Development of Novel Anti-CD27 Human Antibodies with Therapeutic Potential

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CD27 Background

- Member of the TNF-receptor superfamily
- Constitutively expressed on the majority of mature T cells, memory B cells, and a portion of NK cells
- T cell co-stimulatory molecule, required for generation and long-term maintenance of T cell immunity
- Role in clonal B cell expansion and germinal center formation, immunoglobulin synthesis
- Role in NK cell cytolytic activity

Rationale for Targeting CD27 in Cancer

- Anti-mouse CD27 mAb previously shown to have anti-tumor activity in several tumor models
- In vivo CD27 stimulation with sodium/ligand (CD70) promotes strong primary and secondary CD8+cytotoxic T cell responses
- Expression of CD70 on dendritic cells improves immunity of dendritic cell vaccines
- Agonist CD27 mAb may bypass the requirement for CD40 activation in immune activation
- CD27 is expressed by some lymphomas and leukemias

Characterization of CD27 HuMAbs Binding and Ligand Blocking

<table>
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<tr>
<th>Clone</th>
<th>KD(M) (BlaSore)</th>
<th>sCD70 blocking (ELISHA)</th>
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<tr>
<td>1G8</td>
<td>0.025-10</td>
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<td>1G6</td>
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<td>3H12</td>
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- A panel of hybridomas were generated from splenocytes of human immunoglobulin transgenic mice after immunization with second harmonic CD27.
- Human CD27 was selected and expanded by ELISA. Fluorescent cytometry and BlaSore for binding and CD27 blocking.
- 8 selected antibodies were cloned, sequenced and expressed in CHO cells.

Development of huCD27 transgenic mice

- A murine model was established to test the activity of anti-human CD27 HuMAbs (1 μg/ml)
- BAC clone containing the CD27 gene was used for microinjection of mice embryos
- Transgenic lines were established for evaluation of human CD27 expression

Fig. 1. CD27 Expression in Tg Mice by Flow Cytometry

The human CD27 gene is expressed in a similar pattern as mouse CD27. The following antibodies were used against the indicated subset.

Panel A - peripheral blood
Panel B - lymph nodes
Panel C - dual staining, CD27/CD19 in peripheral blood, spleen, lymph node

Fig. 2. CD27 Expression in Tg Mice by IHC

Immunostaining with anti-human (0.1 μg/ml) or mouse (0.5 μg/ml) CD27-FITC and isotype on acetone fixed frozen tissue sections. Counterstained with Mayer’s hematoxylin. LN, lymph nodules; HEV, high endothelial venule; RP, red pulp; WP, white pulp; CA, central artery.

Fig. 3. Regulation of CD27 Expression

- CD27 HuMAbs enhance T cell responses to a dendritic cell-targeted vaccine;
- CD27 HuMAbs inhibit CD27+ human lymphoma growth in xenograft model.

Fig. 4. CD27 HuMAbs Enhance Antigen-Specific CD8+ T Cell Response to Vaccine

Fig. 5. CD27 HuMAb enhances Dendritic Cell Targeted Vaccine

Fig. 6. Anti-tumor Activity of CD27 HuMAb in SCID mice

Summary

- Fully human antibodies specific for human CD27 were selected for characterization;
- A Human CD27-Tg mouse model was developed for in vivo testing of CD27 HuMAbs;
- CD27 HuMAbs enhance antigen-specific CD8+ T cells proliferation and activation;
- CD27 HuMAb enhances T cell responses to a dendritic cell-targeted vaccine;
- CD27 HuMAbs inhibit CD27+ human lymphoma growth in xenograft model.