Society for Immunotherapy of Cancer
Annual Meeting
November 6-10th, 2024
Abstract 507

TIGIT Blockade By Monoclonal Antibodies Promotes T Cell Activation and Anti-tumor Activity That is Not Dependent on a Functionalized Fc Domain

Didi S. Zhu[1], Casey G. Mitchell [1], Gabrielle L. Reiner [1], Dana Piovesan [1], Angelo Kaplan[1], Yihong Guan[1], Patrick G. Schweickert[1], Ritu Kushwaha[1], Nigel P. Walker [1], Matthew J. Walters [1], Kelsey E. Sivick[1]

Arcus Biosciences, Hayward, CA USA

Background

- TIGIT (T-cell immunoreceptor with Ig and ITIM domains) is an inhibitory receptor expressed on T cells and natural killer (NK) cells
  - TIGIT competes with activating receptor CD226 for binding to shared ligand CD155/PVR, which is expressed by cancer and antigen-presenting cells[1-4]
  - The CD155-CD226 interaction leads to signaling and CD226 downregulation[5]
- LFA-1, expressed on T cells, drives early activation events, prolongs contact with antigen presenting cells (APCs), and facilitates target cell killing[6]
  - CD226 can activate LFA-1 and induce changes in LFA-1 conformation to enhance cell-cell contact and T cell activation[5,7]
- PD-1 (Programmed cell death 1) is an inhibitory receptor that can suppress cell activation through de-phosphorylation of CD226[8,9]
  - High levels of CD155 on cancer cells have been associated with poor performance of anti-PD-1 antibodies and overall poor prognosis in several cancer settings[10,11]
- Blockade of PD-1 and TIGIT may induce maximal effector cell functionality through CD226 activation on target cells that co-express all three receptors, particularly pre-exhausted or pre-dysfunctional T cells (T_{PEX})
- **Here, we elucidate the anti-tumor activity of an Fc-silent anti-TIGIT antibody, identify target cell populations across cancer indications for PD-1 and TIGIT dual blockade, and characterize the regulation of CD226 and LFA-1 on T cells**

Results

Fc-silent anti-TIGIT in combination with anti-PD-1 induces CD8⁺ T cell-mediated cancer cell killing *in vitro*

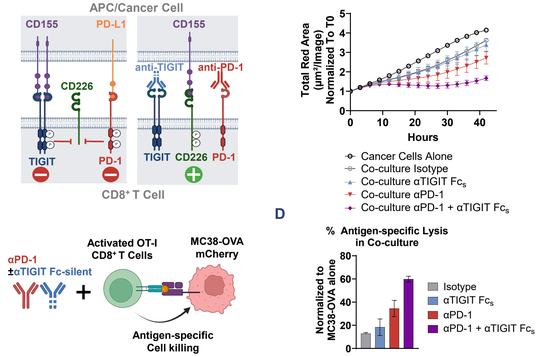

Figure 1. (A) Schematic illustrating mechanism of action of TIGIT and PD-1 dual blockade. **(B)** OT-I CD8⁺ T cells were activated with SIINFEKL for 2 days and treated with isotype control or αPD-1 ± αTIGIT Fc-silent (Fc_s) for 1 hour before co-culturing with MC38 tumors cells that express OVA and mCherry. Tumor cell killing was monitored using an Incucyte. **(C)** Number of MC38-OVA cells during co-culture, as measured through mCherry (red fluorescent) signal over time. **(D)** Percentage of antigen-specific killing after 42 hours of co-culture normalized to MC38-OVA alone.

Results

Human HNSCC tumor infiltrating lymphocytes with exhausted T cell phenotypes co-express CD226, TIGIT and PD-1, while stromal and myeloid cells express CD155 ligand

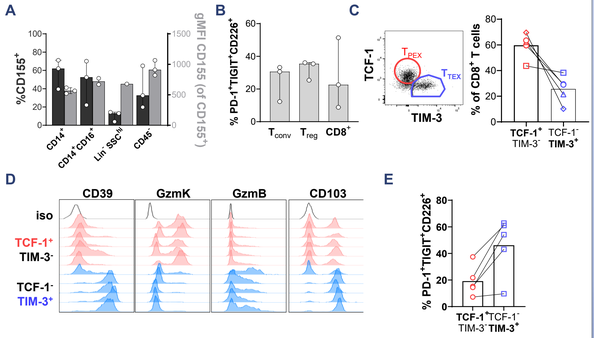

Figure 2. Commercially-sourced dissociated cells from Head and Neck Squamous Cell Carcinoma (HNSCC) patient tumors were profiled using flow cytometry. **(A)** Expression of CD155 on $CD14^+$, $CD14^+CD16^+$, $Lineage^-$ (Lin^-) SSC^{hi} cells, and stromal/cancer cells ($CD45^-$). **(B)** Percentage of $CD4^+$ conventional T cells (T_{conv}), regulatory T cells (T_{reg}) and $CD8^+$ T cells co-expressing PD-1, TIGIT and CD226. **(C)** Dot plot (left) and quantification (right) of pre-exhausted (T_{PEX}:$TCF\text{-}1^+TIM\text{-}3^-$) and terminally exhausted (T_{TEX}:$TCF\text{-}1^-TIM\text{-}3^+$) $CD8^+$ T cells. **(D)** Expression of CD39, granzyme K (GzmK), granzyme B (GzmB) and CD103 on T_{PEX} and T_{TEX} $CD8^+$ T cells. **(E)** Frequency of $PD\text{-}1^+TIGIT^+CD226^+$ cells within T_{PEX} or T_{TEX}.

Similar patterns of PD-1, TIGIT and CD226 co-expression on exhausted CD8⁺ subsets across cancer indications

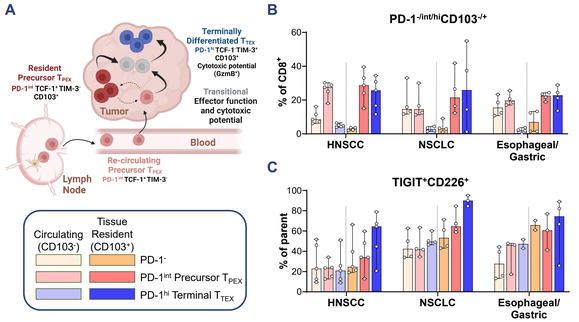

Figure 3. (A) (Top) Illustration of circulating and intratumoral $CD8^+$ T cell exhaustion states. (Bottom) Legend showing six subsets. "Transitional" cells are likely present in both $PD\text{-}1^{int}$ and $PD\text{-}1^{hi}$ subsets. **(B)** Frequency of circulating and tissue-resident T_{PEX} and T_{TEX} in HNSCC, Non-small cell lung cancer (NSCLC) and Esophageal/Gastric tumor samples. **(C)** Percentage of $TIGIT^+CD226^+$ cells within T_{PEX} and T_{TEX}.

Results

Anecdotal inverse association between CD155 and CD226 observed in serial IHC sections

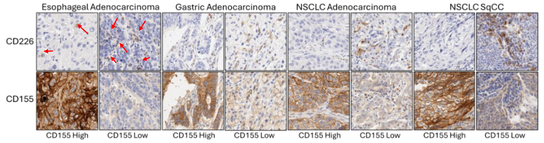

Figure 4. Representative immunohistochemistry (IHC) images of CD226 and CD155 expression from Esophageal Adenocarcinoma, Gastric Adenocarcinoma, NSCLC Adenocarcinoma and NSCLC Squamous Cell Carcinoma (SqCC) tumor samples at 63x magnification. Red arrows indicate examples of CD226 expression on images for Esophageal Adenocarcinoma.

CD155-CD226 engagement leads to downregulation of CD226 on the surface of CD8⁺ T cells

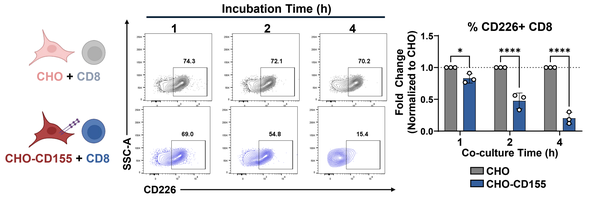

Figure 5. $CD8^+$ T cells isolated from peripheral blood (PB) of healthy donors were co-cultured with control CHO or CD155-expressing CHO (CHO-CD155) cells. Representative dot plot showing CD226 expression on $CD8^+$ T cells after 1, 2 or 4 hour co-culture with CHO or CHO-CD155 with (right) quantification. 2-way ANOVA, Šidák's multiple comparisons test, * $p<0.05$, **** $p<0.0001$.

CD226 partially rebounds on surface of non-proliferating CD8⁺ T cells after removal of CD155 ligand

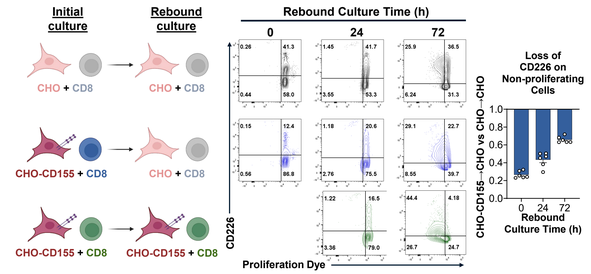

Figure 6. $CD8^+$ T cells from PB of healthy donors were co-cultured with control CHO or CD155-expressing CHO cells for 4 hours (initial culture). $CD8^+$ T cells were then separated from CHO cells and re-cultured with either fresh CHO or CD155-CHO for another 0-72 hours (rebound culture). Representative dot plot (middle) showing CD226 expression on $CD8^+$ T cells after 0, 24 or 72 hours cultured with CHO, CHO-CD155→CHO or CHO-CD155. Frequency of $CD226^+$ non-proliferating (proliferation dye^-) $CD8^+$ T cells from CHO-CD155→CHO co-cultures normalized to CHO→CHO co-cultures shown on right.

Results

CD155-CD226 engagement leads to open LFA-1 conformation on CD8⁺ T Cells

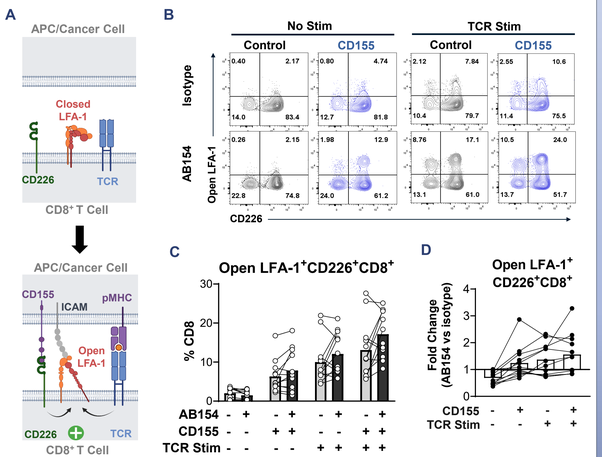

Figure 7. (A) Schematic illustrating LFA-1 conformation change before (top) and after (bottom) CD155-CD226 engagement and T cell receptor (TCR) activation. **(B)** $CD8^+$ T cells, from healthy donor PB, were treated with isotype control or Fc-silent anti-TIGIT (AB154) before incubation with ± plate-bound CD155 ± TCR stimulation (αCD3/αCD28) for 2 hours. Open LFA-1⁺$CD226^+$ $CD8^+$ T cells represented by a dot plot. **(C)** Frequency of open LFA-1⁺$CD226^+$ $CD8^+$ T cells treated as described in (B). **(D)** Changes in frequency of open LFA-1⁺$CD226^+$ $CD8^+$ T cells after treatment with AB154 normalized to isotype treatment.

Summary

- Fc-silent anti-TIGIT can enhance antigen-specific killing of tumor cells by PD-1 blockade *in vitro* (Figure 1)
- HNSCC and other cancer indications contain TIGIT, CD226 and PD-1 co-expressing precursor exhausted $CD8^+$ T cells that can be targeted with PD-1 and TIGIT dual blockade (Figures 2 & 3)
- CD226 is down-regulated on the cell surface in the presence of CD155 ligand (Figure 5)
- Re-expression of CD226 following CD155 interaction suggests opportunity for continued activation through this axis, possibly enhanced through TIGIT blockade (Figure 6)
- CD155-CD226 engagement can induce open LFA-1 conformation on $CD8^+$ T cells, enabling enhanced immune synapse formation (Figure 7)

Some images made with BioRender. References: 1. Jin HS et al. (2020) *Cancer Immuno Res.* 2. Worbys JD et al. (2023) *Nat Comm* 3. Joller N et al. (2011) *J. Immunol*. 4. Yu X et al. (2008) *Nat Immunol*. 5. Weulersse M et al. (2020) *Immunity* 6. Walling BL and Kim M. (2018) *Front Immunol*. 7. Shibuya K et al. (2003) *JEM* 8.Banta KL et al. (2002) *Immunity* 9. Wang B et al. (2018) *Science Immunol*. 10. Jiang C et al. (2022) *Clin Exp Immunol*. 11. Oyama R et al. (2022) *Oncol Lett*.