**Anti-TIGIT antibodies require enhanced FcγR co-engagement for optimal T and NK cell-dependent anti-tumor immunity**

Rebecca Ward1, Elena Paltrinieri1, Marilyn Marques2, Priyadarsini Iyer2, Sylvia Dietrich3, Jeremy D. Waught2, Mark Bushell2, Nicholas S. Wilson4, Jennifer S. Buell1, David A. Savitsky5, and Dhan Chand1

1Agenus Inc. or subsidiary thereof (current or former employee), Lexington, MA

**T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is a critical inhibitor of the immune response to cancer**

An anti-TIGIT Fc enhanced antibody has multiple MOAs in cancer therapy

1. Fc-enhanced anti-TIGIT mAb enhances the quality of the immune synapse between a T cell and a target APC to enable optimal T cell activation.

2. Blocking of TIGIT-FcγR1 interactions on T and NK cells eliminates the inhibitory signaling and promotes the expansion of T and NK cell populations.

3. Fc-enhanced anti-TIGIT mAb enhances superior NK cell activation (NKp46+ NK cells).

4. Relative Treg mediated immune suppression

Fc engineering enhances binding to activating FcγRs

Fc-enhanced TIGIT mAb demonstrates improved binding to activating FcγRIIIA (human) or FcγRIII (mouse)

Table 1: FcγR binding characteristics as determined by surface plasmon resonance or cell binding to FcγRIII

<table>
<thead>
<tr>
<th>FcγR</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRIIIA</td>
<td>Anti-FcγRIIIA (9E9)</td>
</tr>
<tr>
<td>FcγRIII</td>
<td>- TIGIT</td>
</tr>
</tbody>
</table>

Enhanced FcγR co-engagement is required for monotherapy tumor control

Conventional anti-TIGIT mAbs lack single agent activity

- FcγR co-engagement is critical for enhancing anti-TIGIT mAbs responsiveness

**Tumor efficacy by anti-TIGIT antibody is dependent on T and NK cells and NK cell depletion reduces tumor control by anti-TIGIT therapy**

Figure 3: B-16 cells were treated with either isotype control, anti-TIGIT or anti-TIGIT Fc-enhanced antibodies for 72 hours, stained with antibodies against Vβ8 and CD8, and analyzed by flow cytometry. Mean fluorescence intensity (MFI) values are shown as PI.

**FCγR co-engagement is critical for enhancing antigen-specific T cell responses**

Blockade of FCγR decreases CD4 and CD8 T cell responses

Figure 4: GSK263-616-C2 (vβ4.14 transgenic) mice were injected intraperitoneally with 150µg of SEB supernatant together with 50µg of anti-TIGIT or anti-CTLA-4 (clone 30mu) or isotype control in a 1:3 ratio and treated weekly using a digital caliper. Mean tumor size at the start of treatment was approximately 45 mm3.

Antigen-specific T cells were monitored and treated intraperitoneally with 200µg of anti-TIGIT or anti-CTLA-4 antibodies twice a week for 4 weeks per tumor implantation. Tumor growth was monitored and antibody efficacy was determined using tumor volume and standard growth curves (B) (C).

**Conclusions:**

1. Our data describe a novel FcγR-dependent mechanism of action that enhances the therapeutic activity of anti-TIGIT mAbs in preclinical studies.

2. FcγR co-engagement is critical for the activity of anti-TIGIT mAbs.

3. AGEN Fc-enhanced anti-TIGIT antibody shows monotherapy and superior combination potential compared to conventional TIGIT mAbs.

4. AGEN Fc-enhanced anti-TIGIT antibody is expected to extend therapeutic benefits to an additional 40% of patients who express the low affinity FcγR and are less likely to respond clinically to conventional TIGIT antibodies.

References:

1. Waight et al. Cancer Cell 2018

2. Johnston et al., Cancer Cell 2014

3. Waight et al. Cancer Cell 2018

4. Waight et al. Cancer Cell 2018