Development of a Human Anti-CD27 Antibody with Efficacy in Lymphoma and Leukemia Models by Two Distinct Mechanisms

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**CD27 Introduction**
- Member of the tumor necrosis factor (TNF) receptor superfamily
- Constitutively expressed on the surface of majority of mature T cells, memory B cells, and a portion of natural killer (NK) cells
- Also presented in B cell malignancies and adult T cell leukemia/lymphoma
- CD70/CD27 co-stimulatory pathway
  - CD70 activation well-regulated by ligand CD70, which is generally only transiently expressed on activated T cells, B cells, and dendritic cells
  - On T cells: causes activation, proliferation, and maturation of effector and memory cells
  - On human B cells: activates and promotes the generation of plasma cells, proliferation, and the production of immunoglobulin
  - On NK cells: induces cytolytic activity

**Targeting CD27 with Antibodies**
- Agonist anti-CD27 mAb can induce potent anti-tumor immunity through T cell activation (French, RR et al. Blood 2007; Sakahara, T et al. BBRC 2009; Roberts, DJ et al. J. Immunotherapy 2010)
- CD27-targeting antibodies may also provide direct therapeutic effects against tumors with CD27 expression (see Tables below).

**CDX-1127, an agonist anti-CD27 mAb**
- Fully human IgG1κ mAb
- Sub-nanomolar affinity
- Blocks CD70 binding
- Agonist in combination with –CD27
- Cross-reacts with macaque CD27

**CDX-1127 direct killing of CD27+ cells**
- Anti-tumor activity in SCID mice transplanted with human lymphoma cell lines
- Anti-T-cell activity of CDX-1127 agonist activity in mouse tumor models
- Well tolerated in monkey toxicology studies (2.5-25 mg/kg x 5)
- No toxicities reported in Phase I clinical trial
- No significant depletion of PBL (see figure below)
- No cytokine transmembrane elevation
- No activation of human PBMC without TCR engagement
- No cytokine secretion or proliferation
- CD27 safety and toxicology

**CDX-1127 Phase 1 clinical trial**
- Healthy donor study
- Open-label, dose-escalation, safety and pharmacokinetic study of CDX-1127
- ARM 1: Selected solid tumors (n=45)
- ARM 2: Hematologic malignancies (n=45)
- Dose-escalation (mg/kg): 0.1 to 3.0
- Reflected in Kaplan- Meier survival function: 50.1% at 1 year (VIS trials not limited to clinical and molecular subtypes of lymphomas, plasma cell proliferations, or other CD27 positive malignancies)
- Dose-escalation scheme

**Table 1: CD27 expression by IHC**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total CD27</th>
<th>No c-CD3</th>
<th>With c-CD3</th>
</tr>
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<tbody>
<tr>
<td>Bone Marrow</td>
<td>125</td>
<td>121</td>
<td>4</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>23</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Cutaneous SCC</td>
<td>15</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>35</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>40</td>
<td>39</td>
<td>1</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>48</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>16</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Uterine cervical cancer</td>
<td>23</td>
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<td>0</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>22</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>12</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil cancer</td>
<td>41</td>
<td>41</td>
<td>0</td>
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<tr>
<td>Esophageal cancer</td>
<td>21</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Lymph node</td>
<td>20</td>
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</tr>
<tr>
<td>HEV</td>
<td>150</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>RP</td>
<td>41</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>WP</td>
<td>41</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>366</td>
<td>356</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 2: CD27 expression by Flow Cytometry**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CD27+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human CD27+ Tg mice</td>
<td>20/20 (100%)</td>
</tr>
<tr>
<td>Human B-cell lines</td>
<td>20/20 (100%)</td>
</tr>
<tr>
<td>Human T-cell lines</td>
<td>20/20 (100%)</td>
</tr>
<tr>
<td>Human NK-cell lines</td>
<td>20/20 (100%)</td>
</tr>
</tbody>
</table>

**Development of huCD27 transgenic mouse**
- Human CD27 gene (6819 bp)
- Mice generated in the laboratory of Dr. Van Oers, MHJ et al Blood 1993
- Two transgenic lines (L8 and L54) are established, and transgene expression was consistent with Wt expression in huCD27 Tg mice
- No c-CD3, 4% c-CD3

**CDX-1127 safety and toxicology**
- Well tolerated in monkey toxicology studies (2.5-25 mg/kg x 5)
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**Summary**
- An anti-CD27 fully human antibody (CDX-1127) has been developed
- CDX-1127 inhibited the growth of CD27+ human lymphoma and leukemia cells in SCID mice
- CDX-1127 co-stimulatory activity and anti-tumor efficacy have been demonstrated in a human CD27 transgenic mouse model
- CDX-1127 safety was evaluated by human PBMC cytokine release assay and in monkey toxicology studies
- Phase I clinical trial has been initiated in both CD27+ and CD27 malignancies

All authors are full-time employees of Celldex Therapeutics Inc.