**Anti-TIGIT Antibodies Promote Immune Activation Relevant to Targeting Stem-like and Tumor-specific T Cells in Combination With Anti-PD-1**

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**Abstract**

Background. TIGIT is an inhibitory receptor expressed primarily on NK and T cell subsets, and binding to its conserved receptor ligand, CD155, results in mechanisms of immunosuppression. Blocking the TIGIT-CD155 interaction in the context of cancer promotes anti-tumor immunity.

Methods. We characterized cellular subsets that TIGIT blockade may impact and the pharmacology of two anti-TIGIT antibodies - representing functional (AB308) and non-functional (AB154, domvanalimab, dom) FC domain classes - undergoing clinical evaluation. Surrogate antibodies were leveraged to interrogate TIGIT biology in mouse syngeneic tumor models. Human tumor-infiltrating lymphocytes from a variety of cancer types expressed appreciable levels of TIGIT on relevant immune populations, including Tm, Th, and CD8+ T cells.

Results. Both antibodies potently bound TIGIT and blocked the TIGIT-CD155 interaction as well as displayed the predicted phenotypes in terms of Fc receptor (FcyR) engagement. In line with FcyRIIIB binding, AB308 promoted activation and effector function. Thickness of arrows indicates binding affinity; white lines in dom signify mutated residues.

Conclusions. These data provide a rationale for combination with immune-activating agents and support ongoing clinical evaluation of AB154 and AB308 with biomarker strategies focused on understanding the role of Fc functionality.

**TIGIT Blockade Promotes T and NK Cell Activation**

Inhibited State

Activated State

**PD-1, TIGIT, and CD226 are Expressed on Human Tumor Infiltrating T cells Representing Various Phenotypic Profiles and Differentiation States**

**Immune-related Adverse Events (irAEs) for the Combination of Domvanalimab + Zimbelimab**

<table>
<thead>
<tr>
<th>Phase 1 (n=16)</th>
<th>dose (mg/kg)</th>
<th>dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroidism</td>
<td>5 (9.5%)</td>
<td>2 (3.6%)</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>2 (3.5%)</td>
<td>3 (4.7%)</td>
</tr>
<tr>
<td>Infusion-related</td>
<td>4 (6.3%)</td>
<td>3 (4.7%)</td>
</tr>
<tr>
<td>Rash</td>
<td>2 (3.1%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Maculopapular rash</td>
<td>3 (4.5%)</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>1 (1.5%)</td>
<td>1 (1.5%)</td>
</tr>
</tbody>
</table>

**Case Studies from Phase 1 Patients Exhibiting Partial Responses in the Absence of Peripheral Treg Depletion**

**Summary and References**

- **PD-1, TIGIT, and CD226 are co-expressed on tumor-resident and circulating CD8+ T cells that represent stem-like populations (akin to reported cellular targets of anti-PD1) and terminally differentiated dysfunctional populations in gastrointestinal and lung tumor samples (Figure 2).**
- **In mice, Fc-silent anti-TIGIT antibody enhances tumor control without intratumoral Treg depletion** (Figure 3) in combination with anti-PD-1.
- **Domvanalimab binds human TIGIT, blocks the TIGIT-CD155 interaction, and does not exhibit Fc-dependent cytostaticity of TIGIT+ cells in vitro** (Figure 4) or in vivo (Figure 5).
- **Competitor antibodies with Fc domain functionality have reported depletion of peripheral T lymphocyte populations in mice.** In contrast, domvanalimab does not deplete peripheral lymphocyte populations, including regulatory and activated T cell subsets.
- **The incidence of pruritus, rash, maculopapular rash and infusion-related reactions for domvanalimab** in an ongoing Phase 1 trial in combination with anti-PD-1 (zimbelimab) appear to be lower than Fc-enabled anti-TIGIT monoclonal antibodies (Table 1).
- **Phase 1 patients unable to respond to single-agent anti-PD-1 have had deep partial responses to dom + pD1** without evidence of peripheral lymphodepletion (Figure 6) supporting continued clinical development of the dom + anti-PD-1 combination.

**Figure 1. Schematic depicting primary mechanism of action of anti-TIGIT antibodies.** On the surface of NK and T cell subsets, TIGIT outcompetes CD226/CD155 for binding to CD155/PVR, delivering inhibitory signals and blocking activation. When TIGIT is blocked, CD226 promotes activation and effector function. Thickness of arrows indicates binding affinity; white lines in dom signify mutated Fc domain to silence antibody effector function. Created with BioRender.

**Figure 2. Evaluation of TIGIT, CD226, and CD155 expression in the tumor microenvironment.** Cell subsets from frozen-dissected gastrointestinal/esophageal (GE) and lung (NSCLC) cancer patient tumors (sourced from Discovery Life Sciences) were phenotyped using flow cytometry. (A) CD155 and PD-L1 ligand expression on CD25+ monocytes (CD45+CD3-CD16+CD56+CD14+) and cancer infiltrating cells (CD45+CD3-CD16+CD56+CD14+) and on cancer infiltrating cells (CD45+CD3-CD16+CD56+CD14+) and CD8+ T cells. (B) Contour plot (NSCLC subject) of PD-1 expression on CD8+ T cells encompassing various states of activation and differentiation. Each population was further characterized into tissue resident (CD103+) and circulating (CD103) CD8+ populations, resulting in six subsets. (C) Co-expression of TIGIT and CD226 on the six CD8+ T cell populations from (B) and co-expression of PD-1, TIGIT, and CD226 on broad T cell populations.

**Figure 3. Diffeferential effects of surrogate mouse Fc-enabled (WT) and Fc-silent (FcS) anti-TIGIT antibodies in the MC38 syngeneic tumor model.** Mice with established MC38 tumors were dosed with 5 mg/kg anti-PD-1 twice and 10 mg/kg anti-TIGIT four times as indicated by solid triangles (left). The percentage of intratumoral Treg (CD4+FoxP3+CD25+) was quantified by flow cytometry 3 days post-dose (right). Lines or bars and error denote the mean ± standard error of the mean.

**Figure 4. Characterization of anti-TIGIT antibody Fc functionality in vitro.** (A) Antibody-dependent cell-mediated cytotoxicity (ADCC) Luciferase Reporter Assay Kit (Promega). Lines and error denote the mean ± standard deviation (SD). (B) NK-mediated ADCC against TGF target cells isolated from peripheral blood mononuclear cell suspensions with representative histograms showing TIGIT expression. Paired symbols indicate treatment with human IgG isotype (●) or anti-TIGIT antibodies (✱) or anti-TIGIT antibodies (†) triamgulab (tira), AB308, or domvanalimab (dom) for each NK-T cell donor pair. Tira was produced by Arcus using sequences disclosed in the WHO Drug Information proposed INN publication.

**Figure 5. Peripheral lymphocyte and absolute cell counts were assessed in whole blood (WB) samples from Phase 1 patients (NCT03628677). (A) Representative gating strategy. Lin, images (B) Population and per cell TIGIT expression levels at baseline. Bars denote median and each symbol an individual subject. gMFI, geometric mean fluorescence intensity. (C) Fold change in peripheral CD8+, Tm, PD-1+ (non-Tm) and NK cell frequencies for patient 027 (esophageal cancer) and 029 (gastroesophageal junction cancer, GEJ) treated with 10 mg/kg domvanalimab (dom) Q3W + 360 mg zimberelimab (zim) Q3W. Paired symbols indicate treatment with human IgG isotype (●) or anti-TIGIT antibodies (✱) or anti-TIGIT antibodies (†) triamgulab (tira), AB308, or domvanalimab (dom) for each NK-T cell donor pair. Tira was produced by Arcus using sequences disclosed in the WHO Drug Information proposed INN publication.