

Development of novel bispecific immune modulating antibodies

Laura A. Vitale, Lawrence J. Thomas*, Li-Zhen He, Thomas O'Neill, Jenifer Widger, Laura Mills-Chen, Andrea Crocker, Anna Wasiuk, James Testa, Karuna Sundarapandiyan, Eric Forsberg*, James Boyer*, Jeffrey Weidlick, James Storey, Joel Goldstein, Henry C. Marsh, Jr.*, Tibor Keler Celldex Therapeutics, Inc., Hampton, NJ and *Needham, MA

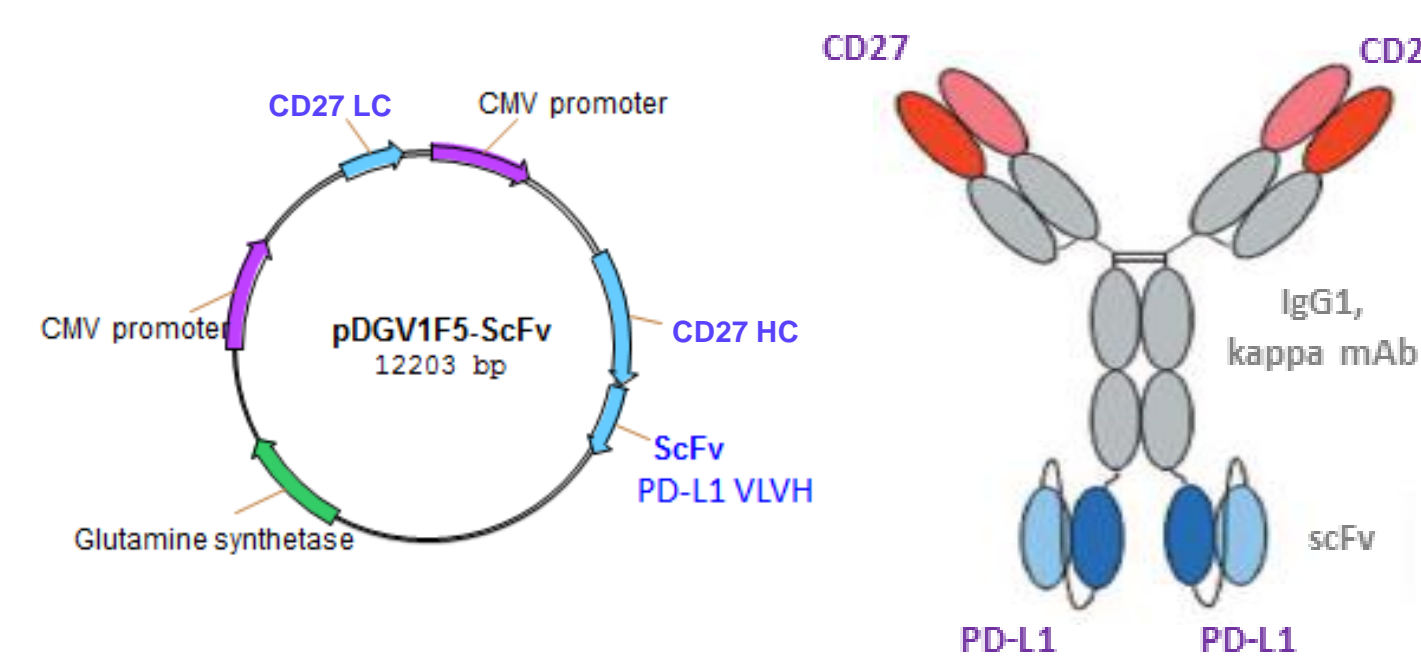


5624

Background and Rationale

- Multiple pathways and receptor/ligand interactions have been shown to be important in controlling the immune response to cancer.
- The use of bispecific antibodies (BsAbs) provides opportunities to engage two pathways with a single molecule and may provide advantages over combination therapy with separately administered antibodies.
- In addition to simplifying development activities, combining two antibodies into one molecule can enhance the efficacy and improve the safety profile compared to separately administered antibodies.
- Our initial strategies include blocking the PD-1 checkpoint pathway combined with our proprietary antibodies targeting various immune receptors
- We have previously shown the benefit of combining PD-1 blockade with CD27 activation in preclinical tumor models. Here we describe a novel anti-CD27 x anti-PD-L1 BsAb with favorable characteristics for cancer immunotherapy.

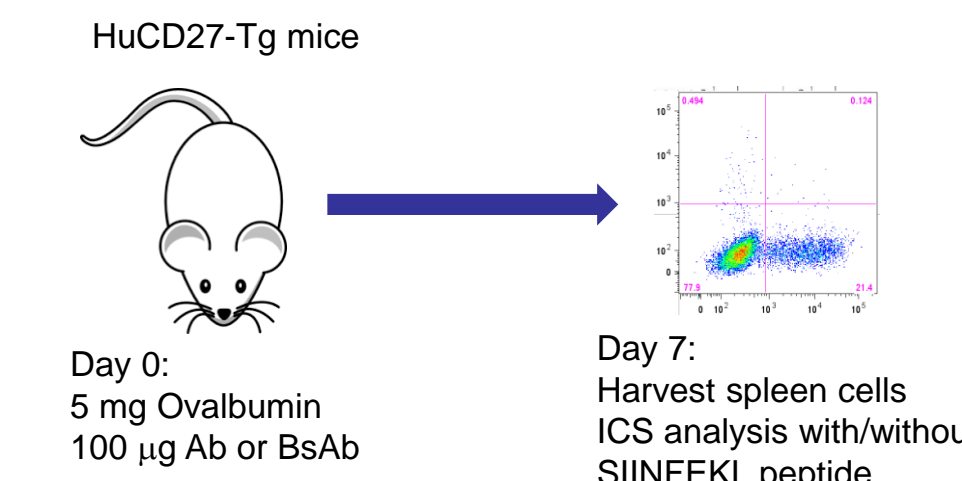
Anti-CD27 x Anti-PD-L1 BsAb



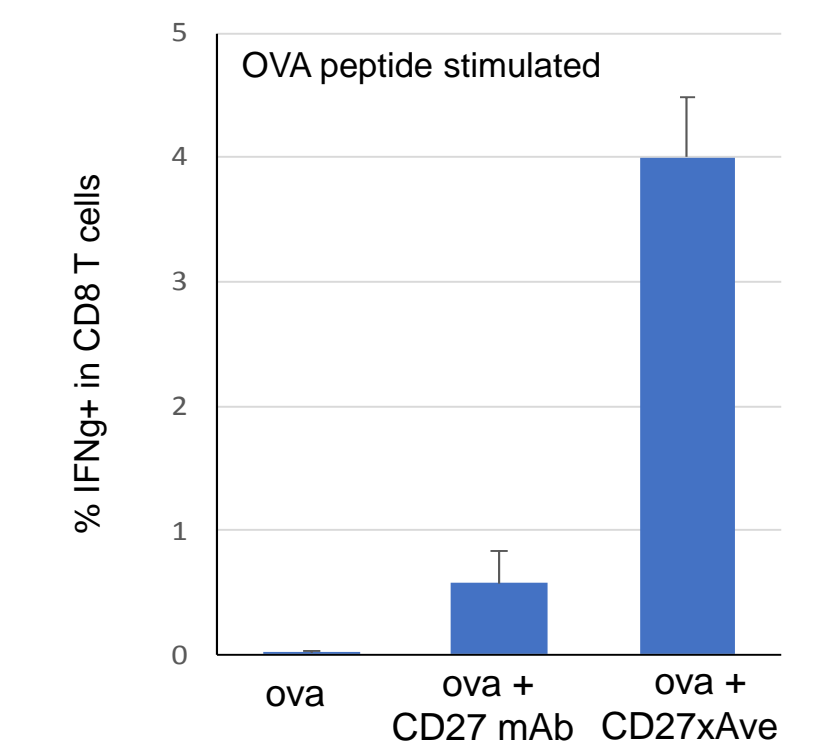
BsAb constructs generated

| BsAb | Anti-CD27 mAb | Anti-PD-L1 mAb | Reactivity with mouse PD-L1 |
|----------|---------------|----------------|-----------------------------|
| CD27xAve | 1F5 | Avelumab | Yes |
| CD27x8B1 | 2B3 | 8B1 | No |
| CD27x9H9 | 2B3 | 9H9 | No |

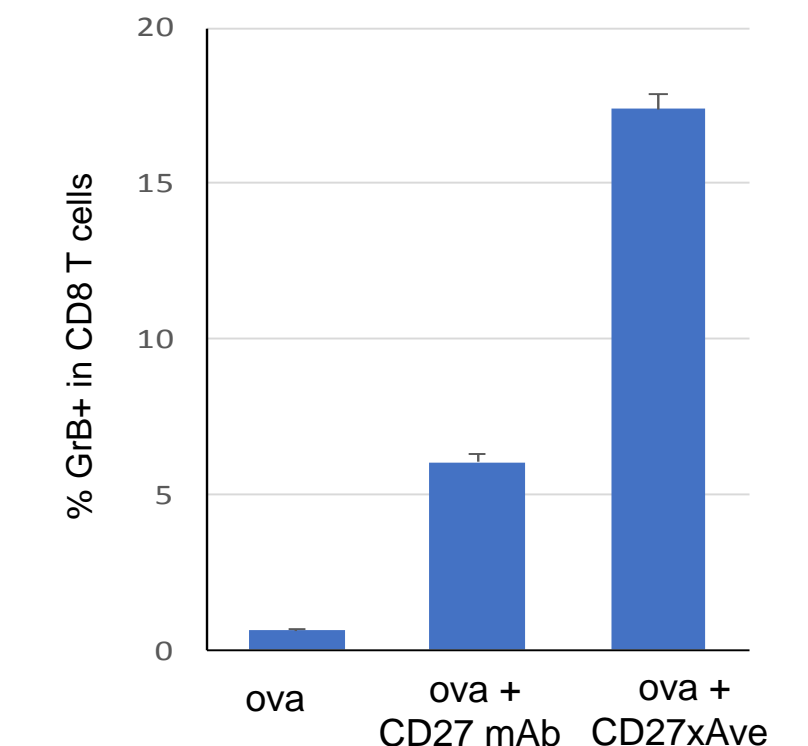
In Vivo Activity (CD27xAve)



Antigen-specific CD8 T cell response



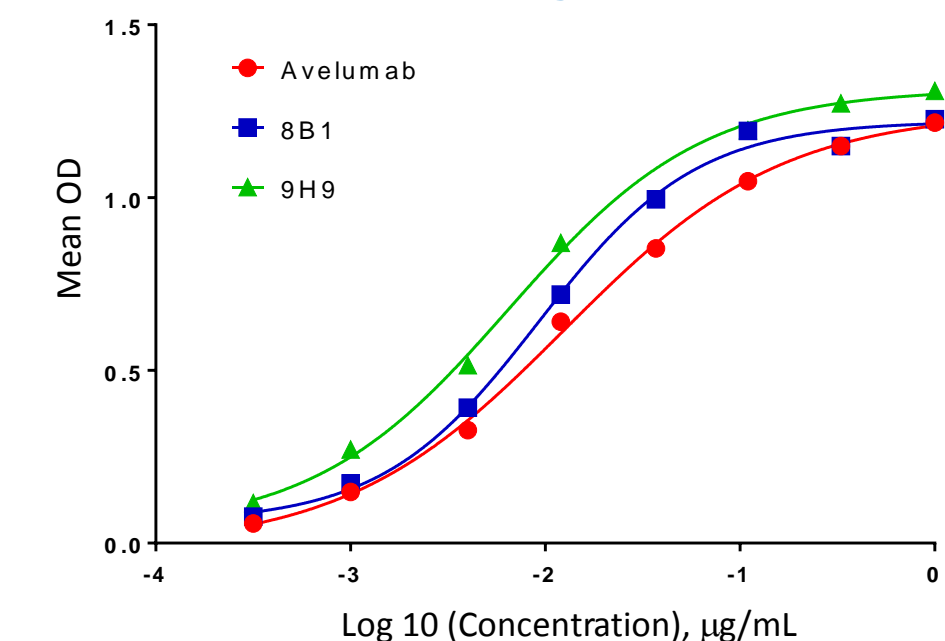
Overall CD8 T cell response



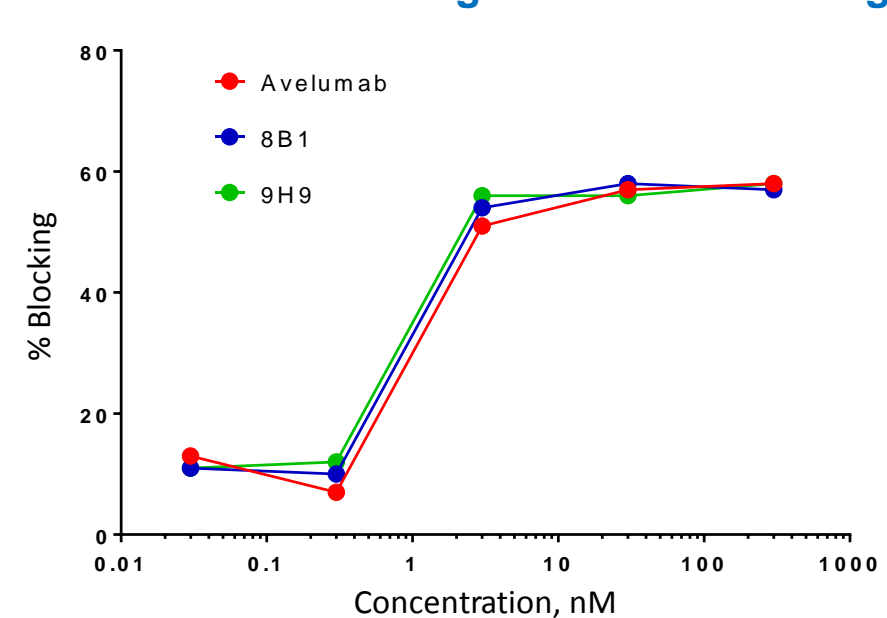
Development of Novel Human Anti-PD-L1 mAbs

Anti-PD-L1 monoclonal antibodies (mAbs) were generated by immunization of human Ig transgenic mice (H2L2 strain of Harbour® transgenic mice) with recombinant human PD-L1. Lead candidates were cloned into a human IgG1κ expression vector. Comparisons are presented with anti-PD-L1 mAb, avelumab, produced from the sequence for A09 246-2 (US 2014/0341917).

Binding to PD-L1



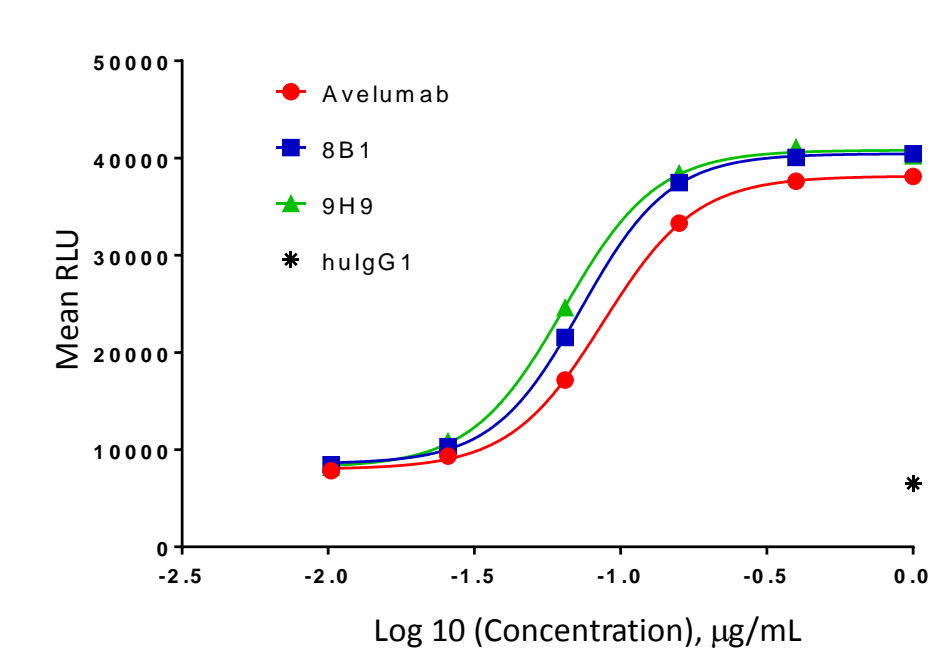
Blocking PD-L1/PD-1 binding



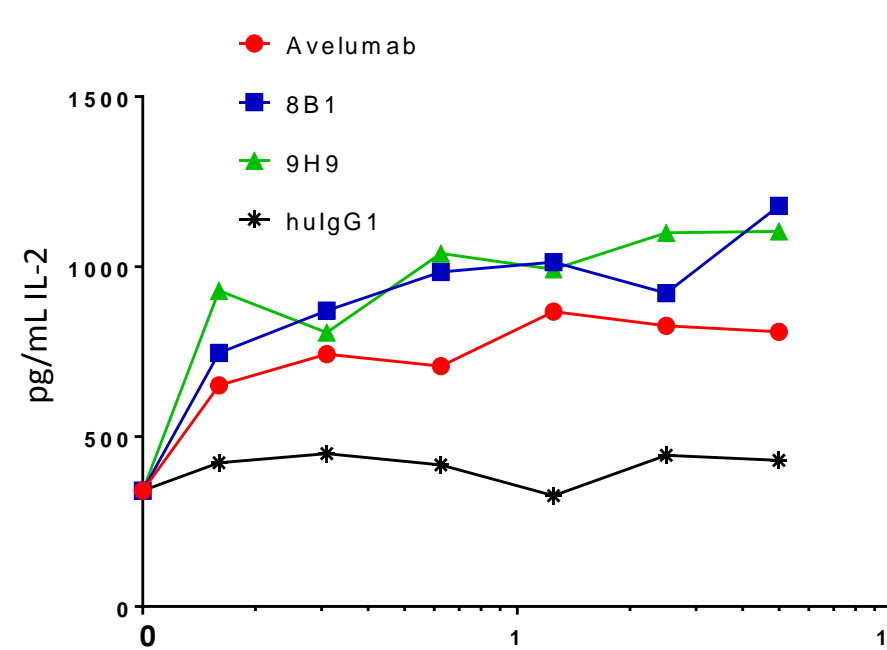
Microtiter plate was coated with recombinant human PD-L1-msFc. Antibody binding was detected with an HRP labeled goat anti-human IgG (Fc specific) antibody.

293 cells expressing human PD-L1 were incubated with the antibodies for 10 minutes, followed by a co-incubation with biotinylated human PD1. Binding to PD-L1 was detected with streptavidin PE.

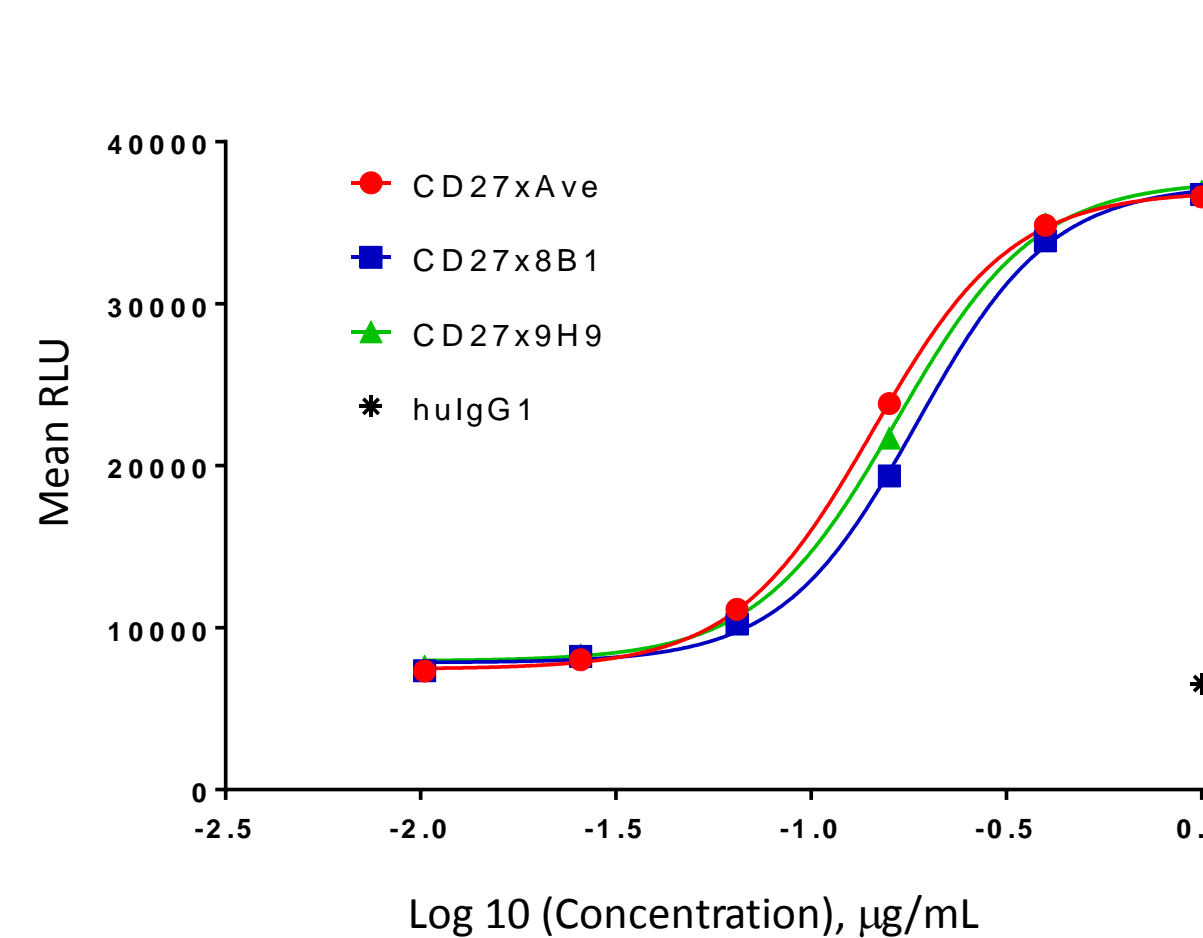
NFAT reporter assay



Mixed lymphocyte reaction

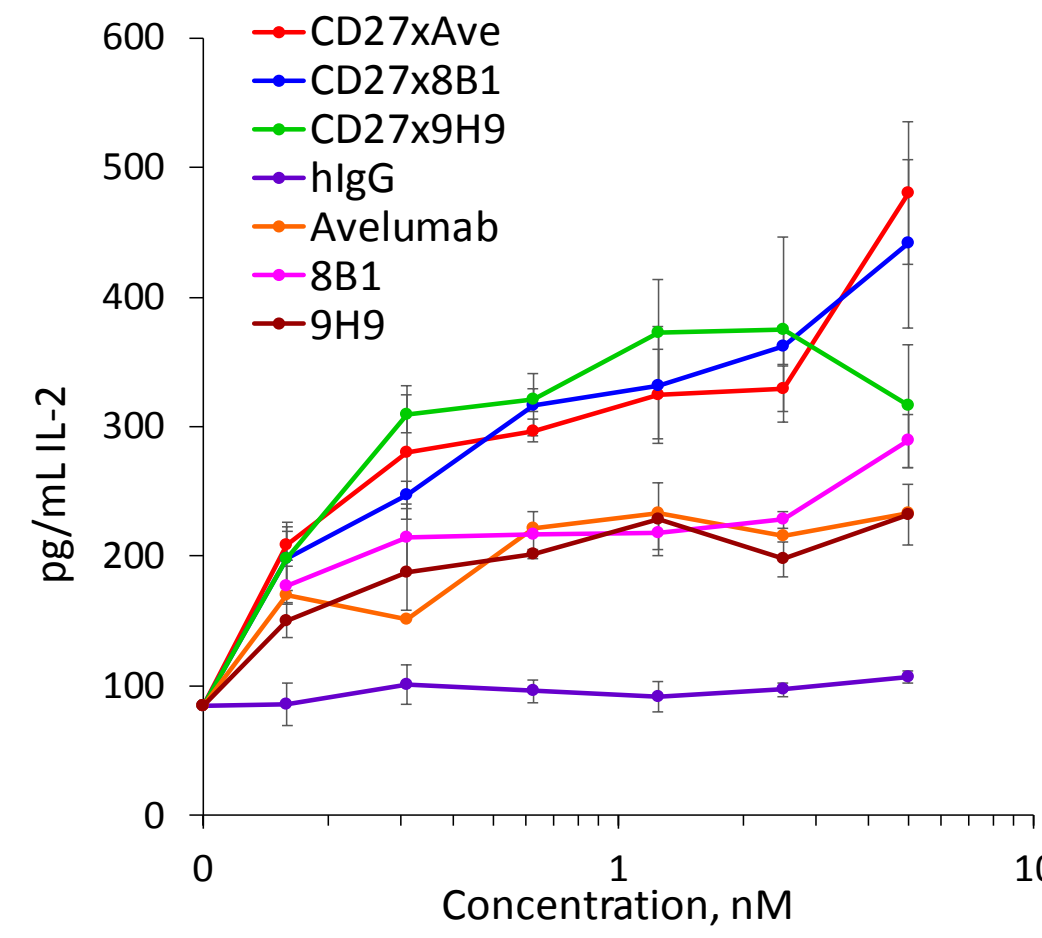


NFAT reporter assay (PD-1 signal blockade)



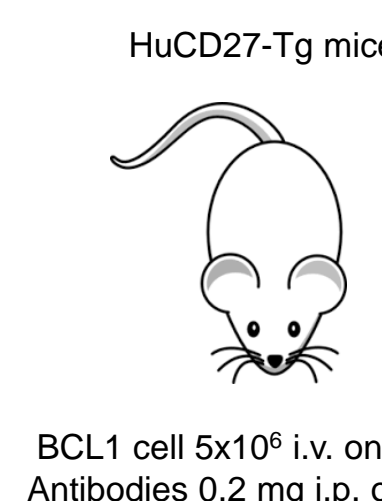
PD-1 Effector cells and PD-L1 aAPC cells were co-cultured in the presence of dilutions of the BsAbs. Activation of the NFAT pathway via PD-L1/PD-1 blockade is detected by addition of Bio-Glo™ reagent (kit available from Promega).

Mixed lymphocyte reaction

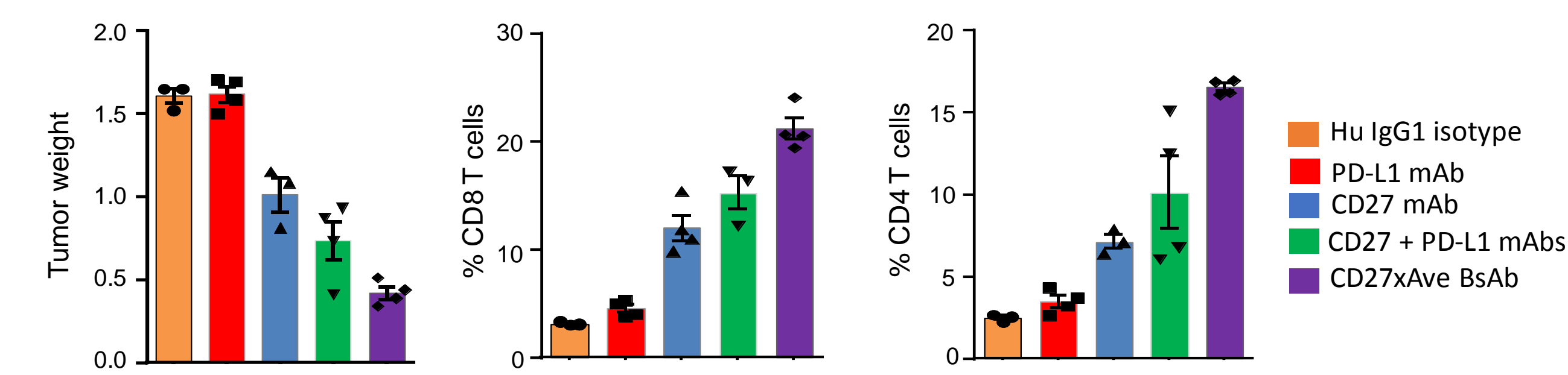
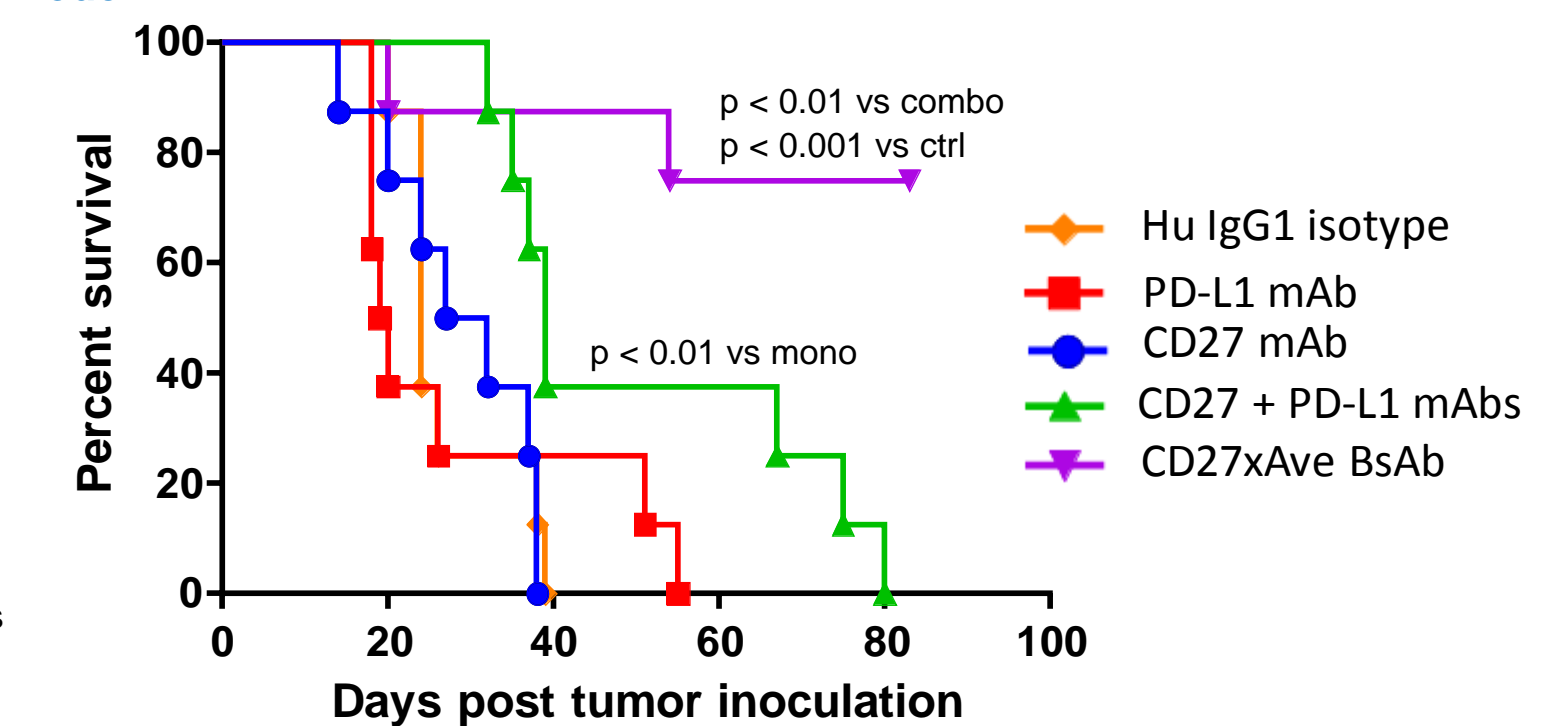


CD4 cells were incubated in the presence of allogeneic dendritic cells and dilutions of antibodies for 3 days. Supernatants were harvest and IL-2 levels were assessed by ELISA (R&D Systems).

BCL1 disseminated lymphoma model



Followed for survival (n=8)
Tumor analysis (day 11)



Summary and Next Steps

- Human PD-L1 antibodies were developed as backbone for developing novel BsAb for cancer immunotherapy
- Tetravalent aCD27xaPD-L1 BsAb were developed using a full human IgG1 backbone for the CD27 mAb and the scFv of the PD-L1 mAb genetically linked to the c-terminus of the heavy chain
- The BsAbs had the following properties:
 - High affinity binding to both CD27 and PD-L1
 - Enhanced CD27 signaling relative to parental CD27 mAbs
 - Potent blockade of PD-L1-driven PD-1 signaling
 - Enhanced MLR activity relative to parental PD-L1 mAbs
 - Enhanced priming of T cell responses compared to parental CD27 mAb
 - Enhanced anti-tumor efficacy compared to combination of CD27 and PD-L1 mAbs
- The data support further evaluation of aCD27xaPD-L1 BsAbs, and provide a platform for additional BsAb combinations



View Poster

Effector cells (Jurkat T cells expressing human PD-1 and a luciferase reporter driven by an NFAT response element) were co-cultured with APCs (CHO-K1 cells expressing human PD-L1 and an engineered cell surface protein designed to activate cognate TCRs) in the presence of dilutions of the antibodies. Blocking the PD-1 negative regulation allows TCR activation and induces luminescence that is detected by addition of Bio-Glo™ reagent (kit available from Promega).

CD4 cells were incubated in the presence of allogeneic dendritic cells and dilutions of antibody for 3 days. Supernatants were harvested and IL-2 levels were assessed by ELISA (R&D Systems).