Society for Immunotherapy of Cancer
Annual Meeting, November 2021
Abstract 258

AB308 is an Anti-TIGIT Antibody That Enhances Immune Activation and Anti-Tumor Immunity Alone and in Combination With Other I-O Therapeutic Agents

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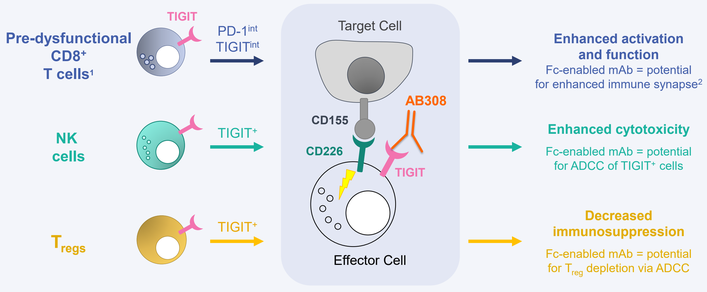
OVERVIEW

- 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}).
- TIGIT competes with an activating receptor, DNAM-1/CD226, for shared receptor ligands (mainly CD155) that are expressed by cancer and antigen-presenting cells (Chauvin & Zarour, 2020 JITC).
- When TIGIT is blocked, binding of CD155 to CD226 promotes immune activation and anti-tumor immunity through multiple mechanisms and shows promising preclinical and clinical activity (Cityscape: Rodriguez-Abreu *et al*, 2020 ASCO Abs 9503; Johnston *et al*., 2014 Cancer Cell; Jin *et al*., 2020 Cancer Immunol Res).
- We describe the preclinical characterization of AB308, a humanized wild-type (Fc-enabled) IgG1 anti-TIGIT antibody that is currently undergoing clinical evaluation (NCT04772989).

TIGIT Blockade Promotes Anti-tumor Immunity Through Several Cellular Contexts



[1]Van der Leun *et al*., 2020 Nat Rev Cancer; [2]Waight *et al*., 2018 Cancer Cell; int = intermediate

RESULTS

Combination of Either Fc-Silent or Fc-Enabled Anti-TIGIT with Anti-PD-1 Results in Enhanced Tumor Control in Mice, and Tumor Infiltrating Lymphocyte (TIL) Analysis Indicates Differential Reshaping of the Tumor Microenvironment

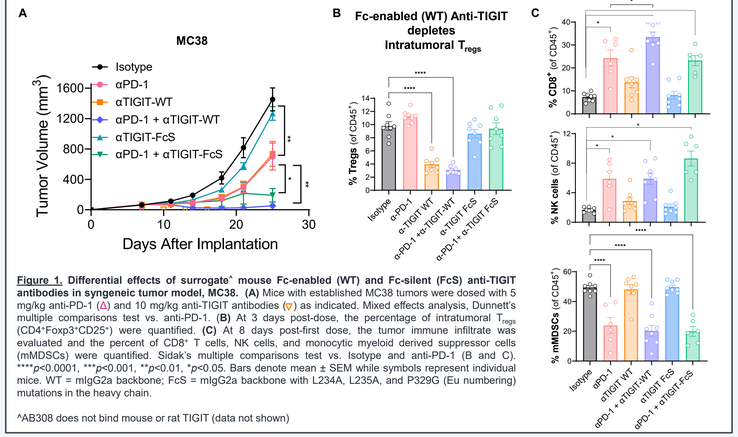


Figure 1. Differential effects of surrogate^ mouse Fc-enabled (WT) and Fc-silent (FcS) anti-TIGIT antibodies in syngeneic tumor model, MC38. **(A)** Mice with established MC38 tumors were dosed with 5 mg/kg anti-PD-1 (Δ) and 10 mg/kg anti-TIGIT antibodies (▽) as indicated. Mixed effects analysis, Dunnett's multiple comparisons test vs. anti-PD-1. **(B)** At 3 days post-dose, the percentage of intratumoral T_{regs} (CD4⁺Foxp3⁺CD25⁺) were quantified. **(C)** At 8 days post-first dose, the tumor immune infiltrate was evaluated and the percent of CD8⁺ T cells, NK cells, and monocytic myeloid derived suppressor cells (mMDSCs) were quantified. Sidak's multiple comparisons test vs. Isotype and anti-PD-1 (B and C). ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05. Bars denote mean ± SEM while symbols represent individual mice. WT = mIgG2a backbone; FcS = mIgG2a backbone with L234A, L235A, and P329G (Eu numbering) mutations in the heavy chain.

^AB308 does not bind mouse or rat TIGIT (data not shown)

RESULTS

AB308 Binds to Human TIGIT With High Affinity and Effectively Blocks the TIGIT-CD155 Interaction

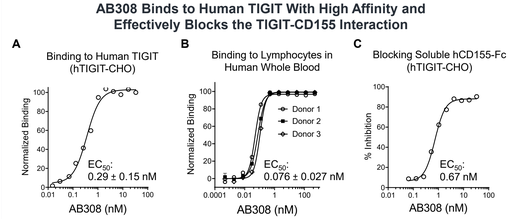


Figure 2. Representative examples of AB308 characterization. **(A)** Binding affinity of AB308 to human TIGIT expressed on CHO cells (hTIGIT-CHO) was determined via flow cytometry by measuring the MFI of a secondary Alexa488 conjugated anti-human IgG antibody. MFI values were normalized to the highest concentration of AB308. **(B)** Binding of AB308 to TIGIT⁺ lymphocytes in human whole blood was measured via flow cytometry using a fluorescently labeled competitive anti-TIGIT antibody clone MBSA43. Values were normalized to % TIGIT on surface detected by MBSA43 in AB308-free (0 nM) sample. **(C)** The ability of AB308 to block the interaction between hTIGIT-CHO and a human soluble CD155-Fc fusion protein (hCD155-Fc) was determined via flow cytometry by measuring the MFI of a fluorescently labeled anti-CD155 antibody. Inhibition normalized to the AB308-free (0 nM) sample.

TIGIT and CD226 are Co-Expressed on Tumor Infiltrating CD8⁺ T cells at Various Stages of Dysfunction

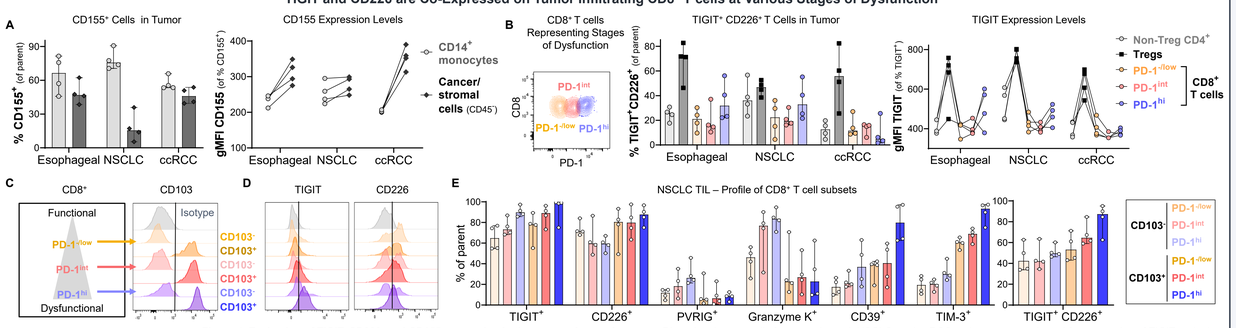


Figure 4. Evaluation of TIGIT, CD226, and CD155 expression in the tumor microenvironment. Dissociated tumor cells from esophageal, NSCLC, and ccRCC patients were evaluated for expression of TIGIT and other relevant markers by flow cytometry. **(A)** CD155 expression on CD14⁺ monocytes (CD45⁺CD3⁻CD16⁻CD56⁻CD14⁺) and cancer/stromal cells (CD45⁻); percent positive and geometric MFI (gMFI) of percent positive cells. **(B)** Example flow plot (NSCLC subject) of CD8⁺ T cells representing various stages of dysfunction[3] (PD-1^{-low}, PD-1^{int}, PD-1^{hi}), co-expression of TIGIT and CD226 and gMFI of TIGIT⁺ cells on CD4⁺ T cells (CD45⁺CD3⁺CD56⁻CD8⁻), including T_{reg} (FoxP3⁺), Non-T_{reg} CD4⁺ (FoxP3⁻), and CD8⁺ T cells (PD-1^{-low}, PD-1^{int}, PD-1^{hi}). **(C)** Schematic depicting identification of tissue resident (CD103⁺) and circulating (CD103⁻) CD8⁺ T cell subsets and **(D)** example histograms of TIGIT and CD226 expression on these subsets from a NSCLC subject. **(E)** Expression of TIGIT family members (TIGIT, CD226, PVRIG), terminal exhaustion markers (CD39, TIM-3), circulating pre-dysfunctional T cell markers (Granzyme K), and co-expression of TIGIT and CD226. Each symbol represents a unique subject while bars denote median ± range. Lines connect populations from the same subject. [3]Thommen *et al*., 2018 Nat Med.

AB308 Has the Capacity to Induce FcγR-mediated Signaling and Enables NK Cell-Mediated Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Against a Subset of TIGIT-Expressing Target Cells

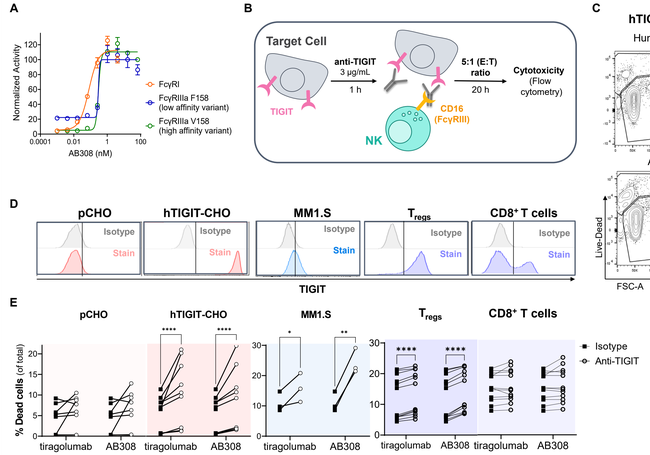


Figure 3. Characterization of ADCC capability by AB308. **(A)** Using an ADCC Luciferase Reporter Assay Kit (Promega) and hTIGIT-CHO cells, AB308 induces signaling via FcγRI, FcγRIIIa variant F158 and FcγRIIIa variant V158. Activity normalized to highest concentration of AB308 (100 nM). **(B)** Schematic of assay used to assess NK cell-mediated ADCC of TIGIT⁺ target cells. Target cells include parental CHO (pCHO), hTIGIT-CHO, MM1.S (multiple myeloma) cell line, healthy donor T_{regs} (CD4⁺CD25⁺CD127^{lo}) and healthy donor CD8⁺ T cells. **(C)** Representative flow plots quantifying cell death (cytotoxicity) of hTIGIT-CHO cells ± AB308 treatment. **(D)** Representative histograms showing TIGIT expression on target cells. **(E)** Cell death (cytotoxicity) quantified for each target cell as indicated. Paired symbols indicate treatment with human IgG1 isotype (■) or anti-TIGIT antibodies AB308 or tiragolumab (○) for each NK cell donor (pCHO, hTIGIT-CHO, MM1.S) or NK/T cell donor pairs (T_{regs}, CD8⁺ T Cells). ****p<0.0001, ***p<0.001, **p<0.01, 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).

AB308 Enhances T Cell Signaling and Primary T Cell Functionality Alone or in Combination with Zimberelimab (Zim, anti-PD-1)

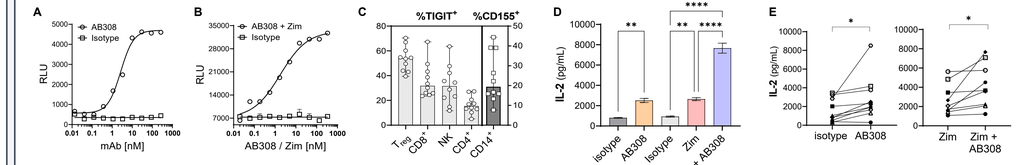


Figure 5. Assessment of AB308 in combination with anti-PD-1 antibody Zimberelimab (Zim). The functional consequences of blocking TIGIT **(A)** and TIGIT/PD-1 **(B)** with AB308 and Zim were assessed using Jurkat-TIGIT or Jurkat-TIGIT/PD-1 luciferase reporter kits (Promega), respectively. **(C)** Percentage of TIGIT⁺ and CD155⁺ cells in healthy donor peripheral blood mononuclear cell (PBMC) populations. **(D, E)** IL-2 secretion by PBMCs stimulated with staphylococcal enterotoxin A (SEA) superantigen (1 ng/mL) ± AB308 (10 μg/mL) ± Zim (1 μg/mL) for 4 days. (D) A representative donor. Sidak's multiple comparisons test vs. Isotype controls and Zim treatment. (E) Paired conditions from 10 donors. Paired *t*-test. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05. Bars denote median ± range (C) or mean ± SEM (D).

Combination of AB308 and Dual Adenosine $A_{2a/2b}$R Antagonist Etrumadenant (Etruma) is Required for Maximal Signaling Through the CD155-CD226 Axis in the Context of Adenosine-Mediated Suppression

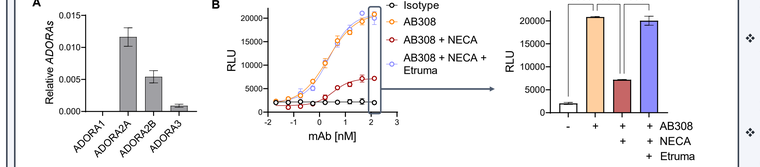


Figure 6. Assessment of AB308 in combination with Etrumadenant (Etruma). **(A)** Relative expression ($2^{-\Delta Ct}$) of adenosine receptors *ADORA1*, *ADORA2A*, *ADORA2B*, *ADORA3* on Jurkat-TIGIT cells from Jurkat-TIGIT luciferase reporter kit (Promega). **(B)** Jurkat-TIGIT cells were co-cultured with CD155-expressing CHO cells in the presence of AB308 ± adenosine agonist NECA (5 μM) ± Etruma (1 μM). Luciferase reporter activity was measured after 6 hours incubation at 37°C. Sidak's multiple comparisons test vs. isotype control and AB308+NECA treatment.****p<0.0001, ***p<0.001. Bars denote mean ± SEM.

SUMMARY

- AB308 is a high affinity anti-TIGIT blocking antibody that has the capacity to induce ADCC (Fig. 2 and 3).
- PD-1, TIGIT and CD226 are co-expressed on tumor-resident and circulating CD8⁺ T cells that may represent pre-dysfunctional/progenitor populations akin to reported cellular targets of anti-PD-(L)1[4,5] and thus probable targets for anti-TIGIT (Fig. 4).
- AB308 can enhance T cell functionality alone and in combination with Zim (Fig. 5) and Etruma (Fig. 6), providing rationale for combinations with immune checkpoint and adenosine pathway blocking agents in the clinic.

[4]Blackburn *et al*., 2008 PNAS; [5]Miller *et al*. 2019 Nat Immunol