CDX-585, a Bispecific Antibody with Dual Targeting of ILT4 and PD-1 Checkpoint Pathways

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Background

- Novel approaches are needed to improve outcomes for patients whose tumors are not responsive or develop resistance to checkpoint inhibition (CPI).
- The ILT4/LILRB2 is an ITIM-bearing receptor expressed by myeloid cells.
  - Engagement of ILT4 receptor by its cognate ligands (HLA-G and HLA Class I) inhibits myeloid cell activation.
  - Various tumors upregulate ILT4 and/or its ligands and their expression correlates with poor outcome.
  - ILT4 signaling has been postulated as a resistance mechanism for CPI of PD-1 and CTLA-4.
  - Early clinical data with the ILT4 antagonist MK-4830 demonstrated good tolerability and promising clinical activity when combined with pembrolizumab, including in patients with CPI-refractory disease[1].
- Bispecific antibodies (bsAbs) provide a promising strategy for dual inhibition of receptors that suppress myeloid and T cell compartments using a single molecule.
- Herein we describe the discovery and characterization of CDX-585, a bsAb developed from novel ILT4 (7B1) and PD-1 (E1A9) antagonist mAbs.

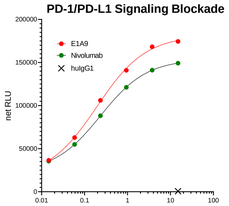
[1] Siu LL, Wang D, Hilton J, Geva R, Rasco D, Perets R, Abraham AK, Wilson DC, Markevnikhv JF, Lunceford J, Suttner L, Siddiqi S, Altura RA, Maurice-Dror C. First-in-Class Anti-Immunoglobulin-Like Transcript 4 Myeloid-Specific Antibody MK-4830 Abrogates a PD-1-Resistance Mechanism in Patients With Advanced Solid Tumors. Clin Cancer Res. 2021 Oct 1:clincanres.CCR-21-2160-A-2021. doi: 10.1158/1078-0432.CCR-21-2160.

αILT4 and αPD-1 mAb Characterization

αPD-1 mAb E1A9

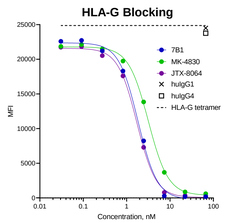
- E1A9 is a novel αPD-1 humanized IgG1k mAb, harboring Fc modifications to eliminate FcγR binding (L234A, L235Q, K322Q, referred to as AQQ)
- E1A9 has sub-nanomolar affinity for human and cynomolgus macaque PD-1
- E1A9 is a potent inhibitor of PD-1 signaling

PD-1 effector cells and PD-L1 APCs were co-cultured in the presence of mAbs for 6 hours before measuring luminescence. When co-cultured, the PD-1/PD-L1 interaction inhibits TCR-mediated luminescence, when this interaction is disrupted TCR activation induces luminescence via the NFAT pathway (Promega kit J1250).

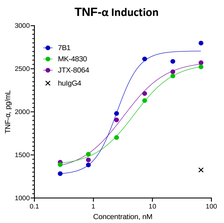


αILT4 mAb 7B1

- 7B1 is a novel αILT4 humanized mAb, expressed as an IgG1κ with Fc modifications that prevent FcγR binding (AQQ)
- 7B1 has sub-nanomolar affinity for human ILT4 and cross-reacts with cynomolgus macaque ILT4 with lower affinity
- 7B1 is a potent inhibitor of HLA-G and HLA-A2 (data not shown) binding to ILT4 and enhances TNF-α production by macrophages stimulated with LPS



Titrated mAbs were incubated with ILT4-expressing 293 cells and then exposed to fluorescently-labeled HLA-G tetramer.



Monocyte-derived macrophages (M-CSF) were incubated overnight with mAbs and LPS. Supernatant was harvested and analyzed for TNF-α production by ELISA.

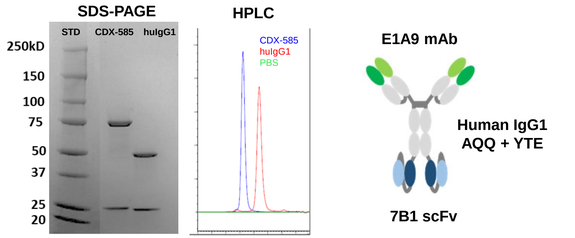
CDX-585 Development and Characterization

αPD-1 mAb E1A9 heavy chain was genetically linked to single chain variable domains of αILT4 mAb 7B1 and expressed as full length IgG1κ

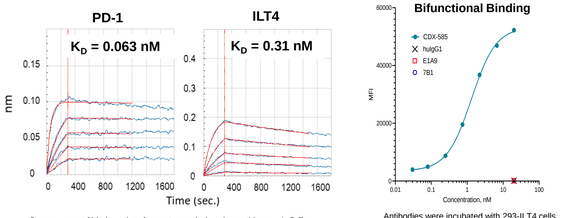
- Modified to eliminate FcγR binding and effector function (AQQ)
- Improved PK through enhanced FcRn binding (M252Y, S254T, T256E, referred to as YTE)
- Tetravalent antigen binding
  - Bivalent for ILT4 and PD-1 for high affinity binding

CDX-585 Characterization

- CDX-585 displays antibody-like characteristics with dual antigen engagement



Reducing SDS-PAGE on 4-15% Tris-HCL gel. SEC-HPLC performed on TSK3000 column with 20 µg injections in PBS

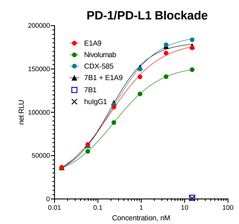


Sensorgrams of bio-layer interferometry analysis using anti-human IgG-Fc sensors to capture the antibodies followed by incubation with HIS–tagged human ILT4 or human PD-1.

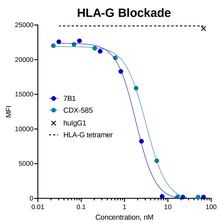
Antibodies were incubated with 293-ILT4 cells, followed by soluble human PD-1-mouse Fc. Bifunctional binding was detected with a PE labeled goat anti-mouse IgG.

Potent Blockade of PD-1/PD-L1 Signaling and HLA-G Ligand Binding

- CDX-585 retains the ability to potently block PD-1/PD-L1 signaling and HLA-G ligand binding



PD-1 effector cells and PD-L1 APCs were co-cultured in the presence of antibodies. Activation of the NFAT pathway via PD-1/PD-L1 blockade was detected by increasing luminescence using Bio-Glo™ reagent (Promega kit J1250).

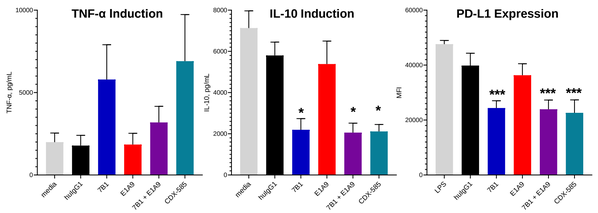


Titrated antibodies preincubated with ILT4-expressing 293 cells, block fluorescently-labeled HLA-G tetramer binding.

CDX-585 *In Vitro* Activity

ILT4 Inhibition with CDX-585 Drives M1 Macrophage Polarization

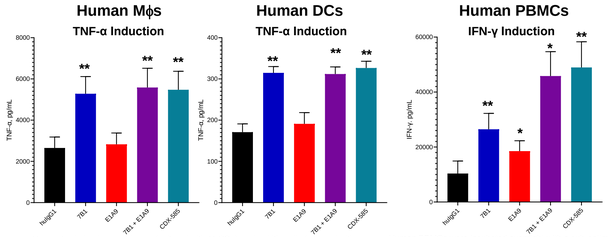
- Human macrophages differentiated in the presence of CDX-585 lead to an enhanced proinflammatory phenotype and downregulation of IL-10 secretion and PD-L1 surface expression



Human monocytes were incubated for 6 days with M-CSF in the presence of antibodies (6.7 nM). After differentiation, cells were activated with LPS overnight. Supernatant was harvested and analyzed for TNF-α and IL-10 production by ELISA. The cells were stained for CD274 expression and analysis by flow cytometry. Statistical significance vs. huIgG1 control measured by student's paired T-test, * = p < 0.05, *** = p < 0.001.

CDX-585 Potentiates Proinflammatory Phenotype in Myeloid and T Cells

- Enhanced cytokine production through ILT4 blockade by human macrophages and DCs in response to LPS
- T cell activation by CDX-585 via dual inhibition of ILT4 and PD-1

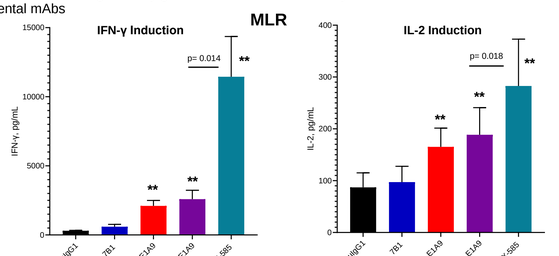


Monocyte-derived macrophages (cultured with M-CSF) or dendritic cells (cultured with GM-CSF/IL-4) were incubated overnight with antibodies (33 nM) and LPS. Supernatant was harvested and analyzed for TNF-α production by ELISA. Statistical significance vs. huIgG1 control measured by student's paired T-test, ** = p < 0.01.

Human PBMCs were incubated overnight with a sub-optimal concentration of anti-CD3 antibody (OKT3) before addition of antibodies (33 nM) and then incubated for 3 days. Supernatant was harvested and analyzed for IFN-γ production by ELISA. Statistical significance vs. huIgG1 control measured by student's paired T-test, * = p < 0.05, ** = p < 0.01.

CDX-585 Exhibits Synergistic Effects in Mixed Lymphocyte Reaction

- Co-inhibition of ILT4 and PD-1 receptors via CDX-585 leads to a synergistic upregulation of IFN-γ and IL-2 secretion by T cells in mixed lymphocyte reactions (MLR)
- These effects are significantly greater than those exhibited by a combination treatment with parental mAbs

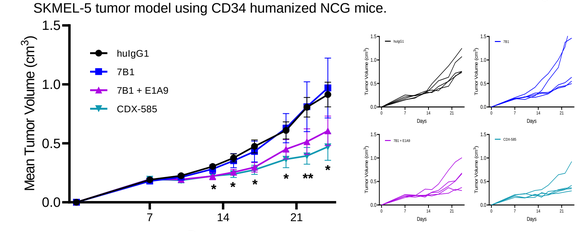


Purified CD4+ T cells and dendritic cells were prepared from independent donor PBMCs (n = 8). Dendritic cells were activated overnight with LPS, then co-cultured with allogeneic CD4+ T cells in the presence of antibodies (5 nM) for 4 days. Supernatant was harvested and analyzed for IFN-γ and IL-2 production by ELISA. Statistical significance vs. huIgG1 control measured by student's paired T-test, ** = p < 0.01.

CDX-585 *In Vivo* Activity

CDX-585 Exhibits Antitumor Activity in Tumor Model

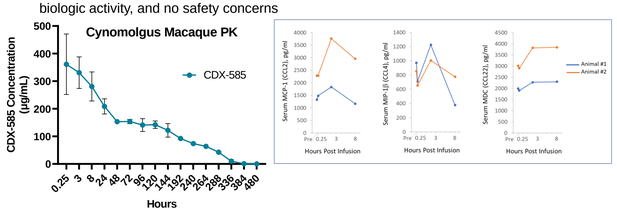
- Co-inhibition of ILT4 and PD-1 receptors via CDX-585 leads to enhanced tumor killing in SKMEL-5 tumor model using CD34 humanized NCG mice.



Twenty HuCD34-NCG mice (Charles River Laboratories), from two donors, were divided into four groups of five mice each. Mice were implanted with 2 x 10^7 SKMEL-5 cells subcutaneously. Starting on the day following implantation, mice were treated as follows: Group 1: Human IgG1 AQQ (0.5 mg/mouse), Group 2: CDX-585 (0.5 mg/mouse), Group 3: 7B1 (0.375 mg/mouse), and Group 4: E1A9 (0.375 mg/mouse) and 7B1 (0.375 mg/mouse). Mice were dosed once a week for 5 weeks. Tumor volumes were measured periodically. Statistical significance vs. huIgG1 control measured by student's T-test, * = p < 0.05, ** = p < 0.01.

CDX-585 Exhibits a Favorable Pharmacokinetic Prolife in Cynomolgus Macaques

- A pilot study in cynomolgus macaques demonstrates a good pharmacokinetic profile, biologic activity, and no safety concerns



- Animals were given single i.v. dose (10 mg/kg) of CDX-585
- No significant adverse laboratory or clinical findings
- Serum concentrations of CDX-585 were measured by ELISA
- Serum concentrations of cytokine/chemokines were analyzed by MesoScale Discovery (MSD)

Conclusions

- **Simultaneous inhibition of ILT4 and PD-1 checkpoints with CDX-585 leads to myeloid and T cell activation and may be of clinical utility, particularly in the CPI refractory setting.**
  - CDX-585 binds to ILT4 and PD-1 with sub-nanomolar affinity and is a potent competitor of their respective ligands.
  - In response to toll like receptor activation with LPS, primary cultures of human monocyte-derived macrophages and dendritic cells treated with CDX-585, exhibited enhanced production of inflammatory cytokines and chemokines.
  - **CDX-585 promoted T cell activation as measured by mixed lymphocyte reactions in a manner not achieved by the combination of ILT4 and PD-1 mAbs.**
  - **CDX-585 demonstrated anti-tumor activity in a humanized mouse model of melanoma.**
- Pilot studies in cynomolgus macaques demonstrated a favorable pharmacokinetic profile without adverse effects of treatment noted in clinical observations or clinical chemistry.
- **CDX-585 effectively combines ILT4 and PD-1 blockade into one molecule with favorable biophysical and functional characteristics supporting the initiation of development activities including manufacturing and IND-enabling studies.**