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# Anti-TIGIT Antibodies Promote Immune Activation in Combination with Immunotherapeutic Agents

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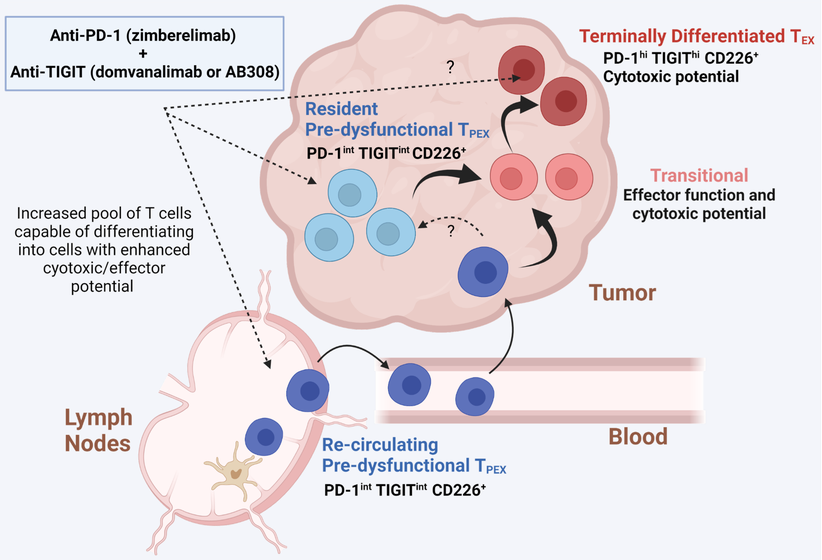
## OVERVIEW

- TIGIT (T-cell immunoreceptor with Ig and ITIM domains) is an inhibitory receptor expressed on CD8$^+$ T cells, CD4$^+$ T cells, natural killer (NK) cells and regulatory T cells ($T_{regs}$).
- It competes with an activating receptor, DNAM-1/CD226, for shared receptor ligands (mainly CD155) that are expressed by cancer and antigen-presenting cells.
- Current data indicate that intratumoral expansion of TCF-1$^+$ PD-1$^{int}$ pre-dysfunctional ($T_{PEX}$) cells are critical for maintaining an ongoing anti-tumor response and are reported to be cellular targets of anti-PD-(L)1 therapy[1-6].
- TIGIT and CD226 are co-expressed on intratumoral CD8$^+$ T cells that may represent pre-dysfunctional TCF-1$^+$ PD-1$^{int}$ populations and thus are probable targets for anti-TIGIT therapy.
- AB308 (Fc-enabled) and domvanalimab (Fc-silent) are high affinity anti-TIGIT blocking antibodies that are currently undergoing clinical evaluation.

**Checkpoint Blockade Promotes Anti-tumor Immunity by Targeting Pre-dysfunctional ($T_{PEX}$) CD8$^+$ T Cells**




int = intermediate

## RESULTS

**Combination of Either Fc-Silent or Fc-Enabled Anti-TIGIT with Anti-PD-1 Enhances Tumor Control Over Anti-PD-1 Alone, But Activity of the Fc-Enabled Anti-TIGIT is Associated With Intratumoral $T_{reg}$ Depletion in Mice**

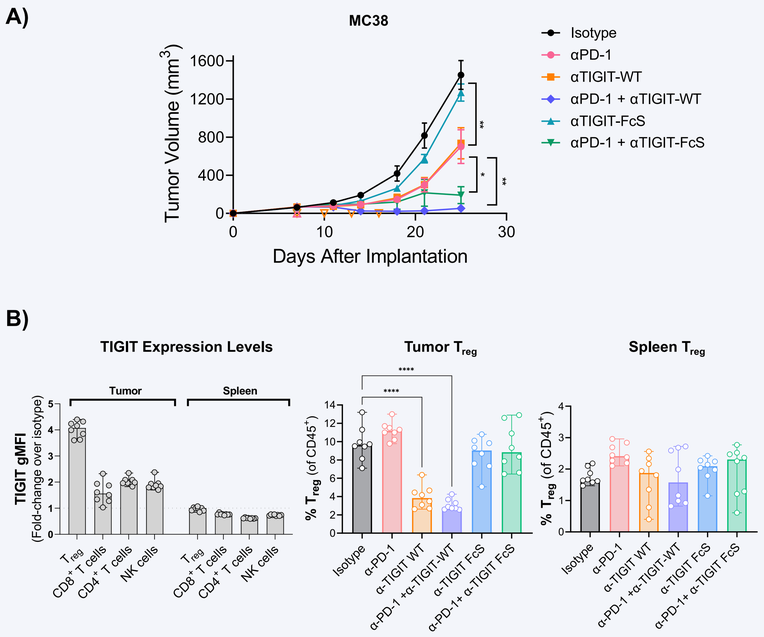



**Figure 1. Differential effects of surrogate^ mouse Fc-enabled (WT) and Fc-silent (FcS) anti-TIGIT antibodies in MC38 syngeneic tumor model.** **(A)** Mice with established MC38 tumors were dosed with 5 mg/kg anti-PD-1 (Δ) and 10 mg/kg anti-TIGIT antibodies (▽) as indicated. Mixed effects analysis, Dunnett's multiple comparisons test vs. anti-PD-1. **(B)** At 3 days post-dose, expression levels of TIGIT (gMFI fold-change over isotype) and the percent of intratumoral $T_{regs}$ (CD4$^+$Foxp3$^+$CD25$^+$) in the tumor and spleen were quantified. Sidak's multiple comparisons test vs. Isotype and anti-PD-1 (B and C). ****$p$<0.0001, **$p$<0.01, *$p$<0.05. Error bars denote median ± range while symbols represent individual mice. WT = mIgG2a backbone; FcS = mIgG2a backbone with L234A, L235A, and P329G (Eu numbering) mutations in the heavy chain.

^AB308 and domvanalimab do not bind mouse or rat TIGIT (data not shown)

## RESULTS

**Fc-enabled AB308 and tiragolumab Induce FcγR-mediated Signaling and Promote NK-mediated ADCC Against TIGIT-Expressing Target Cells**

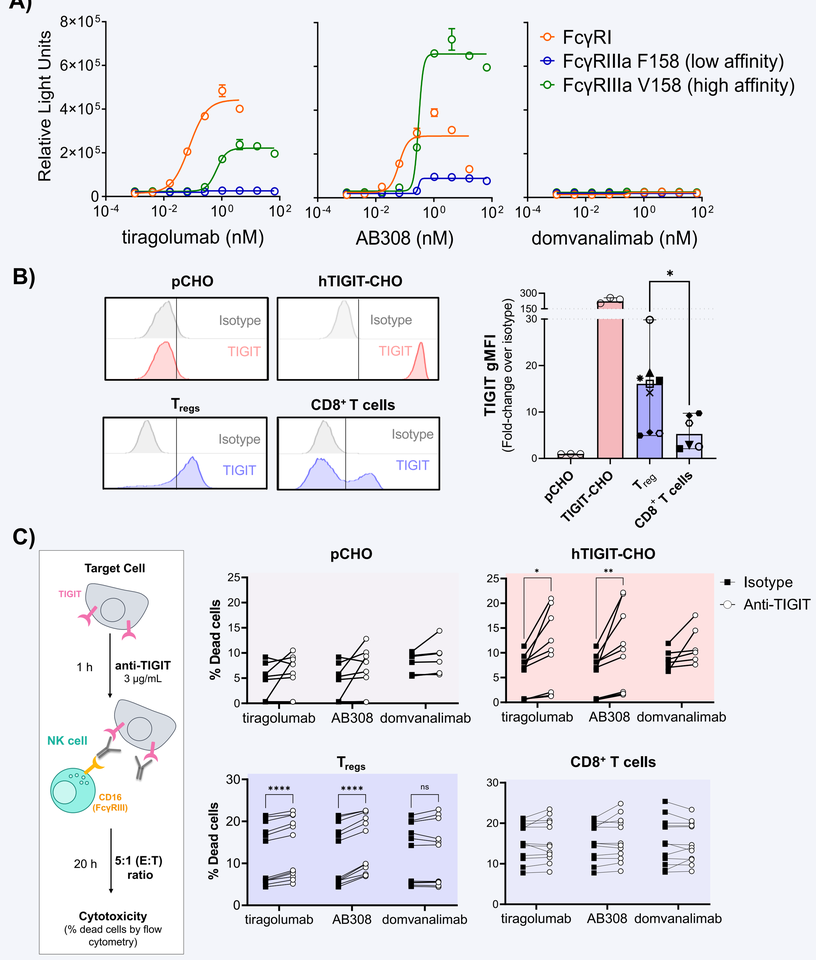



**Figure 2. Characterization of FcγR binding and ADCC capability by AB308, tiraglolumab, and domvanalimab.** **(A)** Using an ADCC Reporter Assay (Promega) and CHO cells expressing human TIGIT (hTIGIT-CHO), AB308 induces signaling via FcγRI and FcγRIIIa F158 and V158 varients. Activity normalized to highest concentration of AB308 (100 nM). **(B)** Representative histograms showing TIGIT expression and quantification (gMFI fold-change over isotype) on target cells. **(C)** (Left) Schematic of assay used to assess NK-mediated ADCC of TIGIT$^+$ target cells. Target cells include parental CHO (pCHO), hTIGIT-CHO, healthy donor $T_{regs}$ (CD4$^+$CD25$^+$CD127$^{lo}$) and healthy donor CD8$^+$ T cells. (Right) Cytotoxicity quantified for each target cell. Paired symbols indicate treatment with human IgG1 isotype (■) or anti-TIGIT antibodies (○) for each NK donor (pCHO, hTIGIT-CHO) or NK/T donor pair ($T_{reg}$, CD8$^+$). ****$p$<0.0001,**$p$<0.01, *$p$<0.05, Sidak's multiple comparisons test vs. human IgG1 Isotype control. Tiragolumab was manufactured by Arcus using sequences disclosed in the WHO Drug Information proposed INN publication (List 117; Vol 31 No. 2, 2017).

**Evaluation at the Single-cell Transcriptional Level of PD-1, TIGIT and CD226 on Tumor Infiltrating CD8$^+$ T cells, Including Those with Pre-dysfunctional ($T_{PEX}$) Phenotypes**

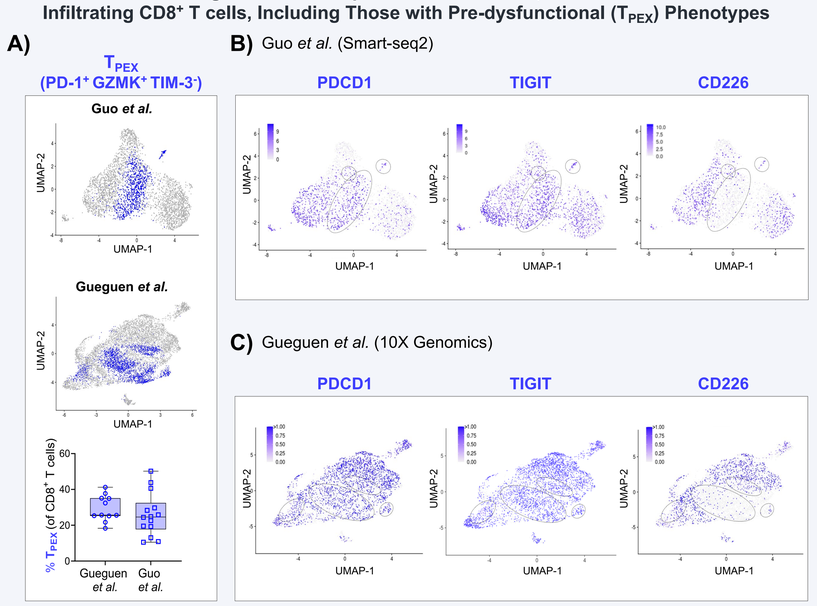



**Figure 3. Evaluation of TIGIT and CD226 gene expression in putative pre-dysfunctional CD8$^+$ T cells in human NSCLC single-cell RNA sequencing (scRNAseq) datasets.** Using two publicly available human NSCLC scRNAseq datasets (Guo *et al.* 2018[7] & Gueguen *et al.* 2020[8]), pre-dysfunctional CD8$^+$ T cells ($T_{PEX}$) were identified by applying filters to single-cell clusters in the following order: medium to high expression of PD-1, negative expression of TIM-3, and positive expression of granzyme K (GZMK). **(A)** UMAP plots mapping $T_{PEX}$ cells, and percent of $T_{PEX}$ of CD8$^+$ T cells in NSCLC subjects within each dataset. Box plots with symbols representing a unique NSCLC subject. **(B and C)** UMAP plots mapping PDCD1, TIGIT, CD226 expression to total CD8$^+$ T cells in (B) Guo *et al.* and (C) Guegen *et al.* datasets. Dotted lines within each plot outline $T_{PEX}$ clusters for reference.

## RESULTS

**PD-1, TIGIT, and CD226 are Co-Expressed on Tumor Infiltrating T cells, Including Those with Stem-like (TCF-1$^+$) and Terminally Differentiated (TIM-3$^+$) Phenotypes**

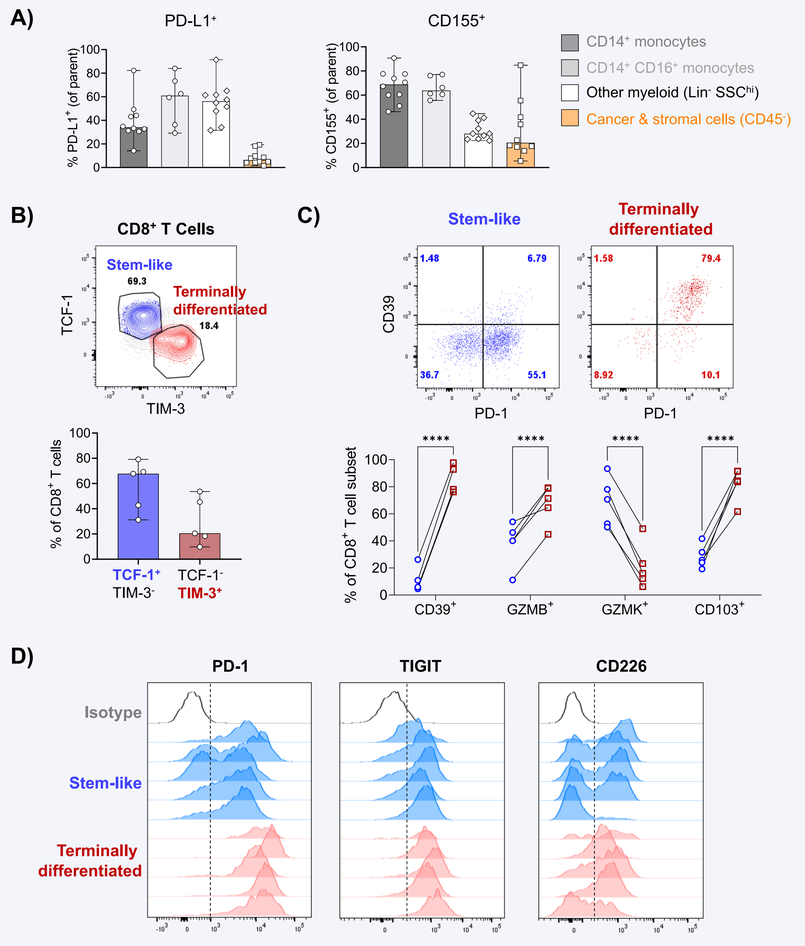






**Figure 4. Evaluation of TIGIT, CD226, and CD155 protein expression in the NSCLC tumor microenvironment.** Commercially-sourced dissociated cells from NSCLC patient tumors were evaluated for expression of TIGIT and other markers by flow cytometry. **(A)** CD155 and PD-L1 expression on CD14$^+$ monocytes (CD45$^+$CD3$^-$CD16$^-$CD56$^-$CD14$^+$ CD16$^-$), CD14$^+$CD16$^+$ monocytes (CD45$^+$CD3$^-$CD16$^-$CD56$^-$CD14$^+$ CD16$^+$), other myeloid cells (CD45$^+$CD3$^-$CD56$^-$CD14$^-$ CD16$^-$CD19$^-$SSC$^{hi}$) and cancer & stromal cells (CD45$^-$). **(B)** Example contour plot (top) and quantification of CD8$^+$ T cells (bottom) representing stem-like (TCF-1$^+$TIM-3$^-$) and terminally differentiated (TCF-1$^-$TIM-3$^+$) cells[5,9]. **(C)** Example dot plots (top) and quantification (bottom) of markers associated with terminal exhaustion (CD39), cytotoxicity (Granzyme B; GZMB), circulating pre-dysfunctional T cells (Granzyme K; GZMK) and tissue residency (CD103). **(D)** Example histograms of PD-1, TIGIT, and CD226 expression on stem-like and terminally differentiated CD8$^+$ T cells. **(E)** Percent positive and expression levels (determined by gMFI of percent positive cells) of PD-1, TIGIT and CD226 in $T_{PEX}$ and terminally exhausted CD8$^+$ T cells. **(F) Top:** Example flow plot of TIGIT, CD226 co-expression in $T_{PEX}$ and terminally exhausted CD8$^+$ T cells and **Bottom:** Percent of PD-1, TIGIT and CD226 co-expression cells. Each symbol represents a unique subject. Bars denote median ± range while lines connect populations from the same subject.

## SUMMARY

- Fc-enabled AB308, but not Fc-silent domvanalimab, has the capacity to bind Fcγ receptors and promote NK-mediated ADCC (Fig. 2).
- PD-1, TIGIT, and CD226 are co-expressed on both stem-like and terminally exhausted intratumoral CD8$^+$ T cell subsets in NSCLC subjects at both the transcriptional (Fig. 3) and protein level (Fig. 4).
- TIGIT & CD226 co-expressing $T_{PEX}$ are probable targets for anti-TIGIT therapy, akin to reported cellular targets of anti-PD-(L)1.

[1]Siddiqui *et al.* 2019 Immunity [2]Sade-Feldman *et al.* 2018 Cell [3]Connolly *et al.* 2021 Science Immunol [4]Jansen *et al.* 2019 Nature [5]Miller *et al.* 2019 Nat Immunol. [6]Van der Leun *et al.*, 2020 Nat Rev Cancer [7]Guo *et al.* 2018 Nat Med [8]Gueguen *et al.* 2020 Science Immunol [9]Thommen *et al.* 2018 Nat Med.