Development of novel bispecific immune modulating antibodies

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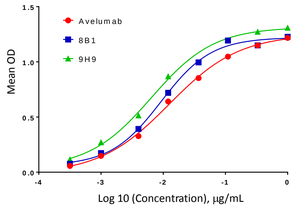
Background and Rationale

- 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.

Development of Novel Human Anti-PD-L1 mAbs

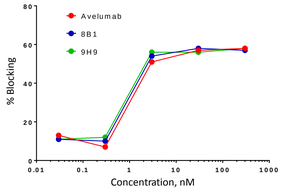
Anti-PD-L1 monoclonal antibodies (mAbs) were generated by immunization of human Ig transgenic mice (H2L2 strain of Harbour® transgenic mice) with recombinant human PD-L1. Lead candidates were cloned into a human IgG1κ expression vector. Comparisons are presented with anti-PD-L1 mAb, avelumab, produced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

Binding to PD-L1



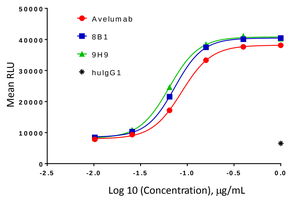
Microtiter plate was coated with recombinant human PD-L1-msFc. Antibody binding was detected with an HRP labeled goat anti-human IgG (Fc specific) antibody.

Blocking PD-L1/PD-1 binding



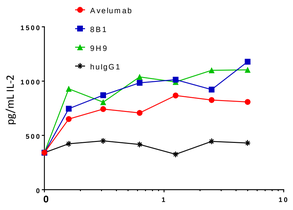
293 cells expressing human PD-L1 were incubated with the antibodies for 10 minutes, followed by a co-incubation with biotinylated human PD1. Binding to PD-L1 was detected with streptavidin PE.

NFAT reporter assay



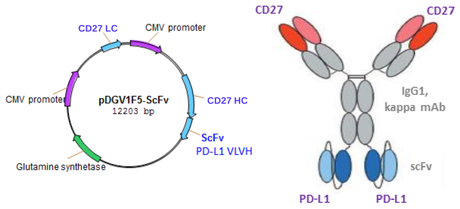
Effector cells (Jurkat T cells expressing human PD-1 and a luciferase reporter driven by an NFAT response element) were co-cultured with APCs (CHO-K1 cells expressing human PD-L1 and an engineered cell surface protein designed to activate cognate TCRs) in the presence of dilutions of the antibodies. Blocking the PD1 negative regulation allows TCR activation and induces luminescence that 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 antibody for 3 days. Supernatants were harvested and IL-2 levels were assessed by ELISA (R&D Systems).

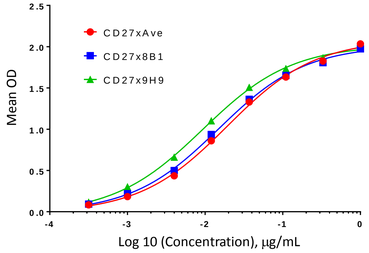
Anti-CD27 x Anti-PD-L1 BsAb



BsAb constructs generated

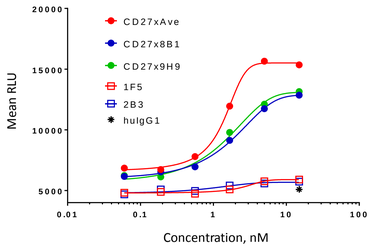
BsAb	Anti-CD27 mAb	Anti-PD-L1 mAb	Reactivity with mouse PD-L1
CD27xAve	1F5	Avelumab	Yes
CD27x8B1	2B3	8B1	No
CD27x9H9	2B3	9H9	No

Bifunctional ELISA



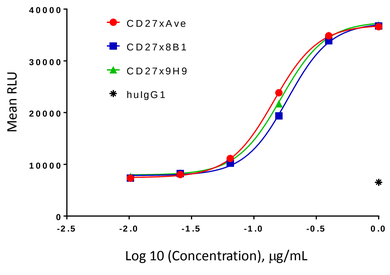
Microtiter plate was coated with human CD27-FLAG-HIS. Dilutions of the BsAbs were allowed to bind before adding human PD-L1-msFc that was detected with an HRP labeled goat anti-mouse IgG (Fc specific) antibody.

NFkB reporter assay (CD27 signaling)



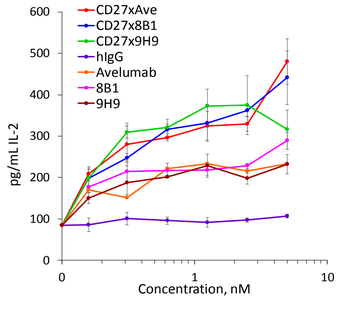
CD27 was transfected into a NFkB-luciferase reporter cell line (Signosis). The cells were incubated for 6 hours with the antibodies and luciferase expression was detected with the Brite-Glo™ system (Promega). Note: the reporter cell line is positive for human PD-L1.

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).

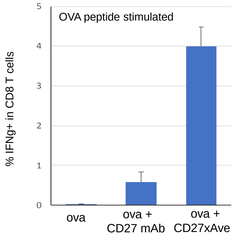
Mixed lymphocyte reaction



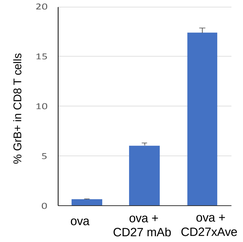
CD4 cells were incubated in the presence of allogeneic dendritic cells and dilutions of antibodies for 3 days. Supernatants were harvest and IL-2 levels were assessed by ELISA (R&D Systems).

In Vivo Activity (CD27xAve)

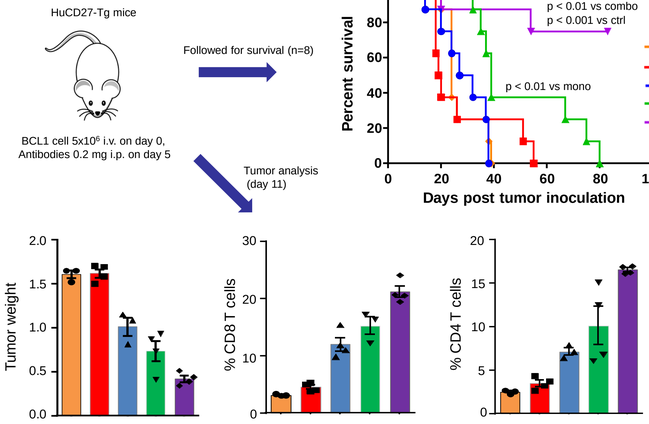
Antigen-specific CD8 T cell response



Overall CD8 T cell response



BCL1 disseminated lymphoma model



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

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