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Presented at the
American Association for Cancer Research Annual Meeting
Atlanta, GA, USA • March 29– April 3, 2019

FcγR Co-Engagement by Anti-TIGIT Monoclonal Antibodies Enhances T cell Functionality and Antitumor Immune Responses

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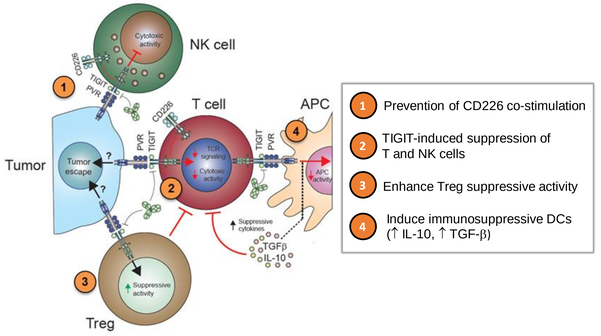
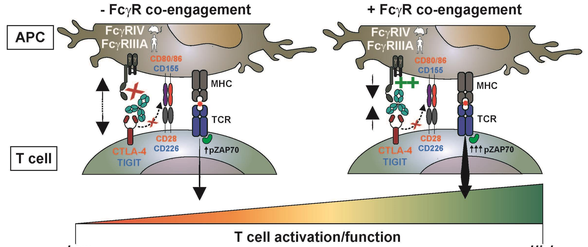
TIGIT: A Key Regulator of the Immune Synapse



1. Prevention of CD226 co-stimulation
2. TIGIT-induced suppression of T and NK cells
3. Enhance Treg suppressive activity
4. Induce immunosuppressive DCs (↑ IL-10, ↑ TGF-β)

Fc:FcγR Co-Engagement Enhances T cell Responsiveness

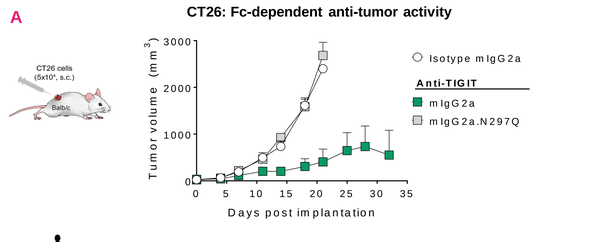


Hypothesis: Optimizing Fc-FcγR co-engagement enhances the activity of anti-TIGIT and anti-CTLA-4 antagonist antibodies[1]. TIGIT and CTLA-4 antibodies with increased binding affinities to activating Fcγ receptors FcγRIV (CD16-2, mouse) or FcγRIIIA (CD16a, human) augment T cell priming by improving the quality of the immune synapse between a T cell and an antigen presenting cell (APC).

FcγR Binding Characteristics of Antibody Fc Variants Used

Anti-TIGIT	Fc Isotype	Fc mutations	Characteristics
Mouse	mIgG2a	-	-
	mIgG2a.N297Q	N297Q	Reduced FcγR binding (“Fc silent”)
	mIgG2a.DLE	S241D.A332L.I334E	> FcγR binding (“Fc enhanced”)
Human	IgG1	-	-
	IgG1.N297A	N297A	Reduced FcγR binding (“Fc silent”)
	IgG1.DLE	S239D.A330L.I332E	> FcγRIIIA binding (“Fc enhanced”)

Anti-TIGIT Requires Intact FcγR Interactions for Anti-Tumor Activity and Improved T cell Stimulation



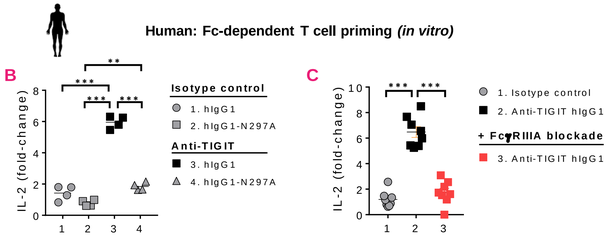


Figure 1. **A.** BALB/c mice with established CT26 tumors (~50mm³) were administered twice weekly intraperitoneally (i.p.) for two weeks with 200 µg of anti-TIGIT clone 10A7 (mIgG2a ■ or mIgG2a-N297Q ■) or mIgG2a isotype control (●). **B.** IL-2 production (day 4) by human PBMCs stimulated with 100 ng/ml of SEA peptide and 10 µg/ml of anti-TIGIT clone 10A7 (hIgG1 ■ or hIgG1-N297A ■) or corresponding isotype controls (● and ■, respectively). **C.** IL-2 production (day 4) by PBMCs stimulated with SEA peptide (100 ng/ml) and anti-TIGIT hIgG1 (10 µg/ml) with (■) or without (■) pre-blockade of FcγRIIIA.

Anti-TIGIT Antagonist Activity Depends on FcγRIV Engagement to Mediate Optimal Immune Modulation *In Vivo*

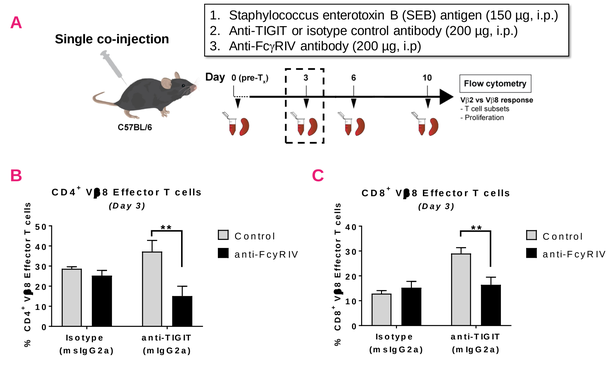


Figure 2. **A.** Experimental model (tumor-free system). C57BL/6 mice were administered i.p. with 150 µg of SEB together with a 200 µg dose of anti-TIGIT clone 10A7 (mIgG2a), or isotype control (mIgG2a) in combination with 200 µg FcγRIV blocking antibody (clone 9E9). T cells in the peripheral blood or spleen (not shown) were evaluated by flow cytometry on days 3, 6 and 10. **B.** CD4⁺ Vβ8⁺ T effector cells and **C.** CD8⁺ Vβ8⁺ T effector cells were evaluated on day 3 post-stimulation by flow cytometry. N=4 mice/group, and data are representative of two independent experiments.

Enhanced FcγR Co-Engagement by Anti-TIGIT Antibodies Promotes Better Tumor Control

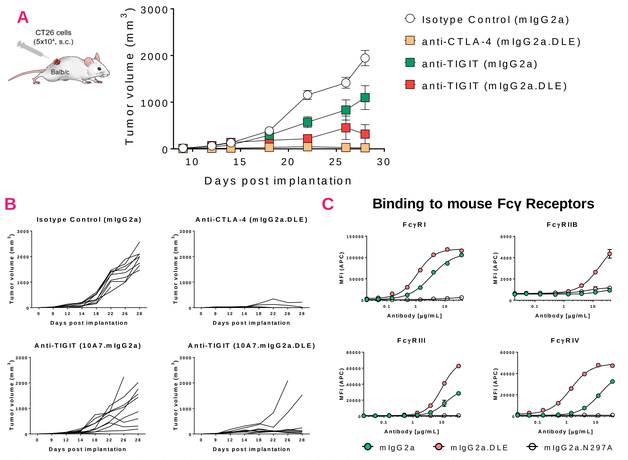


Figure 3. **A.** BALB/c mice (n=10-12/group) with established CT26 tumors (~50mm³) were administered i.p. with 200 µg of anti-TIGIT clone 10A7 (mIgG2a ■), Fc-enhanced anti-TIGIT clone 10A7 (mIgG2a-DLE ■), Fc-enhanced anti-CTLA-4 clone 9D9 (mIgG2a.DLE ■) or isotype control (○) on days 9, 13 and 16. Data representative of 3 independent experiments. **B.** Growth curves showing individual tumor volumes over time of treated CT26-bearing BALB/c mice. **C.** Binding profiles of IgG Fc variants of a non-TIGIT control antibody to CHO-cells stably expressing FcγRI, FcγRIIB, FcγRIII, or FcγRIV. Binding was assessed by flow cytometry and reported as mean fluorescence intensity (MFI).

Anti-TIGIT Has a Novel Mechanism of Immune Modulation that Does Not Include Treg Depletion

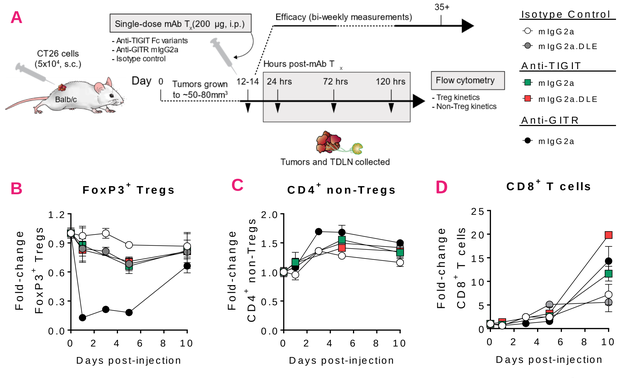


Figure 4. **A.** Experimental model. Balb/c mice with established CT26 tumors (50-80mm³) were administered i.p with a single dose of 200 µg of anti-TIGIT clone 10A7 (mIgG2a ■), Fc-enhanced anti-TIGIT clone 10A7 (mIgG2a-DLE ■), isotype control (mIgG2a ●), Fc-enhanced isotype control (mIgG2a.DLE), or anti-GITR clone DTA-1 (100 µg, mIgG2a ●). Tumors were analyzed on days 1, 3, 5 and 10 post treatment by flow cytometry for changes in T cell frequency. **B.** Frequency of intratumoral FoxP3⁺ Tregs. **C.** CD4⁺ non-Tregs and **D.** CD8⁺ T cells by flow cytometry pre- (t=0 hr), and post-antibody injection. N=3 mice per group per timepoint. Data representative of 3 independent experiments.

Enhanced FcγRIIIA Engagement by Anti-TIGIT Further Improves T Cell Responsiveness *In Vitro* (Human)

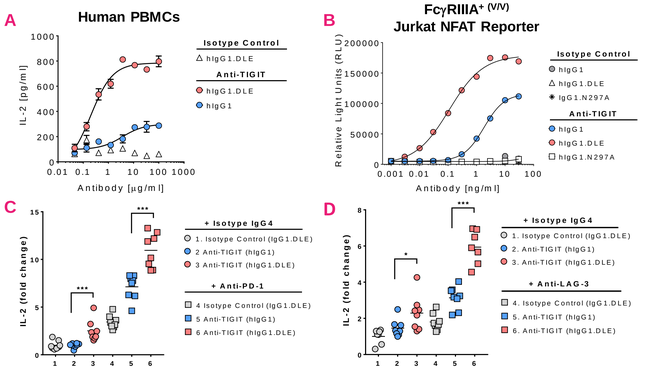


Figure 5. **A.** IL-2 production (day 4) by human PBMCs stimulated with a suboptimal concentration of SEA peptide together with increasing concentrations of anti-TIGIT clone 347_02_A10 (hIgG1 ●), Fc-enhanced anti-TIGIT (hIgG1.DLE ●) or isotype control (hIgG1.DLE △). **B.** Jurkat cells genetically engineered to express FcγRIIIA (V/V) upstream of a NFAT-dependent luciferase reporter were co-cultured with TIGIT-expressing CHO cells and increasing doses of anti-TIGIT or isotype control antibodies. Signaling was assessed based upon luciferase expression shown as relative light units (RLU). **C.** IL-2 production (day 4) by human PBMCs stimulated with a suboptimal concentration of SEA peptide together with 10 µg/ml of anti-TIGIT (hIgG1), Fc-enhanced anti-TIGIT (hIgG1.DLE) or isotype control (hIgG1.DLE) alone or in combination with 10 µg/ml anti-PD-1 (Nivolumab) or **D.** anti-LAG-3 (Clone 25F7)

The Importance of Fc-FcγR Co-Engagement for Improved T Cell Responses Extends to Antibodies Targeting CTLA-4

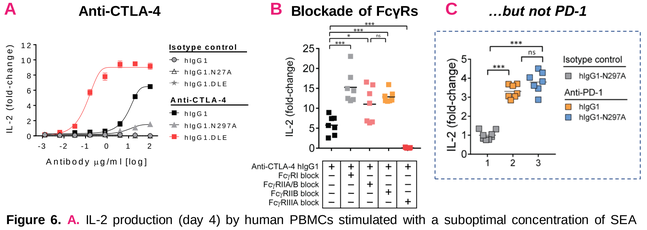


Figure 6. **A.** IL-2 production (day 4) by human PBMCs stimulated with a suboptimal concentration of SEA peptide together with anti-CTLA-4 variants or corresponding hIgG1 isotype controls. **B.** IL-2 production (day 4) by PBMCs following blockade of the indicated FcγRs with FcγR-specific antibodies (10 µg/ml) prior to co-incubation with SEA peptide and anti-CTLA-4 hIgG1 antibody (10 µg/ml). **C.** IL-2 production (day 4) by human PBMCs stimulated with SEA peptide and 10 µg/ml of anti-PD-1 antibodies or isotype control.

Conclusions

- Our data describe a novel FcγR-dependent mechanism of action that enhances the therapeutic activity of anti-TIGIT antibodies in preclinical studies
- Murine FcγRIV and human FcγRIIIA are critical mediators of anti-TIGIT function
- Tumor control is dependent on enhancing CD8 T cell and NK cell function[2] but not Treg depletion
- Enhanced FcγRIIIa co-engagement via Fc engineering further enhances T cell responsiveness
- This novel mechanism extends to antibodies targeting CTLA-4 and could inform the optimal design for a new class of Fc-engineered antibodies for cancer immunotherapy[1].

References
[1]Waight et al., Cancer Cell. 2018; 33(6):1033-1047; [2] Zhang et al., Nat Immunol. 2018; 19(7):723-732

Author Disclosures
Dhan Chand, Jeremy D. Waight, Elena Paltrinieri, Sylvia Dietrich, Mark Bushell, Mathew Costa, Randi Gombos, Jennifer S. Buell, Robert B. Stein, Alexander Duncan, David A. Savitsky: Agenus Inc.: Employment and Stock Ownership. Nicholas S. Wilson: Agenus Inc.: Former Employment and Stock Ownership.