#2392

CDX-527: A novel bispecific immune-modulating antibody targeting CD27 and PD-L1

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Introduction

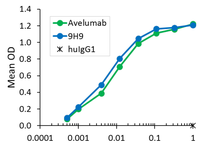
- The use of bispecific antibodies (BsAbs) provides opportunities to engage two independent pathways involved in controlling immune responses to tumors
- Preclinical and clinical studies support the safety and benefit of combining PD-1 blockade with a CD27 agonist[1,2,3]
- CDX-527 is a BsAb that combines blocking the PD-1 checkpoint pathway with CD27-mediated costimulation of T cells
- A full length IgG1 format was used to develop CDX-527 from novel and highly active PD-L1 and CD27 monoclonal antibodies (mAbs)
- CDX-527 was tested for PD-1 blockade and T cell activation *in vitro*
- A pilot study was performed in cynomolgus macaques to study the pharmacokinetics of CDX-527
- Using a surrogate BsAb the immune activation and anti-tumor activity was tested in mice

1. *Buchan et al. Clin Cancer Res. 2018 2. Sanborn, R et al. ASCO 2018 3. Reardon, D et al. SNO 2018*

Characterization of PD-L1 and CD27 Precursor mAbs

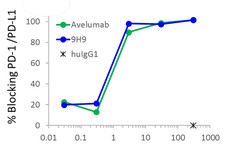
- Anti-PD-L1 and anti-CD27 mAbs were generated by immunization of human Ig transgenic mice (H2L2 strain of Harbour® transgenic mice) with recombinant human PD-L1 and recombinant human CD27
- Lead candidates were cloned into a human IgG1κ expression vector
- Avelumab (PD-L1) and varlilumab (CD27) were used as pos. controls
- The mAbs 9H9 (PD-L1) and 2B3 (CD27) were selected for BsAb based on binding and activity studies

PD-L1 Binding



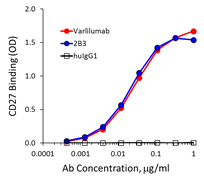
- Recombinant human PD-L1 was bound to microtiter plates
- Abs were added and detected with hu IgG Fc-specific polyclonal reagent

PD-L1/PD-1 Blocking



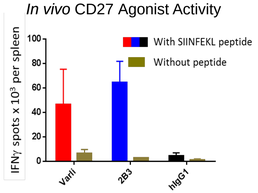
- 293 cells expressing PD-L1 were incubated for 5 minutes with Abs, followed by the addition of human PD1-biotin
- PD1 binding was detected with streptavidin PE and analyzed by flow cytometry

CD27 Binding



- Recombinant human CD27 was bound to microtiter plates.
- Abs were added and detected with human IgG Fc-specific polyclonal reagent

In vivo CD27 Agonist Activity

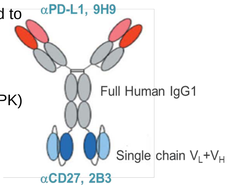


- Human CD27 Tg mice immunized with ovalbumin and administered 50 µg of CD27 mAbs (varlilumab or 2B3 mAb) or control
- After 7 days, spleen cells are stimulated *in vitro* with ovalbumin derived CD8 peptide (SIINFEKL) and IFNγ producing cells analyzed by ELISpot

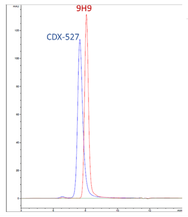
CDX-527 Bispecific Antibody

Full length αPD-L1 mAb 9H9 (human IgG1κ) genetically linked to single chain variable domains of αCD27 mAb 2B3

- Includes human Fc region as part of the BsAb construct
  - Retaining Fc receptor cross-linking for agonist activity
  - Retaining FcRn binding activity for extended half-life (PK)
  - Protein A purification
- Tetravalent molecule
  - Bivalent for CD27 and PD-L1



HPLC



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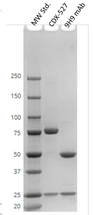
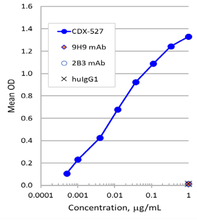


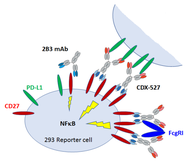
Bifunctional ELISA



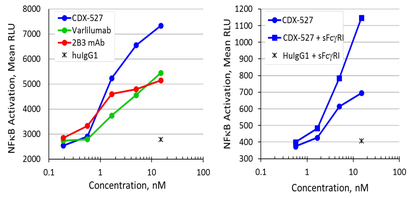
- HPLC performed on TSK3000 column with 20ug injections in PBS
- Reducing SDS-Page on 4-15% Tris-HCl gel
- Bifunctional ELISA: huCD27 was bound to microtiter plate, then antibodies were added, followed by PD-L1-ms Fc. Captured PD-L1 was detected with goat anti-mouse IgG Fc-specific reagent
- Additional biophysical characterization, including DSC, DLS, HUNK and mass spec showed that CDX-527 has no developability issues

Potent FcγRI-Independent CD27 Activation with CDX-527

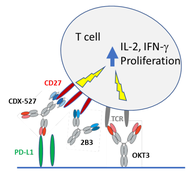
NFκB Reporter Assay

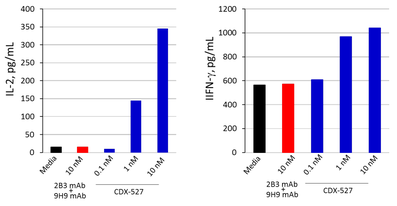


- CD27 activation by mAb 2B3 requires cross-linking for potent CD27 activation
- NFκB reporter cells express PD-L1 that can cross-link CDX-527
- Soluble FcγRI further enhances crosslinking of CDX-527



T Cell Activation

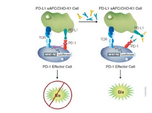




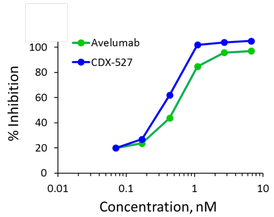
- Plate coated with PD-L1 and sub-optimal amount of anti-CD3 mAb (OKT3)
- Antibodies are added, and then purified T cells
- IL-2 and IFNγ levels in supernatant are measured at 72 hrs. Similar results for proliferation

CDX-527 is a Potent Inhibitor of the PD-1 Pathway

Cell-based PD-1 Blockade Assay

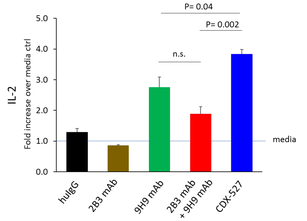


- PD-1 signal blockade was measured using the cell based assay from Promega (Cat # J1250)
- The PD1 Effector cells and PD-L1 cells were co-cultured in the presence of the antibodies for 6 hours before measuring luminescence



Combined PD-1 Blockade and CD27 Activation by CDX-527 Enhances T cell Activation

- CD4 T cells and dendritic cells were prepared from independent PBMCs (n = 3)
- CD4 cells were incubated in the presence of allogeneic dendritic cells and mAbs or CDX-527 for 3 days
- Supernatants were harvest and IL-2 levels were assessed by ELISA (R&D Systems).

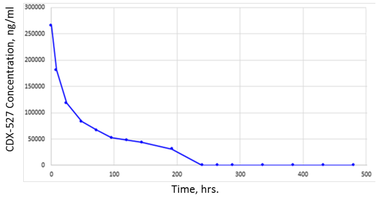


Pharmacokinetics, Pharmacodynamics and Tolerability of CDX-527 in Nonhuman Primates

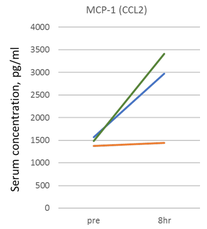
Test Article	Number of Animals	Dose Level	Volume
CDX-527	3	7.0 mg/kg	3.0 ml/kg i.v.

- No significant change was observed in any clinical parameters during the 21 day study

Pharmacokinetics



- Serum levels of CDX-527 were determined by ELISA
- Pharmacokinetic analysis resulted in $t_{1/2}$ of approximately 110 hrs
- Anti-drug antibody responses were observed after 200 hrs impacting CDX-527 levels



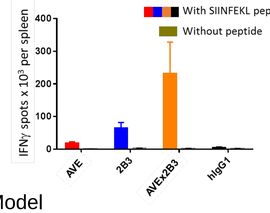
- Chemokine levels for CCL2 were measured by MSD

CDX-527 Demonstrates Enhanced Pharmacodynamic and Antitumor Activity *In Vivo*

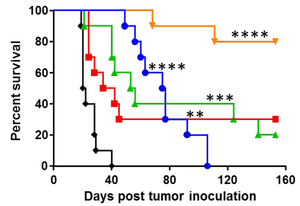
Surrogate construct replaces 9H9 PD-L1 mAb with sequences from avelumab. Avelumab binds to both human and mouse PD-L1

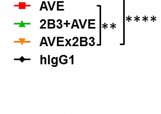
Ovalbumin Vaccination Model

- Human CD27 Tg mice immunized with ovalbumin and administered 200 µg of mAbs or AVEx2B3
- After 7 days, spleen cells are stimulated *in vitro* with ovalbumin derived CD8 peptide (SIINFEKL) and IFNγ producing cells were analyzed by ELISpot



BCL1 Lymphoma Model





- BCL1 cells 1x10⁶ i.v. on day 0; N=10.
- Animals were administered 200 µg mAbs or AVEx2B3 i.p. on day 5.

Conclusions and Next Steps

- Preclinical and clinical studies support combining PD-1 blockade and CD27 costimulation
- CDX-527 is a tetravalent αPD-L1xαCD27 BsAb using a fully human IgG1 backbone for the PD-L1 mAb and the scFv of the CD27 mAb genetically linked to the C-terminus of the heavy chain
- CDX-527 is a potent PD-1 inhibitor, and provides CD27 costimulatory signals that help prime and activate T cell responses
  - Has greater activity than combination of CD27 and PD-L1 mAbs
  - Pilot NHP study demonstrated mAb-like PK profile and was well tolerated
- CDX-527 has initiated manufacturing activities and IND-enabling studies

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