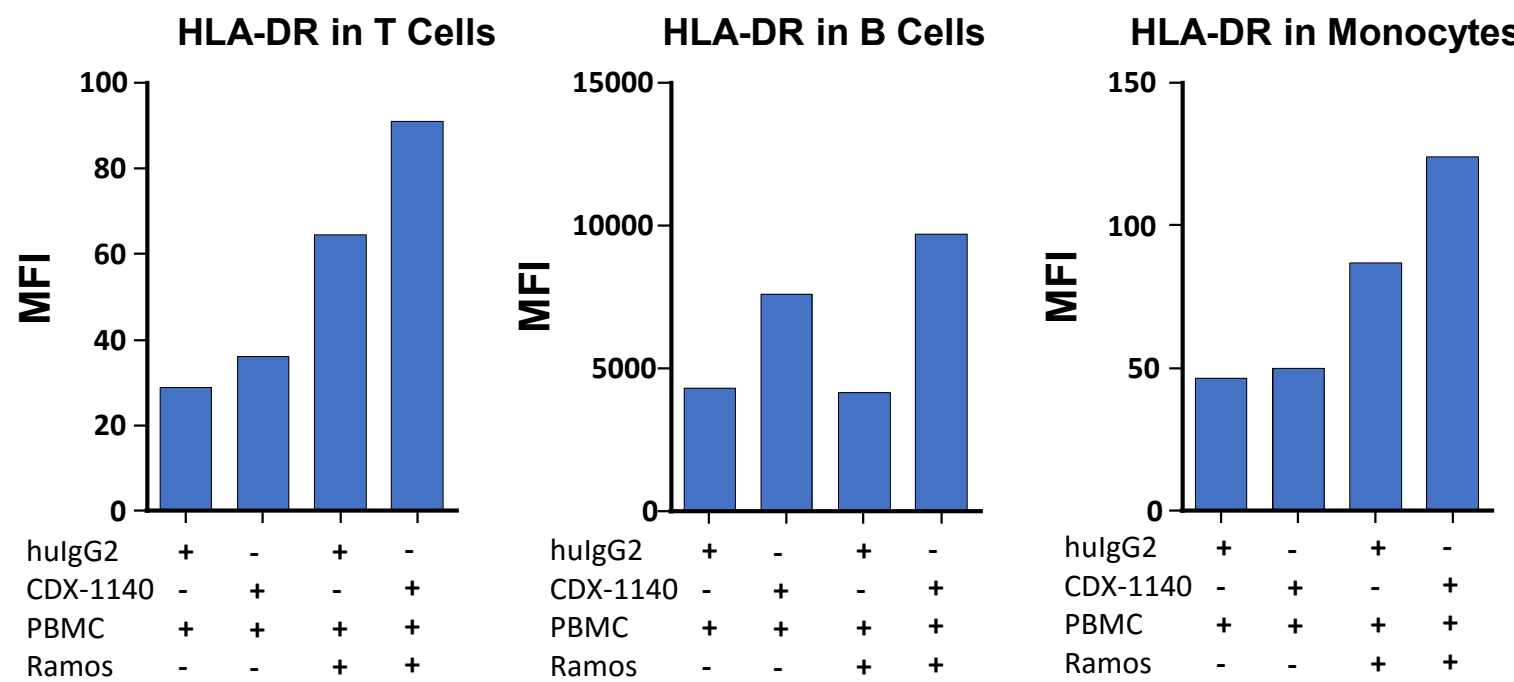


Raji cells 1.0x10<sup>6</sup> were s.c. inoculated on day 0; N=6. CDX-1140 0.3 mg i.p. on day 2, 7, 12; varlilumab 0.1 mg i.p. on day 5, 12, 19; and the combo.

## CDX-1140 in Combination with PBMC



Ramos cells 1.0x10<sup>6</sup> with or without 3x10<sup>6</sup> PBMC were s.c. inoculated on day 0; N=6. CDX-1140 0.3 mg was injected i.p. on day 1, 8 and 15.



PBMC were co-cultured with Ramos cells at a ratio of 5:1 in a 96-well U-bottom plate for 48 hrs in the presence of 0.1 μg/ml CDX-1140 or huIgG2. Cells were stained with antibodies for CD3, CD20 and HLA-DR.

## Summary and Next Steps

- CD40 is a promising and powerful target for immunotherapy, but requires an appropriate balance between antitumor immune activation and harmful side effects of immune stimulation.
- CDX-1140 represents a novel CD40 agonist antibody with unique profile:
  - Potent agonist that functions in the format of human IgG2 isotype and independent of Fc receptors interaction
  - Strong synergy with sCD40L for enhanced activity
  - Activation of human B cells, DC and monocytes in vitro
- CDX-1140 has potent anti-lymphoma efficacy in xenograft models
  - Direct anti-tumor activity against CD40<sup>+</sup> lymphoma
  - Enhanced anti-tumor activity when combined with human immune cells
  - Activation of T cells, B cells, myeloid cells in co-cultures of PBMC with lymphoma cells
  - Synergy with anti-CD27 mAb varlilumab
- Based on these data and a pilot study with non-human primates that confirmed a good safety profile, CDX-1140 is progressing towards clinical trials.
  - A Phase 1 study with CDX-1140 in advanced cancer patients, including lymphoma patients, is planned to initiate in 2017.
  - Following dose escalation of CDX-1140, combinations will be explored with immunotherapy and conventional therapies.