**Background**

- Axl is a member of the TAM (Tyro3/Axl/MaTk) family of receptor tyrosine kinases and a negative regulator of innate immunity.
- Activation of Axl through its ligand Gas6 leads to suppression of myeloid cell activity, while its activation in tumor cells drives tumor growth, metastasis, and is associated with acquired resistance to targeted therapies, radiotherapy and chemotherapy.
- We describe a humanized mAb: Axl-targeting monoclonal antibody (mAb), CDX-0168, that potently inhibits Gas6 binding and activation of Axl in tumor cell lines.
- CDX-0168 blocks a robust inflammatory response in human primary myeloid cells via a FcR-dependent mechanism, leading to T cell activation in mixed lymphocyte reactions.
- Administration of CDX-0168 to tumor cells co-cultured with human PBMCs leads to dose-dependent killing of Axl-expressing tumor cells in vitro and in vivo.
- The pleiotropic effects of Axl activation in cancer support combination of Axl-targeting agents with other targeted agents, either as drug combinations or as part of the same molecule.
- A prototype tetavalent bispecific (bissell) antibody engineered to block both Axl and PD-L1 preserves full Axl and PD-L1 blockade and immune stimulatory activity.

**CDX-0168 Potently Binds to Axl and Blocks Gas6 Binding**

- CDX-0168 binds to tumor cell lines expressing varying amounts of Axl and recently expressed Axl with sub-nanomolar potency.
- Binding to purified Axl domains demarcates binding to Igl, the major Gas6 binding domain.

**Immune Activation is FcR Dependent and Unique to CDX-0168**

- Cytokine induction by CDX-0168 requires binding to Fc receptors.
- CDX-0168 nucleiates with impaired Fc-binding (CDX-0168-FcIgU) fails to elicit a cytokine response in human macrophages.
- Immune activation is unique to CDX-0168 as other Axl inhibitors do not induce cytokine secretion.
- Axl mAbs and Gas6 traps were added at 100 nM, bemcentinib was added at 1 uM.
- CDX-0168 and 9237-62S-8: Axl inhibitory mAbs; sAxl-Fc: Gas6 trap.

**Antitumor Responses in Axl-Expressing Tumor Models**

- Human monocytes were differentiated into macrophages with M-CSF and dendritic cells (DCs) with IL-4 and GM-CSF.
- DCs and macrophages were treated with 100 nM CDX-0168 with or without 5 uM sAxl-Fc or 10 ng/mL LPS for 24 hours.
- Cytokine and chemokine release were measured by ELISA or luminex.

**Conclusion**

- CDX-0168 inhibits T cell activation in an FcR dependent manner in mixed lymphocyte reactions (MLR).
- CDX-0168 co-incubated with allogenic DCs and mAbs at 100 nM for 4 days. Secreted IL-6 levels were measured by ELISA.

- Axl-expressing cells were co-incubated overnight with human donor PBMCs at a 7:1 ratio and treated with increasing doses of CDX-0168. Lysis was measured using CytoTox ONE assay from Promega. Dose-dependent cell killing was observed.

- CDX-0168 blocks Axl and PD-1 signaling in cell-based assays with similar potency to each parent.
- PD-1 signaling was performed using a reporter assay (Promega).

**Inhibition of Gas6-Dependent Signaling**

- Human donor macrophages were treated with control, 100 nM CDX-0168, or LPS for 1 hour. Phospho-proteins were quantified by western blot using an Odyssey CLx instrument and normalized to β-actin.

**Activation of Inflammatory Signaling Pathways**

- Human donor macrophages were treated with control, 100 nM CDX-0168, or LPS for 1 hour. Phospho-proteins were quantified by western blot using an Odyssey CLx instrument and normalized to β-actin.