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Fc-silent Anti-TIGIT Antibodies Potentiate Anti-tumor Immunity Without Depleting Regulatory T Cells

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Background & Summary

- TIGIT is an inhibitory checkpoint receptor primarily expressed on T and NK cell subsets.
- TIGIT suppresses cell activation through intrinsic mechanisms and by outcompeting a key activating receptor, CD226/DNAM-1, for the shared ligand CD155/PVR[1-5].
- Analogous to the TCR and CD28, PD-1 can de-phosphorylate CD226 to suppress cell activation[6,7].
- To achieve maximal effector cell activation and functionality, it is hypothesized that simultaneous blockade of TIGIT and PD-1 is necessary, particularly to target cell subsets that co-express TIGIT and PD-1, namely pre-exhausted T cells (T_{pex}).
- The TIGIT field has largely focused on elucidating mechanisms of action related to blocking antibodies that retain Fc functionality. In mouse models, Fc-enabled anti-TIGIT can deplete regulatory T cells (T_{reg}), an activity associated with single-agent efficacy[8,9]; however, whether such mechanisms will translate to humans is unclear.
- In contrast, Fc-silent anti-TIGIT can enhance efficacy of anti-PD-1 without T_{reg} depletion in mice[10].

➢ **Here, we report pharmacology and mechanisms of action associated with Fc-silent anti-TIGIT antibodies relative to Fc-enabled counterparts. We found:**

- Human and mouse tumor-infiltrating lymphocytes express TIGIT and CD226 on T_{reg}, $CD4^+$ non-T_{reg}, and on $CD8^+$ T cells with tumor-reactive or exhausted/dysfunctional phenotypes (**Figure 2**).
- In mice, in combination with anti-PD-1, Fc-silent anti-TIGIT enhances activation, differentiation, and effector function of tumor-specific $CD8^+$ T cells in a manner dependent upon T cell egress from the tumor-draining lymph node (**Figure 3**).
- *In vitro*, Fc-enabled human TIGIT-specific antibodies facilitated antibody-dependent cell-mediated cytotoxicity (ADCC) against TIGIT-expressing human T_{reg}, with preferential depletion of a $Helios^+$ effector T_{reg} subset (**Figure 4** & **Figure 5**).
- In cancer patients, significant and stable decreases in T_{reg} were measured in the peripheral blood of cancer patients treated with an Fc-enabled (AB308), but not an Fc-silent (domvanalimab), anti-TIGIT antibody (**Figure 6**).
- Cancer patients treated with domvanalimab in combination with anti-PD-1 antibody, zimberelimab, experienced partial responses while maintaining stable peripheral T_{reg} frequencies on treatment[10].

➢ **Silencing the Fc domain of anti-TIGIT antibodies prevents depletion of peripheral T_{reg}, potentially critical for an optimal safety-efficacy profile in cancer patients.**

Some images made with BioRender. Referenced DOIs: [1]10.1158/2326-6066.CIR-19-0877, [2]10.1038/s41467-023-40755-3, [3]10.4049/jimmunol.1003081, [4]10.1172/JCI81187, [5]10.1038/ni.1674, [6]10.1016/j.immuni.2022.02.005, [7]10.1126/sciimmunol.aat7061, [8]10.1158/1535-7163.MCT-20-0464, [9]10.3389/fimmu.2022.828319, [10]10.4049/JIMMUNOL.208.SUPP.120.13; download at: https://arcusbio.com/our-science/publications/, [11]10.1146/annurev-immunol-042718-041717.

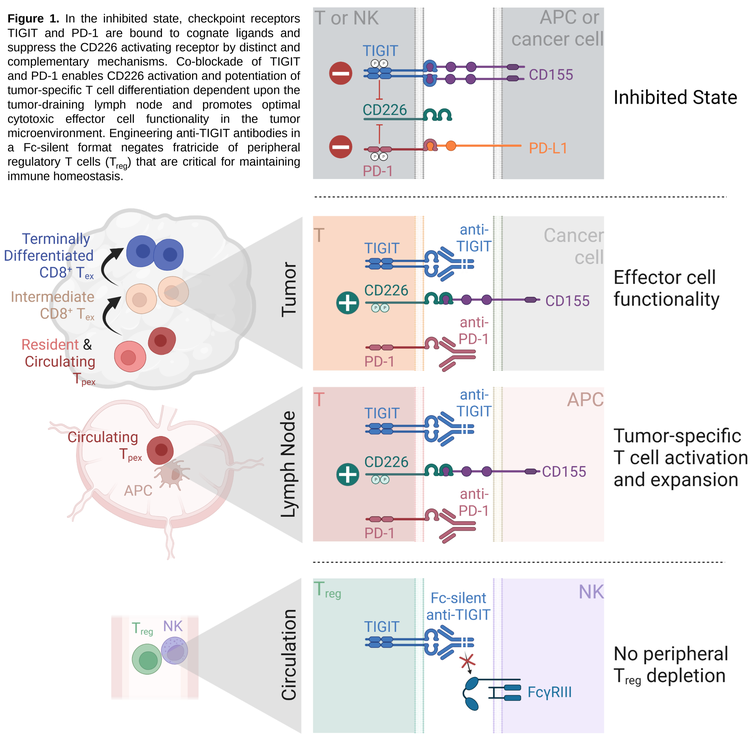

Figure 1. In the inhibited state, checkpoint receptors TIGIT and PD-1 are bound to cognate ligands and suppress the CD226 activating receptor by distinct and complementary mechanisms. Co-blockade of TIGIT and PD-1 enables CD226 activation and potentiation of tumor-specific T cell differentiation dependent upon the tumor-draining lymph node and promotes optimal cytotoxic effector cell functionality in the tumor microenvironment. Engineering anti-TIGIT antibodies in a Fc-silent format negates fratricide of peripheral regulatory T cells (T_{reg}) that are critical for maintaining immune homeostasis.

Results

Fc-enabled Anti-TIGIT Directs NK-mediated ADCC Against TIGIT-expressing Target Cells

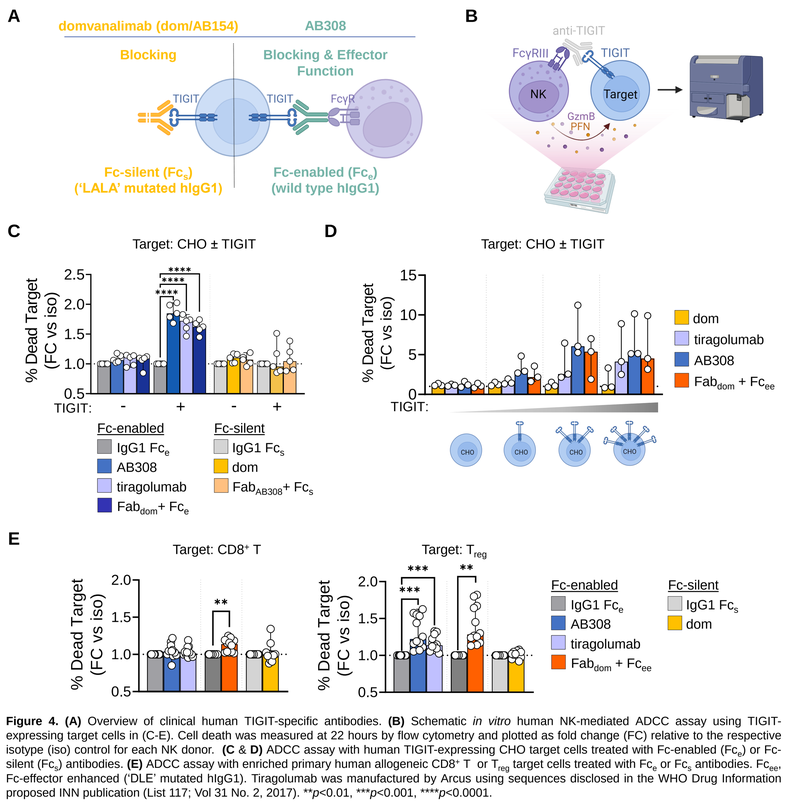

Figure 4. (A) Overview of clinical human TIGIT-specific antibodies. **(B)** Schematic *in vitro* human NK-mediated ADCC assay using TIGIT-expressing target cells in (C-E). Cell death was measured at 22 hours by flow cytometry and plotted as fold change (FC) relative to the respective isotype (iso) control for each NK donor. **(C & D)** ADCC assay with human TIGIT-expressing CHO target cells treated with Fc-enabled (Fc_e) or Fc-silent (Fc_s) antibodies. **(E)** ADCC assay with enriched primary human allogeneic $CD8^+$ T or T_{reg} target cells treated with Fc_s or Fc_e antibodies. Fc_{ee}, Fc-effector enhanced ('DLE' mutated hIgG1). Tiragolumab was manufactured by Arcus using sequences disclosed in the WHO Drug Information proposed INN publication (List 117; Vol 31 No. 2, 2017). $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$.

Peripheral $Helios^+$ Effector T_{reg} (eT_{reg}) are Preferentially Targeted By TIGIT Directed ADCC

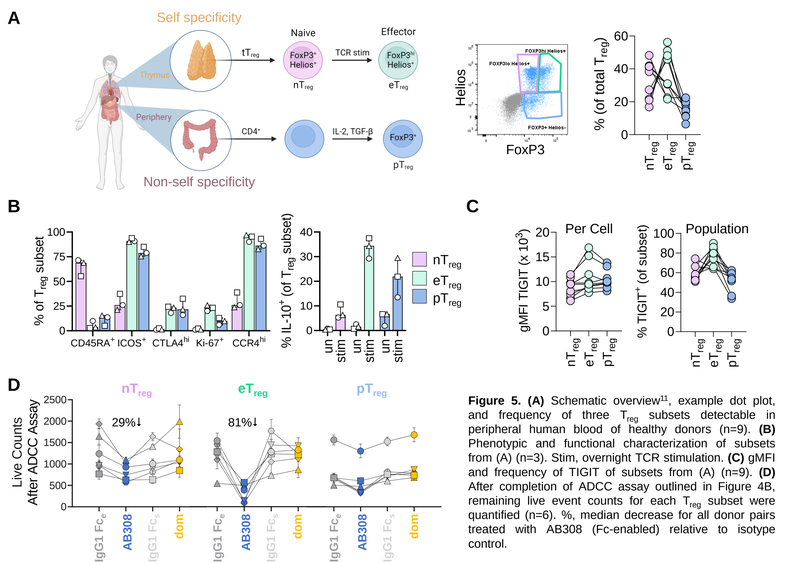

Figure 5. (A) Schematic overview[11], example dot plot, and frequency of three T_{reg} subsets detectable in peripheral human blood of healthy donors (n=9). **(B)** Phenotypic and functional characterization of subsets from (A) (n=3). Stim, overnight TCR stimulation. **(C)** gMFI and frequency of TIGIT of subsets from (A) (n=9). **(D)** After completion of ADCC assay outlined in Figure 4B, remaining live event counts for each T_{reg} subset were quantified (n=6). %, median decrease for all donor pairs treated with AB308 (Fc-enabled) relative to isotype control.

Peripheral T_{reg} Significantly Decrease in Patients Treated with Fc-enabled Anti-TIGIT, But Remain Stable in Patients Treated with Fc-silent Anti-TIGIT

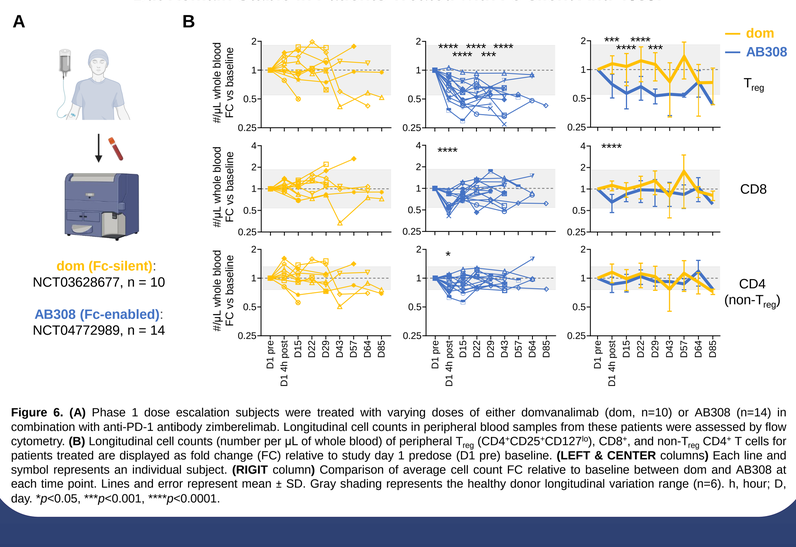

Figure 6. (A) Phase 1 dose escalation subjects were treated with varying doses of either domvanalimab (dom, n=10) or AB308 (n=14) in combination with anti-PD-1 antibody zimberelimab. Longitudinal cell counts in peripheral blood samples from these patients were assessed by flow cytometry. **(B)** Longitudinal cell counts (number per µL of whole blood) of peripheral T_{reg} ($CD4^+CD25^+CD127^{lo}$), $CD8^+$, and non-T_{reg} $CD4^+$ T cells for patients treated are displayed as fold change (FC) relative to study day 1 predose (D1 pre) baseline. **(LEFT & CENTER** columns**)** Each line and symbol represents an individual subject. **(RIGIT** column**)** Comparison of average cell count FC relative to baseline between dom and AB308 at each time point. Lines and error represent mean ± SD. Gray shading represents the healthy donor longitudinal variation range (n=6). h, hour; D, day. $^*p<0.05$, $^{***}p<0.001$, $^{****}p<0.0001$.

Results

Human and Mouse Tumor Infiltrating Lymphocytes Co-express TIGIT, PD-1, and CD226

Figure 2. (A) Exhausted T cell differentiation occurs in the presence of chronic antigen and is a continuum of functionally distinct T cell states. Pre-exhausted (T_{pex}) cells express PD-1 and TIGIT and are the proposed targets of combination blockade. Activation of circulating (c) or resident (r) T_{pex} promotes differentiation to an intermediate (Int) state with cytotoxic potential before eventually terminal (Term) differentiation. **(B)** TCF-1 and TIM-3 can be used to distinguish early and late differentiation stages of exhausted T cells in human **(C-I)** and mouse **(J-P)** tumors by flow cytometry.

Results

Fc-silent Anti-TIGIT Potentiates Tumor-specific T Cell Activation and Differentiation that is Dependent on the Tumor Draining Lymph Node

Figure 3. (A) Experimental overview. MC38 tumor-bearing mice were given 0.25 mg/kg FTY720 daily by oral gavage (PO QD) initiating one day prior to antibody treatments and 10 mg/kg αPD-1 ± αTIGIT (both Fc-silent) every 5 days by intraperitoneal injection (IP Q5D). FTY720 is a functional S1PR antagonist that prevents T cell egress from secondary lymph nodes. The antibody regimen was optimized based on tumor receptor occupancy (not shown). Flow cytometry assessments in (B-F) were done 3 days after the first antibody treatment while tumor outgrowth was assessed in (G-H). **(B-F)** Data are normalized as fold change (FC) relative to the mean of the isotype (iso) + vehicle control group and compiled from three independent experiments each with n=8/group. Representative dot plots **(B)**, frequency and number per mg of $p15E^+$ (endogenous tumor-specific $CD8^+$) T cells in the tumor **(C)** and inguinal tumor-draining lymph node (tdLN) **(D)**. **(E)** Schematic and representative dot plot showing differentiation states of intratumoral exhausted $CD8^+$ T cells defined by TCF-1 and CD69 expression. cT_{pex}, circulating pre-exhausted; rT_{pex}, resident pre-exhausted; Int, intermediate (cytotoxic); Term, terminally differentiated. **(F)** Frequency of $p15E^+$ subsets from (E) in the tumor. **(G)** Tumor growth curves where symbols and error represent the mean ± SEM and **(H)** individual growth curves with number of complete regressions (CR). n=10/group. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$.