Development of novel bispecific immune modulating antibodies

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 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 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 IgG transgenic mice (H2L2 strain of Harbour® Varanese mice) with recombinant PD-L1. Lead candidates were cloned into a human IgG1 expression vector. Comparisons are presented with anti-PD-L1 mAb, avelumab, produced from the sequence for A09 246-2 (US 20140341917).

Anti-CD27 x Anti-PD-L1 BsAbs were generated by fusing CD27 x IgG1 (Avelumab) and CD27 x IgG1 (Avelumab) to create a tetravalent aCD27- BsAb.

**BsAbs constructs generated**

<table>
<thead>
<tr>
<th>BsAb</th>
<th>Anti-CD27 x</th>
<th>Anti-PD-L1 x</th>
<th>Reactivity with moxie PD-L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD27xAve</td>
<td>IFS</td>
<td>Avelumab</td>
<td>Yes</td>
</tr>
<tr>
<td>CD27x9H9</td>
<td>283</td>
<td>851</td>
<td>No</td>
</tr>
<tr>
<td>CD27x8B1</td>
<td>283</td>
<td>859</td>
<td>No</td>
</tr>
</tbody>
</table>

**Bioluminescent ELISA**

- Maximum phenochange was measured with human CD27 x Avelumab. Expression of the CD27 reporter was measured by ELISA after transfection of CD27 into human PBMCs. CD27 expression was detected with a HRP labeled goat anti-human polyclonal antibody.

**NFAT reporter assay (CD27 signaling)**

CD27 were transfected into a NFkB reporter cell line (Signosis). The cells were treated in 1 hour with antibody alone or antibody and a functional CD27 agonist. The cell interaction was detected with the Bio-Glo system (Promega). Here the reporter cell line includes a NFAT promoter.

**Mixed lymphocyte reaction**

CD4 cells were activated in the presence of allogeneic dendritic cells (OVA 257-264) and IL-2. Supernatants were harvested and IL-2 concentration was measured by ELISA

**Antigen-specific CD8 T cell responses**

**Overall CD8 T cell response**

- Human PD-L1 antibodies were developed as backbone for developing novel BsAb for cancer Immunotherapy.
- Toleratization of CD27xPD-L1 BsAb were developed using a 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 mAb
  - 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 tumor efficacy compared to anti-tumorization of CD27 and PD-L1 mAbs
  - The data support further evaluation of aCD27xPD-L1 BsAbs, and provide a platform for additional BsAb combinations.

**Summary and Next Steps**

- High affinity binding to both CD27 and PD-L1
- Enhanced CD27 signaling relative to parental CD27 mAb
- 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 tumor efficacy compared to anti-tumorization of CD27 and PD-L1 mAbs
- The data support further evaluation of aCD27xPD-L1 BsAbs, and provide a platform for additional BsAb combinations.