Simultaneous De-repression of Innate and Adaptive Immune Responses Through Dual Targeting of ILT4 and PD-(L)1 with Bispecific Antibodies

#1865

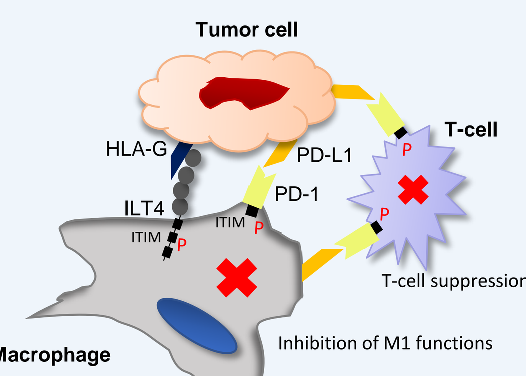
[1]Laura Vitale, [2]Mike Murphy, [1]Anna Wasiuk, [1]Jeff Weidlick, [1]Thomas O'Neill, [1]Jenifer Widger, [1]Laura Mills-Chen, [1]Andrea Crocker, [1]Colleen Patterson, [3]James Boyer, [2]Linda Crew, [2]Edward J. Natoli, [2]Jay S. Lillquist, [1]Joel Goldstein, [3]Lawrence J. Thomas, [3]Henry C. Marsh, [2]Diego Alvarado and [1]Tibor Keler

[1]Celldex Therapeutics, Inc., Hampton, NJ 08827, [2]New Haven, CT 06511, and [3]Fall River, MA 02723

BACKGROUND

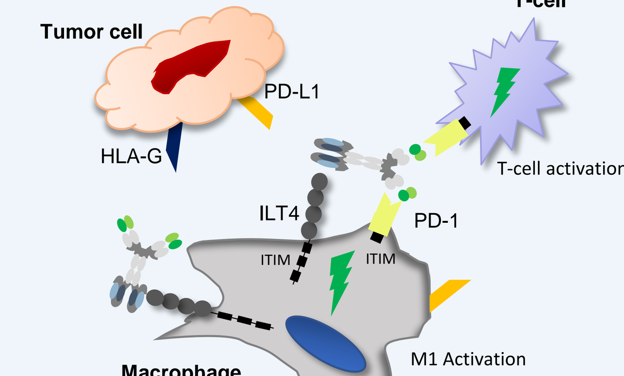
- ILT4 (LILRB2/CD85) is an ITIM containing negative regulator of myeloid cells
- Binding and activation of the receptor by its cognate ligands HLA-G and HLA Class I in myeloid cells has immunosuppressive effects through multiple mechanisms
- Expression of ILT4 in several tumor types is associated with poor outcome
- Antagonist Abs to ILT4 have immune enhancing and antitumor effects in preclinical models and recently demonstrated early clinical activity and safety that can be augmented with PD-1 blockade, including in the checkpoint refractory setting
- We describe the discovery and characterization of ILT4-inhibitory mAbs for engineering bispecific antibodies (bsAbs) that revert myeloid cell suppression by antagonizing ILT4 and activate T-cell responses through PD-(L)1 inhibition

Innate and Adaptive Cell Suppression Via ILT4 and PD-1



- PD-1 can be expressed by many immune cells in the tumor microenvironment, including T cells, macrophages, NK cells, and dendritic cells
- ILT4 is expressed primarily by monocytes, macrophages, dendritic cells, and tumors
- Engagement of either receptor by their ligands results in phosphorylation of their ITIM motif to recruit Src homology domain containing phosphatases and results in inhibition of critical cellular functions such as activation and maturation

Reversion of Innate and Adaptive Cell Suppression

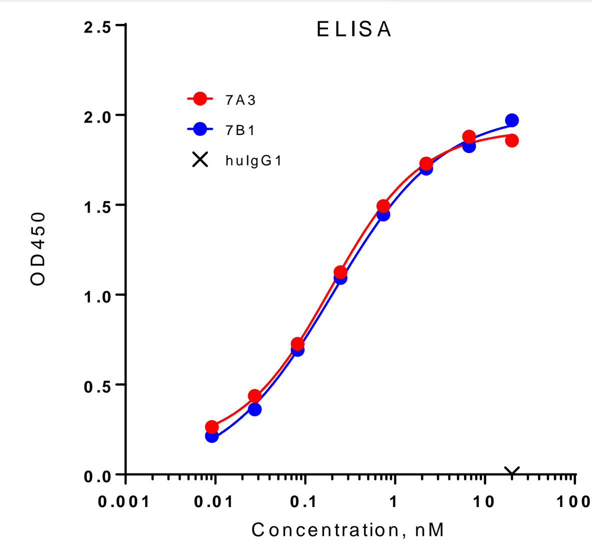


- PD-(L)1-ILT4 bsAbs can act as an antagonist through high affinity binding to either PD-1 or ILT4 similar to mAbs
- The bsAb can also engage both receptors simultaneously either in trans, or in cis when both receptors are expressed on the same cell (e.g. macrophages), leading to M1 macrophage polarization, increased proinflammatory cytokine release and T cell activation

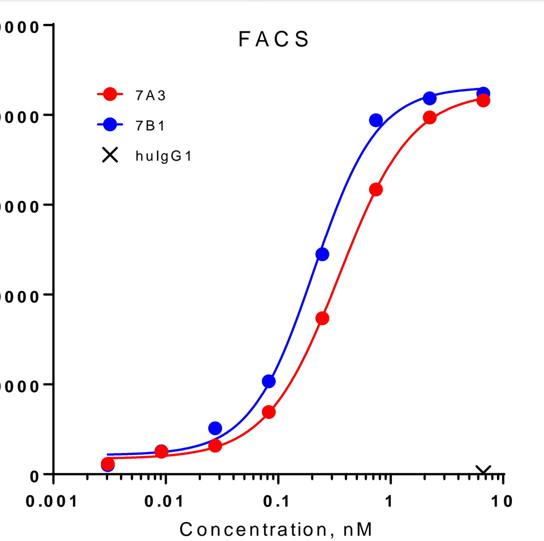
αILT4 mAb Discovery and Characterization

- αILT4 mAbs were generated by immunizing mice with purified human ILT4 extracellular domain
- Initial screening and characterization was done using chimeric antibodies containing human IgG4 (S228P)/kappa constant domains
- αILT4 mAbs 7A3 and 7B1 were humanized and expressed as IgG1 with Fc null mutations
- mAbs 7A3 and 7B1 effectively bind the human ILT4 extracellular domain and cell surface ILT4 on human monocytes, macrophages, dendritic cells and 293 cells overexpressing ILT4 (293-ILT4)
- mAbs 7A3 and 7B1 bind to ILT4 with high specificity and do not bind closely-related ILT family members

ILT4 Binding

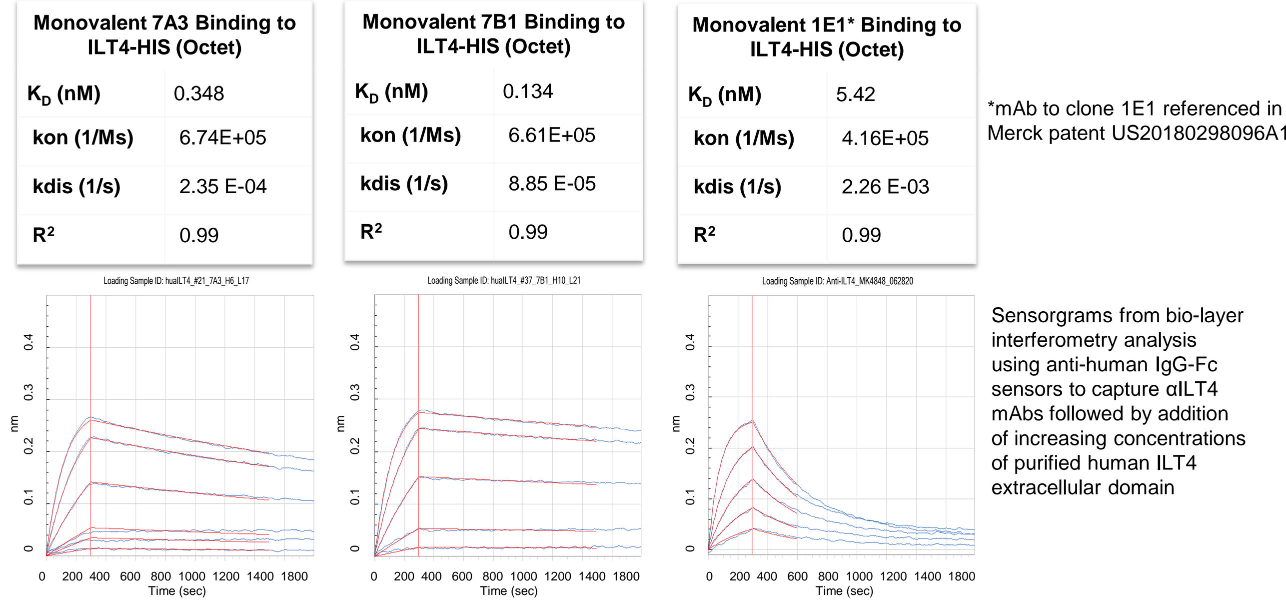


Each αILT4 mAb was titrated onto ELISA plates coated with the purified extracellular domain of human ILT4



Each αILT4 mAb was titrated onto 293-ILT4 cells; detection with goat anti-human IgG PE secondary

αILT4 mAb Discovery and Characterization



Monovalent 7A3 Binding to ILT4-HIS (Octet)	
K_D (nM)	0.348
kon (1/Ms)	6.74E+05
kdis (1/s)	2.35 E-04
R^2	0.99

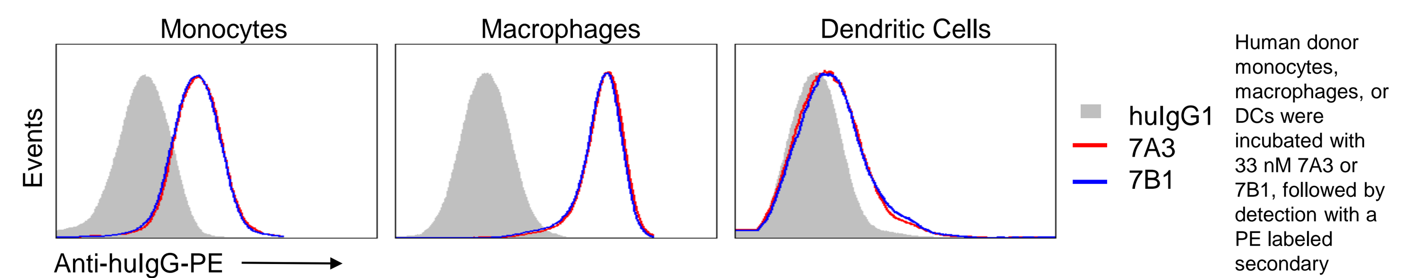
Monovalent 7B1 Binding to ILT4-HIS (Octet)	
K_D (nM)	0.134
kon (1/Ms)	6.61E+05
kdis (1/s)	8.85 E-05
R^2	0.99

Monovalent 1E1* Binding to ILT4-HIS (Octet)	
K_D (nM)	5.42
kon (1/Ms)	4.16E+05
kdis (1/s)	2.26 E-03
R^2	0.99

*mAb to clone 1E1 referenced in Merck patent US20180298096A1

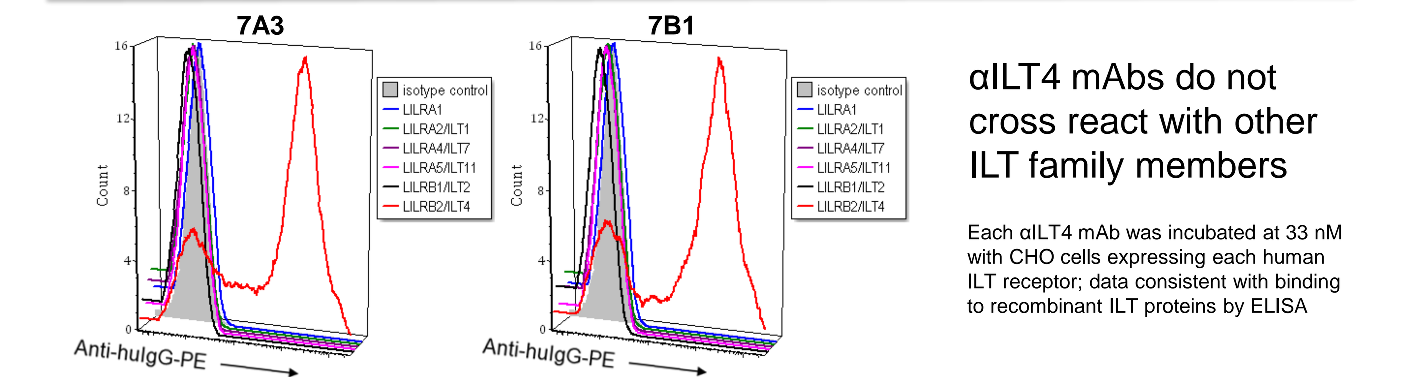
Sensorgrams from bio-layer interferometry analysis using anti-human IgG-Fc sensors to capture αILT4 mAbs followed by addition of increasing concentrations of purified human ILT4 extracellular domain

Binding to Primary Myeloid Cells



Human donor monocytes, macrophages, or DCs were incubated with 33 nM 7A3 or 7B1, followed by detection with a PE labeled secondary

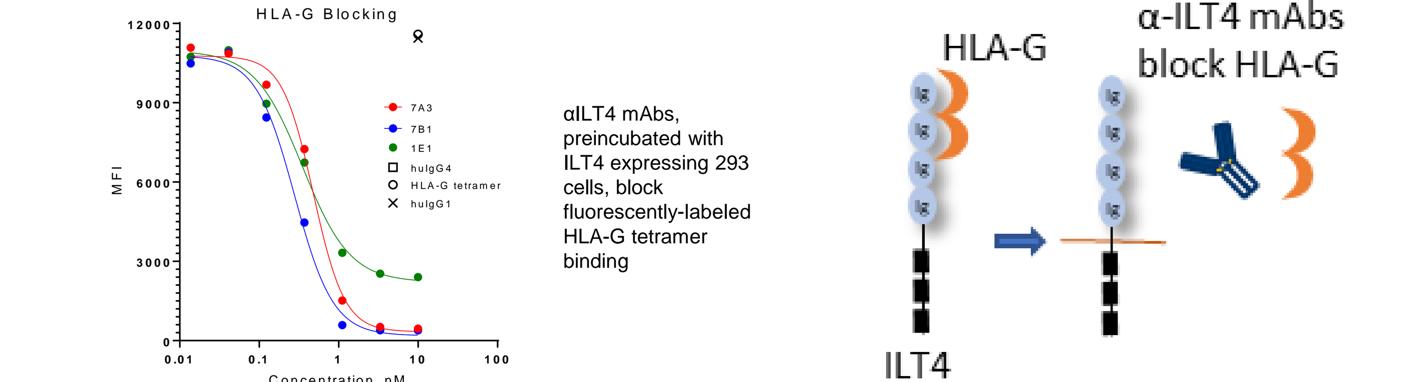
Cross Reactivity to ILT Family Members



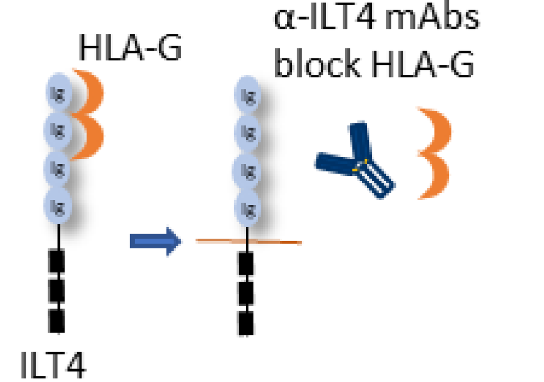
αILT4 mAbs do not cross react with other ILT family members

Each αILT4 mAb was incubated at 33 nM with CHO cells expressing each human ILT receptor; data consistent with binding to recombinant ILT proteins by ELISA

ILT4 mAbs Efficiently Block HLA-G Ligand Binding

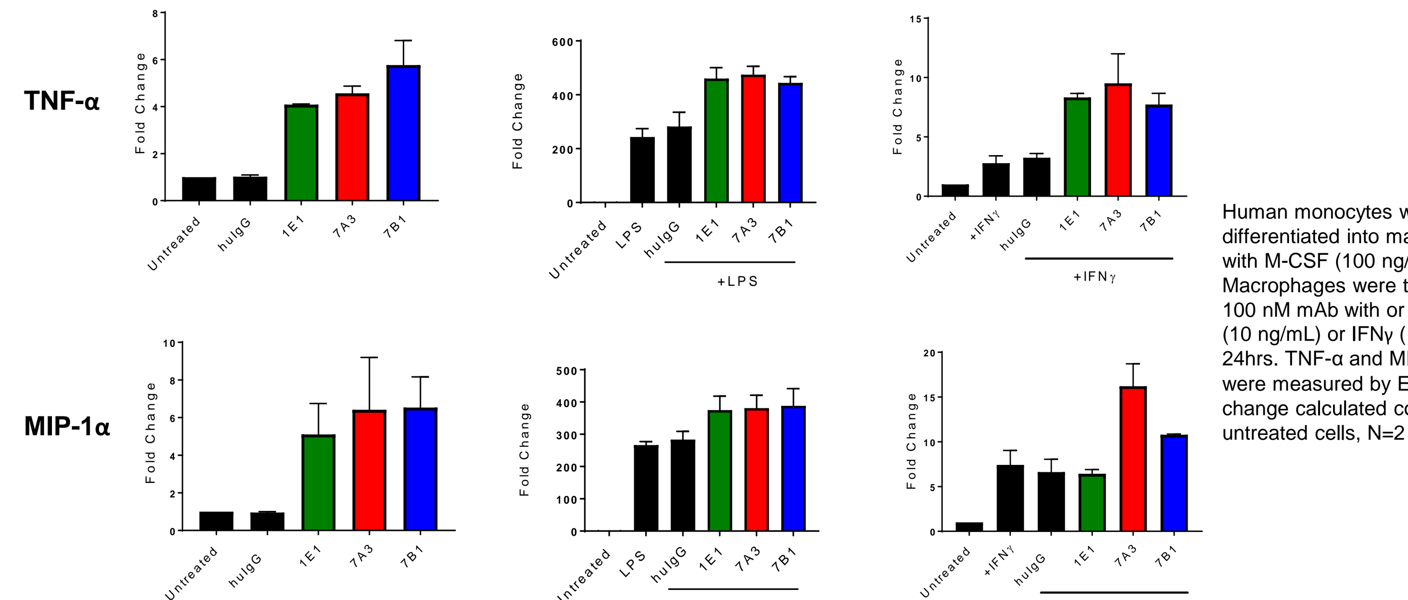


αILT4 mAbs, preincubated with ILT4 expressing 293 cells, block fluorescently-labeled HLA-G tetramer binding



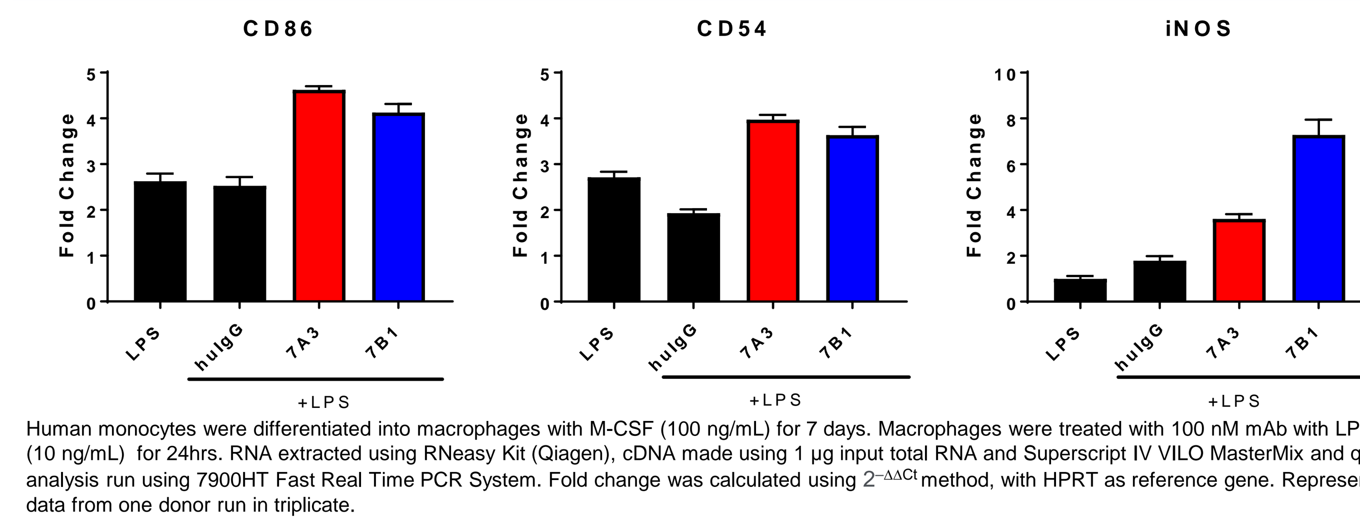
Activation of Immune Responses in Human Mϕs

- Treatment of human macrophages (Mϕs) with αILT4 mAbs can induce cytokine/chemokine release by themselves or can further potentiate the effects of LPS and IFNγ



Human monocytes were differentiated into macrophages with M-CSF (100 ng/mL) for 7 days. Macrophages were treated with 100 nM mAb with or without LPS (10 ng/mL) or IFNγ (10 ng/mL) for 24hrs. TNF-α and MIP-1α release were measured by ELISA; fold change calculated compared to untreated cells, N=2 donors

αILT4 mAbs Promote M1 Mϕ Polarization



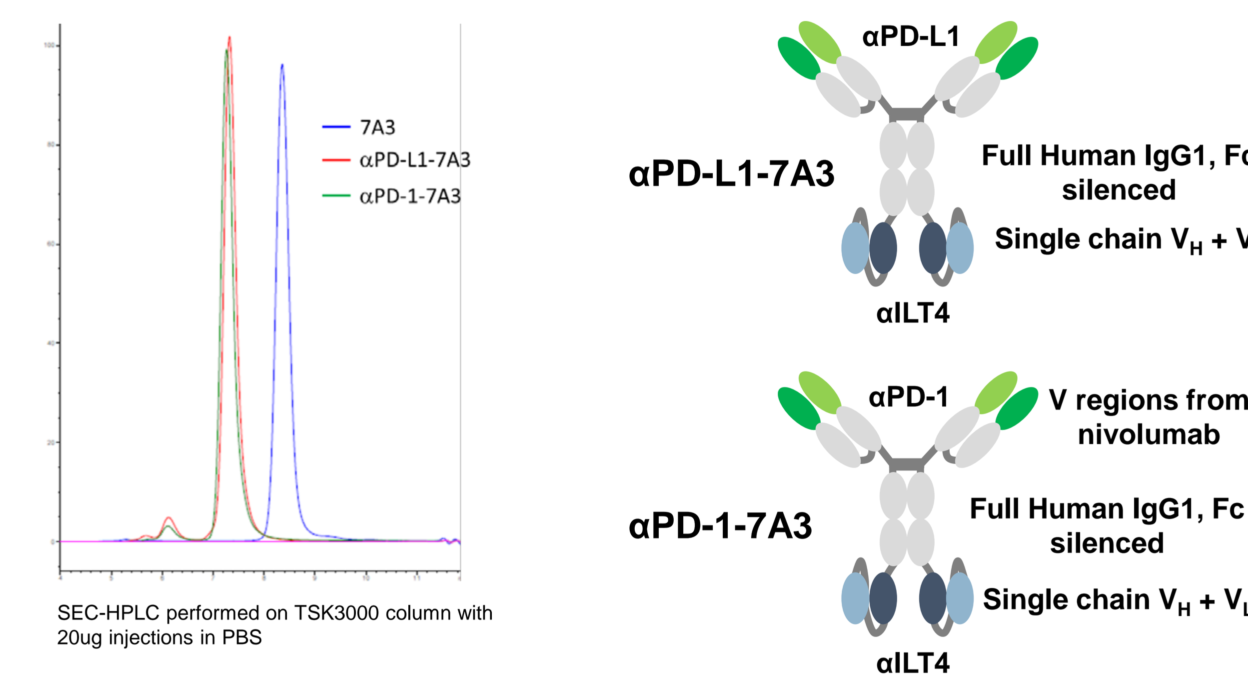
Human monocytes were differentiated into macrophages with M-CSF (100 ng/mL) for 7 days. Macrophages were treated with 100 nM mAb with LPS (10 ng/mL) for 24hrs. RNA extracted using RNeasy Kit (Qiagen), cDNA made using 1 µg input total RNA and Superscript IV VILO MasterMix and qPCR analysis run using 7900HT Fast Real Time PCR System. Fold change was calculated using $2^{-\Delta\Delta Ct}$ method, with HPRT as reference gene. Representative data from one donor run in triplicate.

PD-(L)1-ILT4 Bispecific Antibody Development

αPD-L1 mAb 9H9 (Celldex) or αPD-1 mAb (nivolumab V regions) were genetically linked to single chain variable domains of αILT4 mAb 7A3 and expressed as full length IgG1κ

- Modified to eliminate Fcγ receptor binding
  - No effector function but retains FcRn binding for PK
- Tetravalent antigen binding
  - Bivalent for ILT4 and PD-(L)1 for high affinity binding

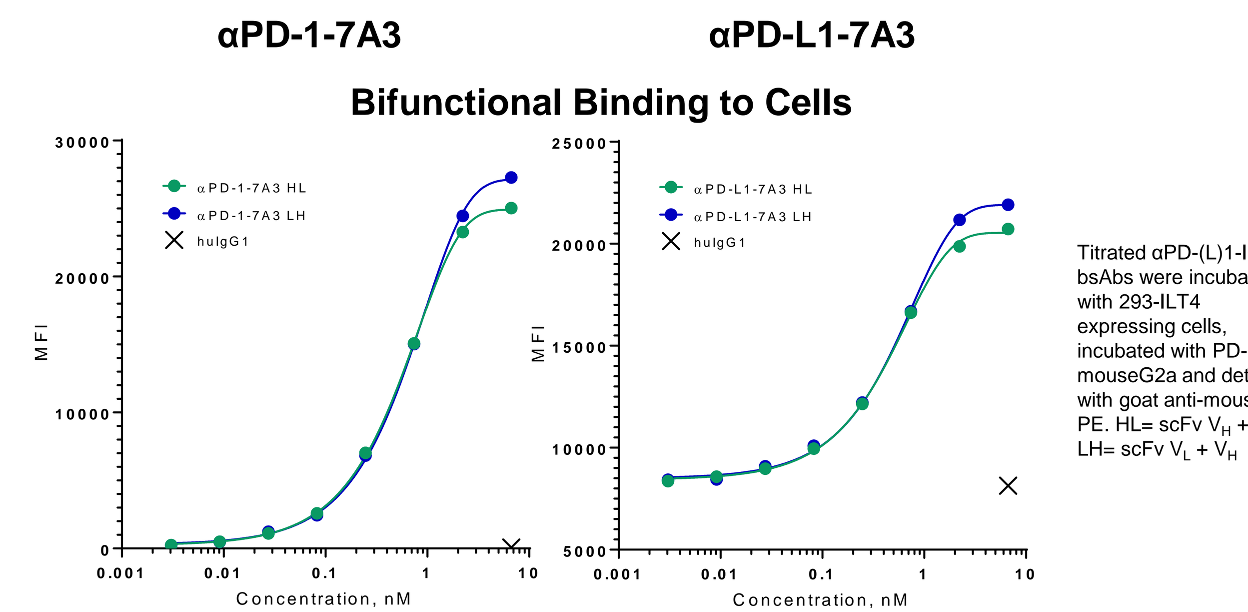
Antibody-like characteristics



SEC-HPLC performed on TSK3000 column with 20ug injections in PBS

PD-(L)1-ILT4 Bispecific Antibodies Retain Parental Antibody Functional Activity

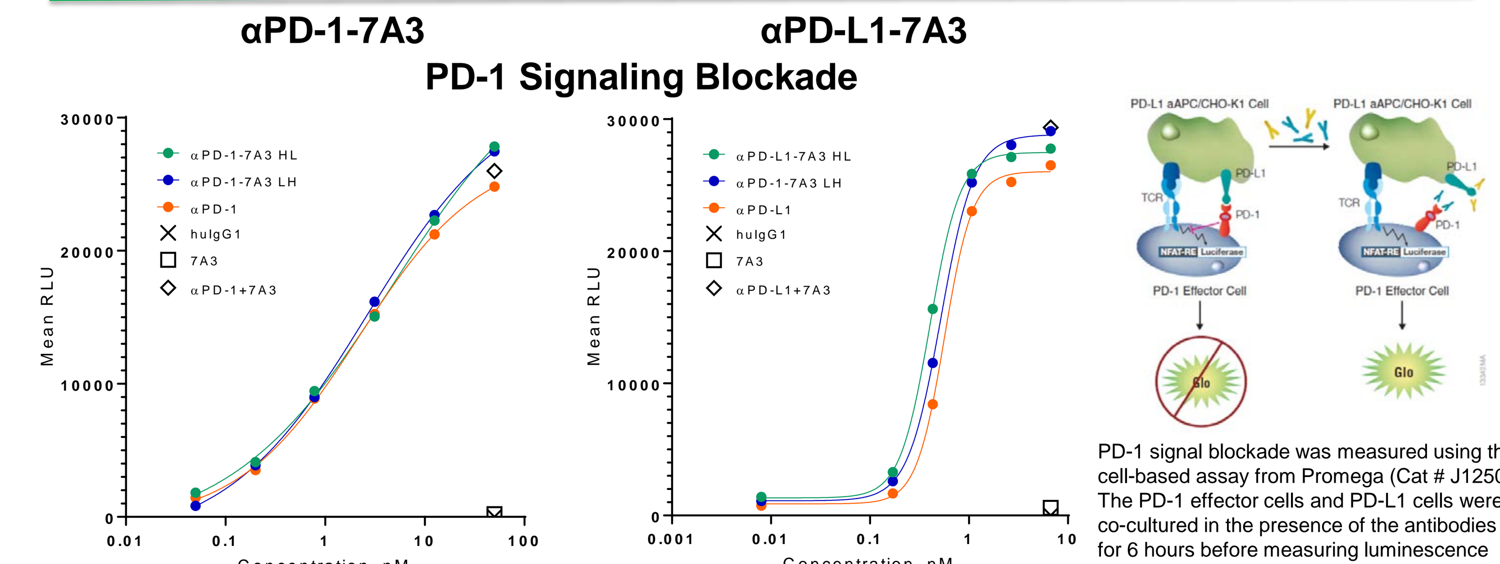
Simultaneous Binding of Both PD-(L)1 and ILT4



Titrated αPD-(L)1-ILT4 bsAbs were incubated with 293-ILT4 expressing cells, incubated with PD-(L)1 mouseG2a and detected with goat anti-mouse Fc PE. HL= scFv V_H + V_L; LH= scFv V_L + V_H

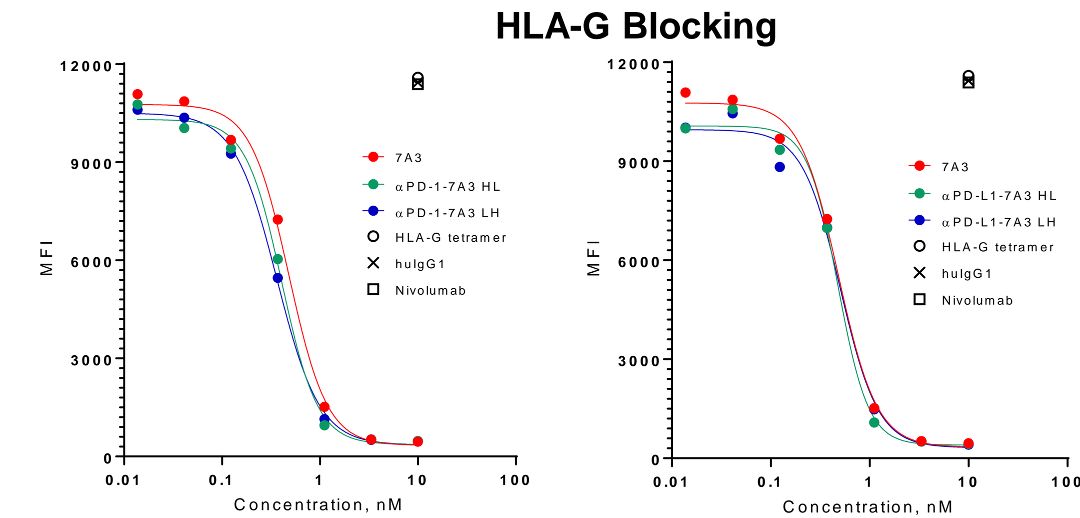
PD-(L)1-ILT4 Bispecific Antibodies Retain Parental Antibody Functional Activity

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



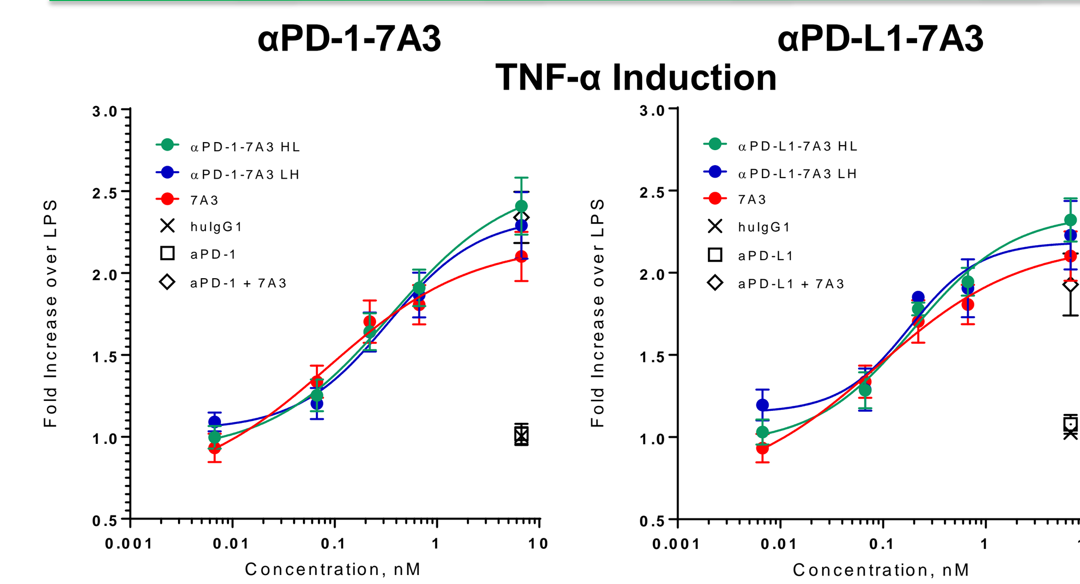
PD-1 signal blockade was measured using the cell-based assay from Promega (Cat # J1250). The PD-1 effector cells and PD-L1 cells were co-cultured in the presence of the antibodies for 6 hours before measuring luminescence

HLA-G Blocking



Titrated αPD-(L)1-ILT4 bsAbs preincubated with ILT4 expressing 293 cells, block fluorescently-labeled HLA-G tetramer binding

Cytokine Release



αPD-(L)1-ILT4 bsAbs induce TNF-α release in human donor macrophages in a dose dependent manner

Conclusions

- **ILT4 receptor inhibition with monoclonal antibodies lead to myeloid cell de-repression**
  - Novel humanized mAbs 7A3 and 7B1 bind to ILT4 with high specificity and efficiently block HLA-G
  - Treatment of human macrophages with 7A3 or 7B1 mAbs leads to enhanced cytokine/chemokine secretion in vitro and M1 polarization
- **Simultaneous de-repression of myeloid and T cell checkpoints with ILT4 and PD-(L)1 bsAbs may be of clinical utility, particularly in the CPI refractory setting**
- Clear evidence that prototype PD-(L)1-ILT4 bsAbs retain all the properties of the parental antibodies
  - Current efforts are focused on developing the clinical candidate for the bsAb co-targeting ILT4 and PD-(L)1