#2963

CDX-585, a Novel Bispecific Antibody Targeting PD-1 and ILT4

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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 immunoreceptor tyrosine-based inhibitory motif (ITIM)-bearing receptor expressed by myeloid cells.
  - Engagement of the ILT4 receptor by its cognate ligands (*e.g.* HLA-G and HLA Class I) inhibits myeloid cell activation.
  - ILT4 and its ligands are upregulated within the tumor microenvironment and expression correlates with poor outcomes in various tumors.
  - ILT4 signaling has been postulated as a resistance mechanism for checkpoint inhibition 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 (Siu, LL et al *Clin. Can. Res*. 2022).
- 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 development of CDX-585, a bsAb developed from novel ILT4 (7B1) and PD-1 (E1A9) antagonist mAbs.

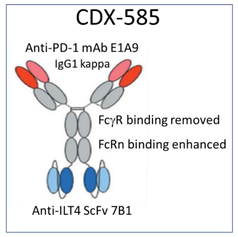
CDX-585 Generation & Characterization

CDX-585 was generated from novel αPD-1 (E1A9) and αILT-4 (7B1) mAbs.

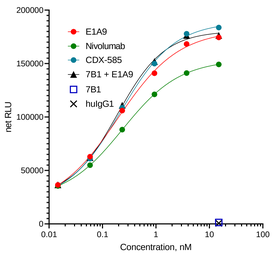
- E1A9 is a novel αPD-1 humanized mAb that potently inhibits PD-1 signaling by PD-L1.
- 7B1 is a novel αILT4 humanized mAb that is a potent inhibitor of HLA-G and HLA-A2 binding to ILT4.

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

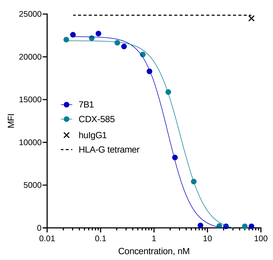
- Modified to eliminate FcγR binding and effector function (AQQ).
- Improved pharmacokinetics through enhanced FcRn binding (M252Y, S254T, T256E, referred to as YTE).
- Tetravalent antigen binding.
  - Bivalent for ILT4 and PD-1 for high affinity binding.



CDX-585 retains comparable potency to parental mAbs in blocking PD-1 signaling and HLA-G ligand binding.



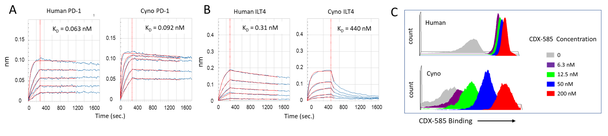
PD-1 effector cells and PD-L1 APCs were co-cultured in the presence of antibodies. Activation of the NFAT pathway via PD-1/PD-L1 blockade was detected by increasing luminescence using Bio-Glo™ reagent (Promega kit J1250).



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

CDX-585 has sub-nanomolar affinity for human PD-1 and ILT4 and cross-reacts with cynomolgus macaque receptors.

- CDX-585 has sub-nanomolar affinity for both human and cynomolgus macaque PD-1.
- CDX-585 has sub-nanomolar affinity for human ILT4 and cross-reacts with cynomolgus macaque ILT4 with lower affinity, but achieves similar saturation on monocytes.

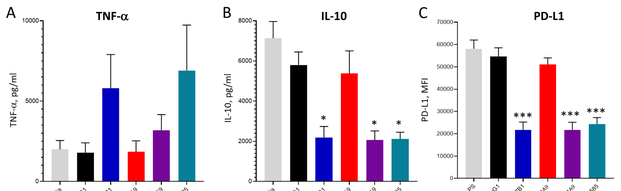


A. Sensorgrams of bio-layer interferometry analysis using anti-human IgG-Fc sensors to capture CDX-585 followed by human or cynomolgus monomeric soluble PD-1. **B.** Sensorgrams of bio-layer interferometry analysis using anti-human IgG-Fc sensors to capture CDX-585 followed by human or cynomolgus soluble ILT4. **C.** PBMCs from human or cynomolgus sources were incubated for 20 minutes at room temperature with biotin-labeled CDX-585 and detected with a streptavidin-PE probe.

CDX-585 *In Vitro* Activity

ILT4 Inhibition with CDX-585 Drives M1 Macrophage Polarization.

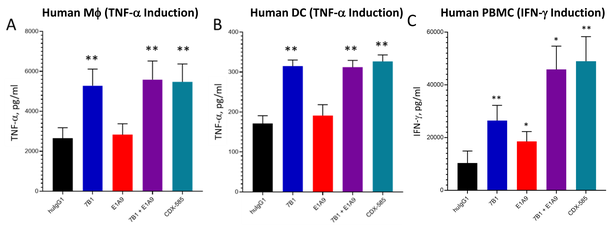
- Human macrophages differentiated in the presence of CDX-585 lead to an enhanced proinflammatory phenotype, downregulation of IL-10 secretion and PD-L1 surface expression.



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 (A) TNF-α and (B) IL-10 production by ELISA. The cells were stained for (C) PD-L1 expression and analysis by flow cytometry. Statistical significance vs. hulgG1 control measured by student's paired T-test, * = p < 0.05, *** = p < 0.001.

CDX-585 Potentiates Proinflammatory Phenotype in Myeloid and T Cells.

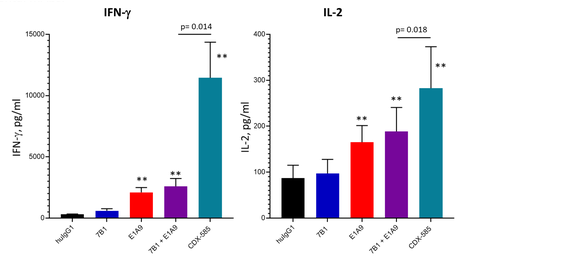
- Enhanced cytokine production through ILT4 blockade by human macrophages and DCs in response to LPS.
- T cell activation by CDX-585 via dual inhibition of ILT4 and PD-1.



Monocyte-derived (A) macrophages (cultured with M-CSF) or (B) dendritic cells (cultured with GM-CSF/IL-4) were incubated overnight with antibodies (6.7 nM) and LPS. Supernatant was harvested and analyzed for TNF-α production by ELISA. (C) 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. hulgG1 control was measured by Student's paired T-test, * = p < 0.05, ** = p < 0.01.

CDX-585 exhibits synergistic effects in mixed lymphocyte reaction.

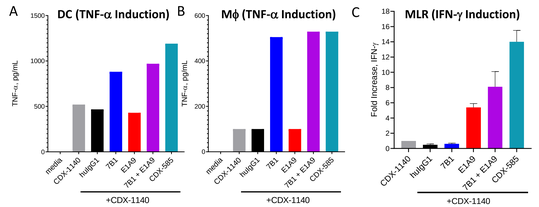
- Co-inhibition of ILT4 and PD-1 receptors via CDX-585 leads to a synergistic upregulation of IFN-γ and IL-2 secretion by T cells in mixed lymphocyte reactions (MLR).
- These effects are significantly greater than those exhibited by a combination treatment with parental mAbs.



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. hulgG1 control was measured by Student's paired T-test, ** = p < 0.01.

CD40 agonism induces potent MLR activity with CDX-585.

- DC activation with anti-CD40 agonist mAb (CDX-1140) promotes potent T cell activation with CDX-585 in an MLR.
- Potential for enhanced activity of CDX-585 in the context of inherent CD40 activation (CD40 ligand) or combination with anti-CD40 agonist mAb (CDX-1140).

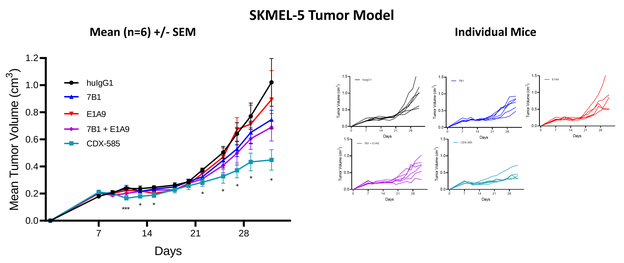


(A) Monocyte derived dendritic cells (cultured with GM-CSF/IL4, panel and (B) monocyte-derived macrophages (cultured with M-CSF) were incubated overnight with antibodies (6.7nM) and CDX-1140 (5μg/mL). Supernatant was harvested and analyzed for TNF-α production by ELISA. (C) Purified CD4⁺ T cells and monocyte-derived dendritic cells were prepared from independent donors. Dendritic cells were activated overnight with CDX-1140 (0.5μg/mL), then co-cultured with allogeneic CD4⁺ T cells in the presence of antibodies (5nM) for 4 days. Supernatant was harvested and analyzed for IFN-γ production by ELISA.

CDX-585 *In Vivo* Activity

CDX-585 demonstrates anti-tumor activity.

- Enhanced anti-tumor activity demonstrated in humanized mouse model of melanoma relative to parental mAbs alone or in combination.



Thirty HuCD34-NCG mice (Charles River Laboratories), were divided into five groups of six 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 Group 5: 7B1 (0.375 mg/mouse) and E1A9 (0.375 mg/mouse). Mice were dosed once a week for 5 weeks. Tumor volumes were measured periodically. Statistical significance vs. hulgG1 control was measured by Student's T-test, * = p < 0.05, *** = p < 0.005.

CDX-585 Toxicology Study

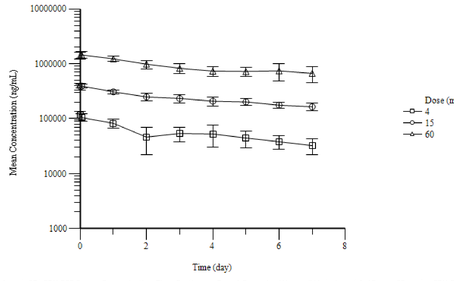
4-week GLP toxicology study in cynomolgus macaques.

Group No.	CDX-585 Dose Level (mg/kg/dose)	Number of Animals: Main Study Males	Main Study Females	Recovery Study Males	Recovery Study Females
1	0	3	3	2	2
2	4	3	3	-	-
3	15	3	3	2	2
4	60	3	3	2	2

- Cynomolgus macaques
- 15 minutes intravenous infusion once weekly on Days 1, 8, 15, 22, and 29
- Main study animals were euthanized on Day 31
- Recovery animals were euthanized on Day 59

- Administration of CDX-585 by once weekly intravenous infusion (5 total doses) was well tolerated in cynomolgus monkeys at levels of 4, 15, and 60 mg/kg.
- CDX-585-related microscopic findings occurred at all dose levels and were limited to:
  - Increased cellularity in lymphoid organs,
  - Increased mononuclear or mixed cell infiltrates in multiple organs,
  - Both changes showing complete/partial resolution following the postdosing recovery period.
- All findings attributed to CDX-585 at ≥ 4 mg/kg were considered non-adverse, as they were generally minimal to mild in the terminal phase of the study and had complete or partial resolution following the dose-free interval.
- Based on the results, under the condition of this study, the no-observed-adverse-effect level (NOAEL) was considered to be 60 mg/kg.

CDX-585 Toxicokinetic Prolife



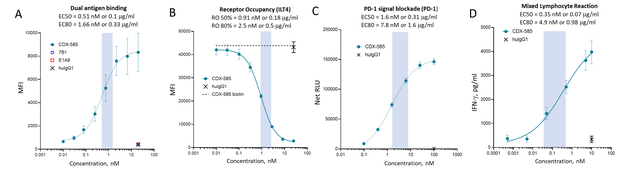
Mean (±SD) CDX-585 Serum Concentration-Time Profiles on Days 1 Following Weekly IV Infusion of 4, 15, and 60 mg/kg CDX-585. (Males and Females Combined)

- Systemic exposure to CDX-585 appeared to be independent of sex.
- Following once-weekly IV infusion of CDX-585, mean C_{max}, $AUC_{0\text{-}2day}$, and $AUC_{0\text{-}7day}$ values for CDX-585 increased with increasing dose in an approximately dose proportional manner following the Day 1 administration.
- Following the Day 1 administration., mean $T_{1/2}$ values were 5.76, 5.43, and 8.60 days at 4, 15, and 60 mg/kg, respectively.
- The presence of anti-CDX-585 antibodies appeared to impact systemic exposure to CDX-585 for ADA positive animals by Day 29.

First in Human Dose Selection

Determination of CDX-585 EC_{50} and EC_{80} from relevant pharmacological studies.

- The *in vitro* concentration dependence of CDX-585 was examined using relevant pharmacological and receptor occupancy studies.
- Overall, the EC_{50} for CDX-585 from relevant pharmacological and receptor occupancy studies is in the range of 0.07 - 0.31 μg/ml, and the EC_{80} is in the range of 0.33 – 1.6 μg/ml.
- The data in totality support a First in Human (FIH) starting dose of 0.01 mg/kg.

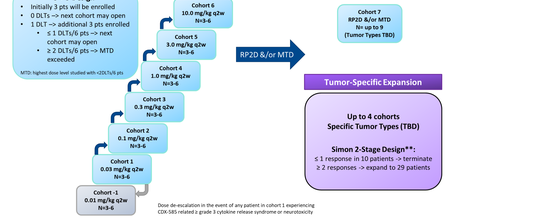


A. Bifunctional binding to ILT4 and PD-1. CDX-585, ILT4 mAb (7B1) and PD-1 mAb (E1A9) were incubated with HEK-293 cells expressing human ILT4. Cells were washed and soluble human PD-1 fused to mouse Fc was added and detected by flow cytometry with a PE-labeled goat anti-mouse IgG polyclonal reagent (n=2). **B.** Receptor occupancy on CD14⁺ monocytes. PBMCs from 4 independent donors were incubated with varying concentrations of unlabeled CDX-585 and then exposed to a saturating concentration of biotin-labeled CDX-585, which was detected by flow cytometry using streptavidin-PE. **C.** PD-1 effector cells and PD-L1 APCs were co-cultured in the presence of dilutions of CDX-585, or human IgG1. Activation of the NFAT pathway via PD-L1/PD-1 blockade was detected by increasing luminescence using the Bio-Glo™ reagent (Promega kit J1250). **D.** Mixed lymphocyte reaction. Purified CD4⁺ T cells and dendritic cells were prepared from independent donor PBMCs (n = 5). Dendritic cells were activated overnight with LPS, then co-cultured with allogeneic CD4⁺ T cells in the presence of varying concentrations of CDX-585 for 4 days. Supernatant was harvested and analyzed for IFN-γ. The light blue shaded areas represent the 50%-80% effect level for each assay.

Clinical Study Design

A Phase 1 dose-escalation and expansion study of the PD-1 x ILT4 bispecific antibody CDX-585 in patients with advanced malignancies.

- Open-label, non-randomized, multicenter, dose-escalation study with expansion cohorts to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamic, and clinical activity of CDX-585 in patients with advanced or metastatic solid tumors that have progressed during or after standard of care (NCT05788484).
- CDX-585 will be administered intravenously every 2 weeks until confirmed disease progression, intolerance, or for a maximum of 2 years.



Conclusions

Simultaneous inhibition of ILT4 and PD-1 checkpoints with CDX-585 leads to myeloid and T cell activation with enhanced activity relative to combination of mAbs.

- Inhibition of ILT4 on myeloid cells promotes a pro-inflammatory phenotype.
- CDX-585 has sub-nanomolar affinity and potently inhibits both PD-1 and ILT4.
- 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.
- CDX-585 effectively combines ILT4 and PD-1 blockade into one molecule with favorable biophysical and functional characteristics which supported the initiation of development activities including manufacturing.
- GMP CMC activities have been successfully executed.

CDX-585 demonstrates good safety profile in a GLP-compliant toxicology study supporting initiation of clinical development.

- A toxicology study demonstrated that the administration of CDX-585 by once weekly intravenous infusion (5 total doses, on Days 1, 8, 15, 22, and 29; 15 minute infusions) was well tolerated in cynomolgus monkeys at levels of 4, 15, and 60 mg/kg.
- The NOAEL in cynomolgus monkeys was considered to be 60 mg/kg.
- A clinical study with CDX-585 in advanced cancer patients will be initiating soon.

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