Bispecific claudin-6 x CD3 antibodies in a 2+1 format demonstrate selectivity and activity on human ovarian cancer cells

Matthew S. Faber, Sung-Hyung Lee, Yoon Kyung Kim, Jing Qi, Kendra N. Avery, Duc-Hanh T. Nguyen, Ruman, Flash, Araz Eivazi, Seung Y. Chu, Juan E. Diaz, Conner Ardila, Ruschelle Love, Alex Nisthal, Norman J. Barlow, Christine Bonzon, Umesh S. Muchhal, Matthew J. Bennett, John R. Desjarlais

Introduction

- Claudin-6 (CLDN6) is a tetraspan membrane protein involved in the formation of tight junctions. IHC and bulk RNAseq data show CLDN6 is differentially expressed in ovarian cancers compared to normal tissue.
- There is a large unmet need for targeted therapies to treat ovarian cancer and other solid tumors, and the differential expression of CLDN6 in cancerous tissue makes it a promising target for CD3 bispecific antibody therapeutics.
- A complicating factor is that many members of the claudin family have high sequence identity, with CLDN9 having the most similarity to CLDN6. CLDN6 and CLDN9 extracellular (EC) loops differ at only 3 out of 76 residues. CLDN6 is highly expressed in some normal tissue, which makes selectivity for CLDN6 over CLDN9 critical.

To overcome these challenges, we engineered a highly selective anti-CLDN6 antibody and formatted it into our XmAb® 2+1 bispecific antibody format. These selective CLDN6 x CD3 bispecifics were tested for in vitro and in vivo activity in multiple models.

RNA-seq reveals CLDN6 as a selective ovarian cancer target, but close homolog CLDN9 is expressed on normal tissues

CLDNs most similar to CLDN6

<table>
<thead>
<tr>
<th>CLDN</th>
<th>Parental Fv Ab</th>
<th>Parental Fv Ab CLDN6 Chimeric Ab</th>
<th>Loop 1 (CLDN4-CLDN6 Chimeric Ab)</th>
<th>Loop 2 (CLDN8-CLDN9 Chimeric Ab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLDN1</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>CLDN3</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
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<tr>
<td>CLDN4</td>
<td>9</td>
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<td>CLDN5</td>
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</tr>
<tr>
<td>CLDN6</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>CLDN7</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>24</td>
</tr>
</tbody>
</table>

Loop 1

CLDN6 and CLDN9 EC loops differ at only 3 positions

Loop 2

A partially-selective α-CLDN6 mAb was humanized and placed into our XmAb® 2+1 bispecific format

Parental 2+1 bispecific has moderate selectivity

XmAb® 2+1 bispecific

Parental 2+1 bispecific

Fv engineering further improves CLDN6 selectivity over CLDN9

Single concentration scan of ~300 variants, MI values normalized to the parental Fv.

Variants with improved selectivity

Selected CLDN6 x CD3 bispecifics induce TDCC of CLDN6+ cell lines and spare cells with normal tissue levels of CLDN9

IHC confirms RNA-seq data: no CLDN6 expression in healthy tissue, but strong expression in ovarian cancer tissue, and CLDN9 activity must be avoided to achieve high normal tissue expression.

CLDN9 x CD3 lead candidates decrease established tumor size in ovarian cancer patient populations.

PK dose response with most potent bispecific

PK of Fv-1 bispecific w/ CD3-high.

Summary

The engineered XmAb® 2+1 CLDN6 x CD3 bispecific antibodies are:

- Highly selective for CLDN6 over all other CLDNs.
- Effective in recruiting T cells to kill cancer cells with CLDN6 levels similar to those observed in ovarian cancer patient populations.
- Effective at reducing tumor growth in vivo xenograft mouse models of ovarian cancer.
- Well-tolerated in non-human primates with favorable PK.

These results support further evaluation of these bispecifics for treatment of ovarian cancer.