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Anti-TIGIT antibodies require enhanced FcγR co-engagement for optimal T and NK cell-dependent anti-tumor immunity

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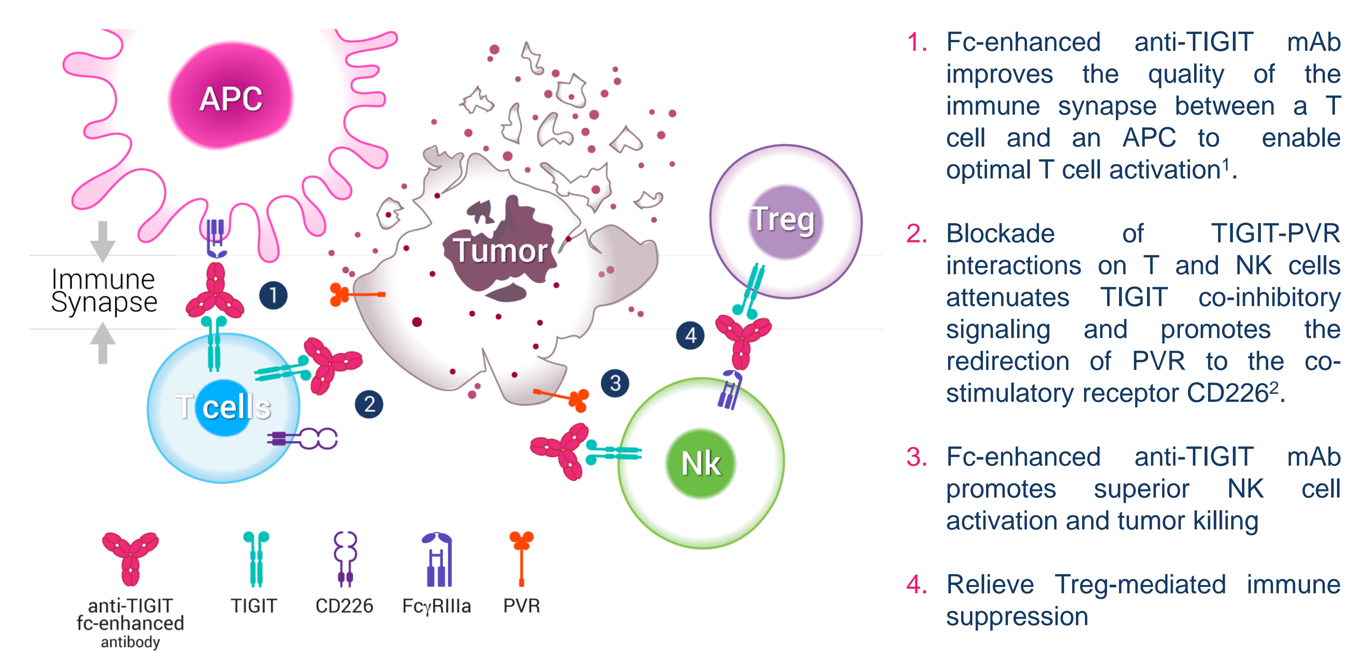
T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is a critical inhibitor of the immune response to cancer

An anti-TIGIT Fc enhanced antibody has multiple MOAs in cancer therapy



1. Fc-enhanced anti-TIGIT mAb improves the quality of the immune synapse between a T cell and an APC to enable optimal T cell activation[1].
2. Blockade of TIGIT-PVR interactions on T and NK cells attenuates TIGIT co-inhibitory signaling and promotes the redirection of PVR to the co-stimulatory receptor CD226[2].
3. Fc-enhanced anti-TIGIT mAb promotes superior NK cell activation and tumor killing
4. Relieve Treg-mediated immune suppression

Fc engineering enhances binding to activating FcγRs

Fc enhanced TIGIT mAb demonstrates improved binding to activating FcγRIIIA (human) or FcγRIV (mouse)

Table 1: FcyR binding characteristics as determined by surface plasmon resonance or cell binding to FcyR-expressing recombinant CHO cells.

Anti-TIGIT	Description	Fc Isotype	Fc mutations	Blocking Properties	FcγR Binding Characteristics
Human (AGEN)	Conventional	IgG1	-	+	+
	Fc-Enhanced Anti-TIGIT	IgG1.DLE	S239D.A330L.I332E	+	> FcγRIIIA binding ("Fc enhanced")
	Fc-silent	IgG1.N297A	N297A	+	Reduced FcyRIIIA binding ("Fc-silent")
Murine Surrogate (10A7)	Conventional	mIgG2b	-	+	+
	Fc-silent	mIgG2a.N297Q	N297Q	+	Reduced FcyRIV binding ("Fc-silent")
	Fc-enhanced Anti-TIGIT	mIgG2b.DLE mIgG2a	S241D.A332L.I334E	+	> FcγRIV binding ("Fc enhanced")

Enhanced FcγR co-engagement is required for monotherapy tumor control

Conventional anti-TIGIT mAbs lack single agent activity

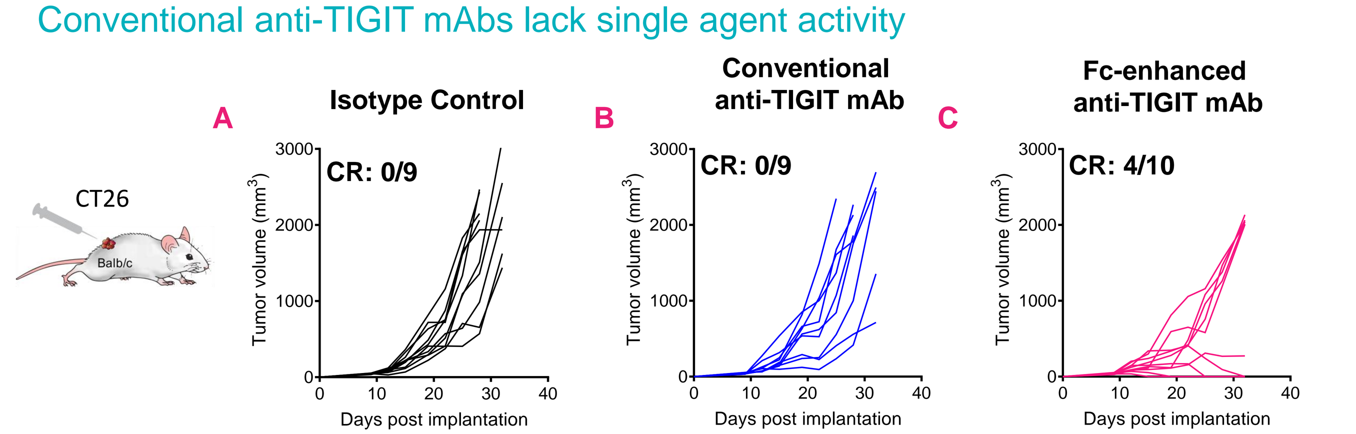


Figure1. Balb/c mice were injected with 10^5 CT26 tumor cells (subcutaneous, n=9-10 mice/group) and treated intraperitoneally with 200 µg of **A.** Isotype control, **B.** conventional anti-TIGIT (mIgG2b) or **C.** Fc-enhanced anti-TIGIT (IgG2b.DLE) antibodies twice a week for 2 weeks post-tumor implantation. Tumor growth was monitored bi-weekly using a digital caliper. Mean tumor size at the start of treatment was approximately 45 mm^3.

Anti-TIGIT monotherapy requires intact Fcγ interactions

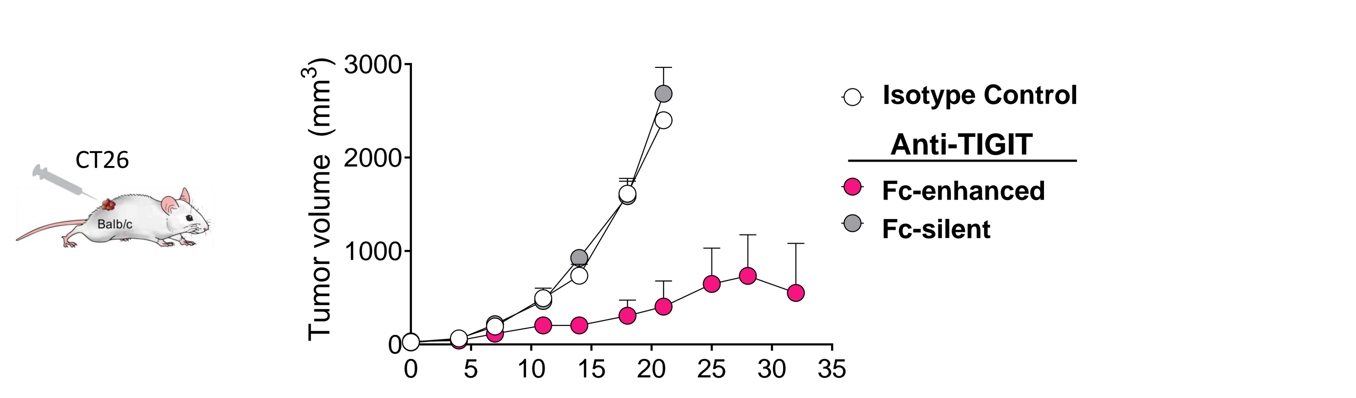


Figure 2. Balb/c mice were injected with 10^5 CT26 tumor cells (subcutaneous, n=5 mice/group) and treated intraperitoneally with 200 µg of Fc-enhanced anti-TIGIT (●), Fc-silent anti-TIGIT (●) or isotype control (O) antibodies twice a week for two weeks post-tumor implantation. Tumor growth was monitored bi-weekly using a digital caliper. Mean tumor size at the start of treatment was approximately 45 mm^3.

Tumor efficacy by anti-TIGIT antibody is dependent on T cells and NK cells

NK depletion reduces tumor control by anti-TIGIT therapy

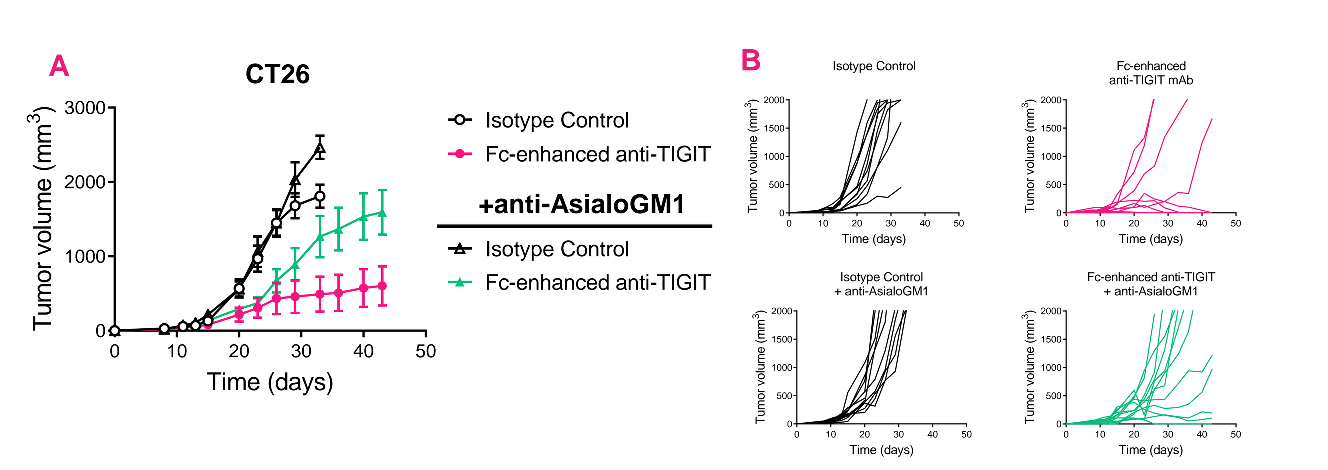


Figure 3. Balb/c mice were injected with 5X10⁴ CT26 tumor cells (subcutaneous, n=12-15 mice/group) and treated intraperitoneally with 200 µg of Fc-enhanced anti-TIGIT or isotype control antibodies on days 3, 6, 9 and 12 post-tumor implantation. To deplete NK cells, 200 µg of anti-asialoGM1 antibody was administered 1 day prior to tumor inoculation and every 3 days post tumor implantation. **A.** Tumor growth was monitored bi-weekly using a digital caliper. **B.** Graphs showing individual tumor volumes from treated mice.

Anti-TIGIT therapy enhances NK cell-dependent tumor control in a T cell deficient model

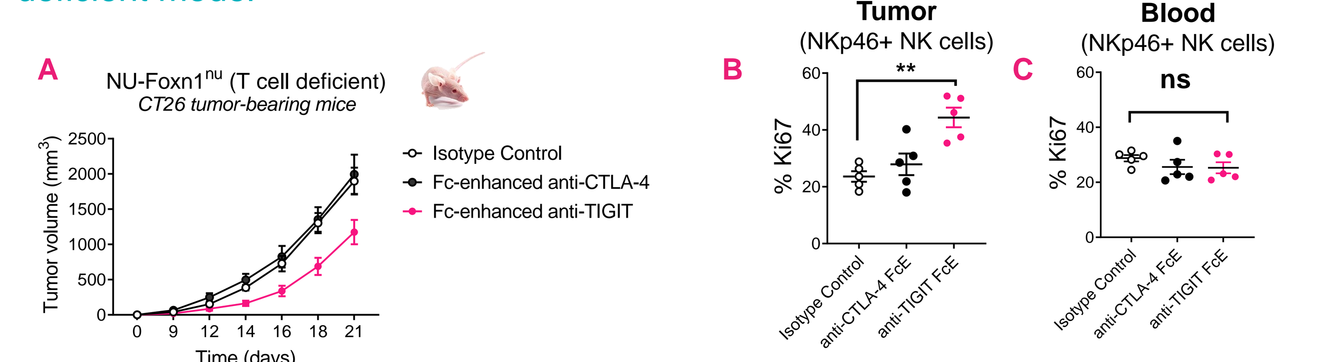


Figure 4. NU-Foxn1ⁿᵘ mice were injected with 5X10⁴ CT26 tumor cells (subcutaneous, n=10 mice/group) and treated intraperitoneally with 200 µg of Fc-enhanced (FcE) anti-TIGIT, FcE anti-CTLA-4 (9D9 msIgG2a.DLE) or isotype control antibodies on days 3, 6, 9 and 12 post-tumor implantation. Tumor growth was monitored bi-weekly using a digital caliper. **A.** Graph showing mean tumor volumes and standard errors. Frequency of NKp46+ Ki67+ NK cells was determined by flow cytometry from single cell suspensions prepared from the **B.** tumor and **C.** peripheral blood collected on day 21.

Tumor control is not dependent on depletion of intratumoral regulatory T cells (Tregs)

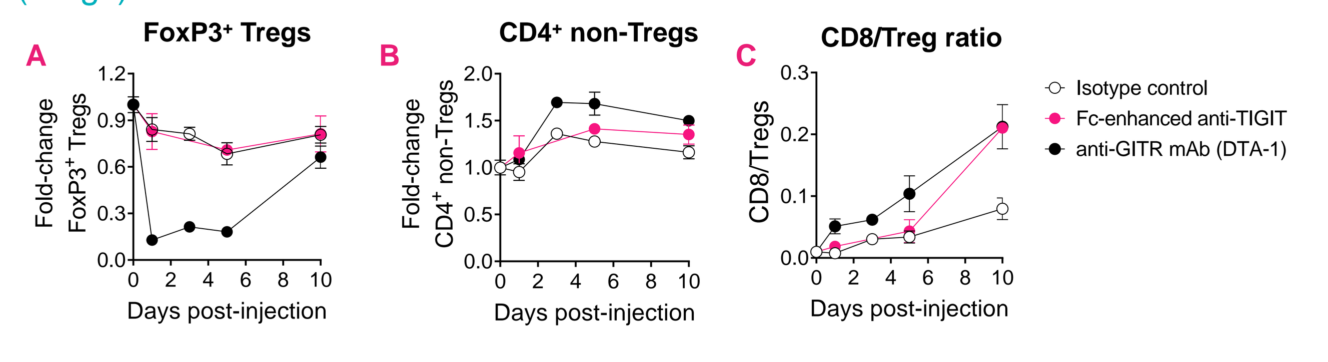


Figure 5. Balb/c mice (n=3 mice per group) with established CT26 tumors (50-80mm^3) were administered intraperitoneally with a single dose of 200 µg of Fc-enhanced anti-TIGIT (●), isotype control (O), or anti-GITR clone (DTA-1 mIgG2a (●)) antibodies. Tumors were analyzed on days 1, 3, 5 and 10 post treatment by flow cytometry for changes in T cell frequency. **A.** Frequency of intratumoral FoxP3⁺ Tregs, **B.** CD4⁺ non-Tregs and **C.** CD8/Treg ratio were assessed by flow cytometry.

FcγR co-engagement is critical for enhancing antigen-specific T cell responses

Blockade of FcγRIV decreases CD4 and CD8 T cell responses

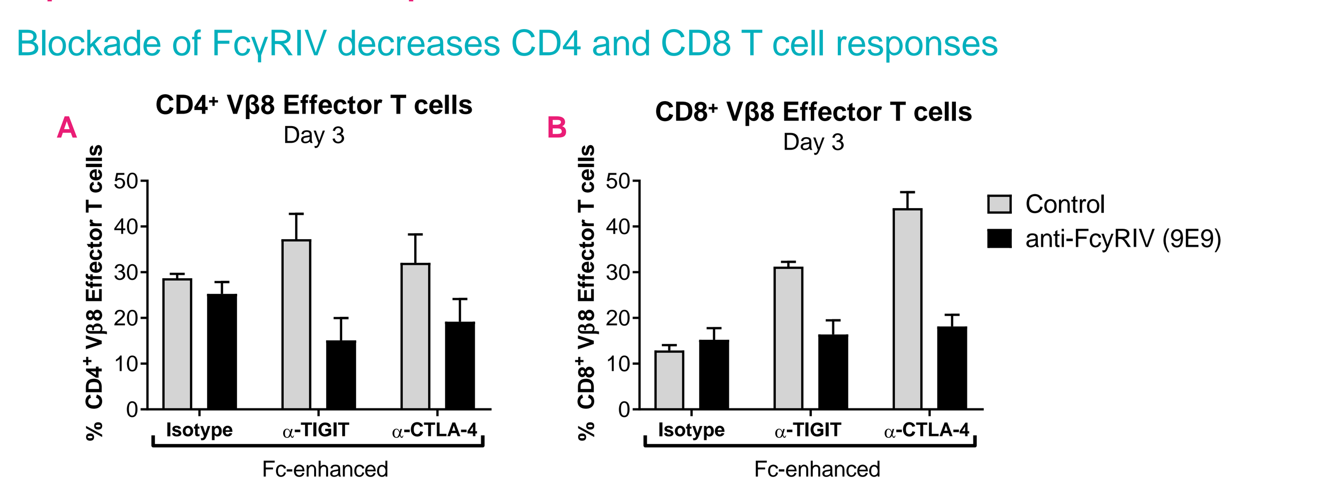


Figure 6. C57BL/6 mice (n=4 mice/group) were injected intraperitoneally with 150 µg of SEB superantigen together with 200 µg of an anti-TIGIT, anti-CTLA-4 (Clone 9D9) or isotype control antibodies alone or in combination with 200 µg of anti-FcγRIV antibody (Clone 9E9). Frequency of SEB-specific **A.** CD4+ (Vβ8) T cells and **B.** CD8+ (Vβ8) T cells were assessed from blood collected on day 3 by flow cytometry.

AGEN Fc-enhanced anti-TIGIT mAb enhances antigen-specific CD8 T cell memory recall

Superior to conventional IgG1 and Fc-silent anti-TIGIT mAbs

CMV memory recall assay

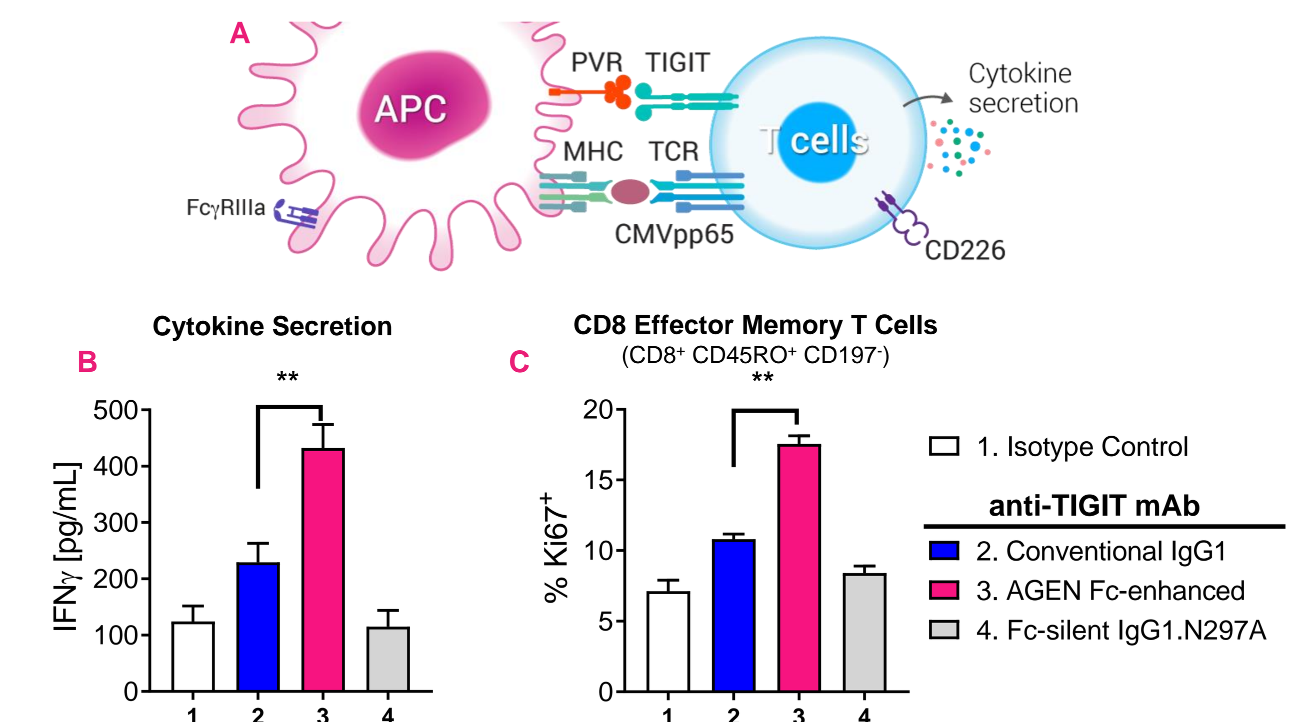


Figure 7. **A.** Primary cytomegalovirus (CMV) whole antigen memory recall assay. Primary, CMV-reactive human donor PBMC were stimulated with a sub-optimal concentration of CMV whole antigens and treated with 10 µg/mL of AGEN Fc-enhanced anti-TIGIT, conventional IgG1 anti-TIGIT, Fc-silent IgG1.N297A anti-TIGIT or isotype control antibodies. **B.** Cytokine secretion was analyzed by AlphaLISA from supernatants collected after 4 days. **C.** Ki67 staining on CD8+CD45RO+CD197- cells was analyzed via flow cytometry.

Enhanced T cell responsiveness by anti-TIGIT therapy is dependent on FcγRIIIA co-engagement

Blockade of FcγRIIIA attenuates T cell activation by anti-TIGIT

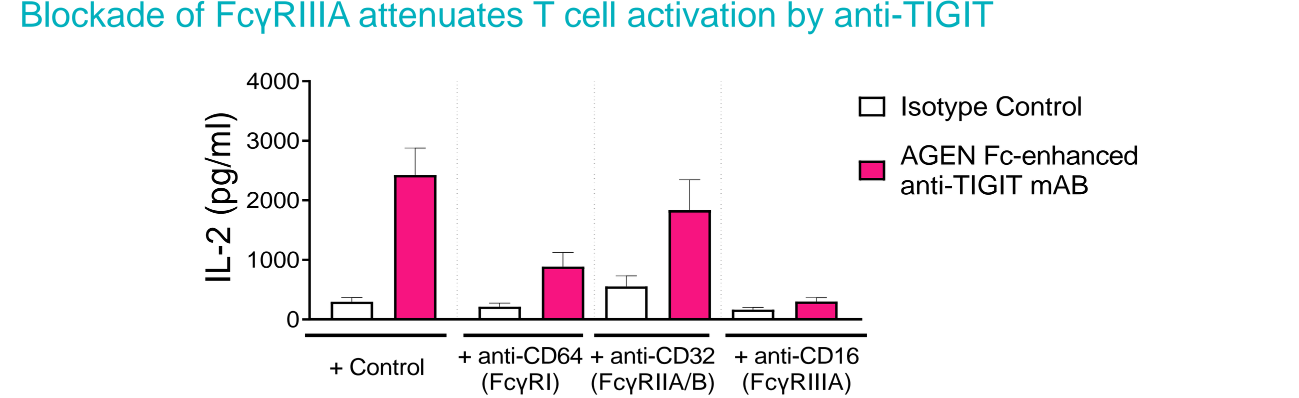


Figure 8. Healthy donor PBMCs were pre-incubated with 10 µg/mL of FcγRI (Clone 10.1), FcγRIIA/IIB (Clone 6C4), FcγRIIIA (Clone 3G8) or an isotype control blocking antibody for 15-30 min, stimulated with a sub-optimal concentration of SEA peptide and treated with 10 µg/mL of AGEN Fc-enhanced anti-TIGIT or isotype control antibodies. Cytokine secretion was analyzed by AlphaLISA from supernatants collected after 4 days.

AGEN Fc-enhanced anti-TIGIT mAb promotes superior NK cell activation and effector function

Superior NK cell activation compared to conventional IgG1 anti-TIGIT mAb

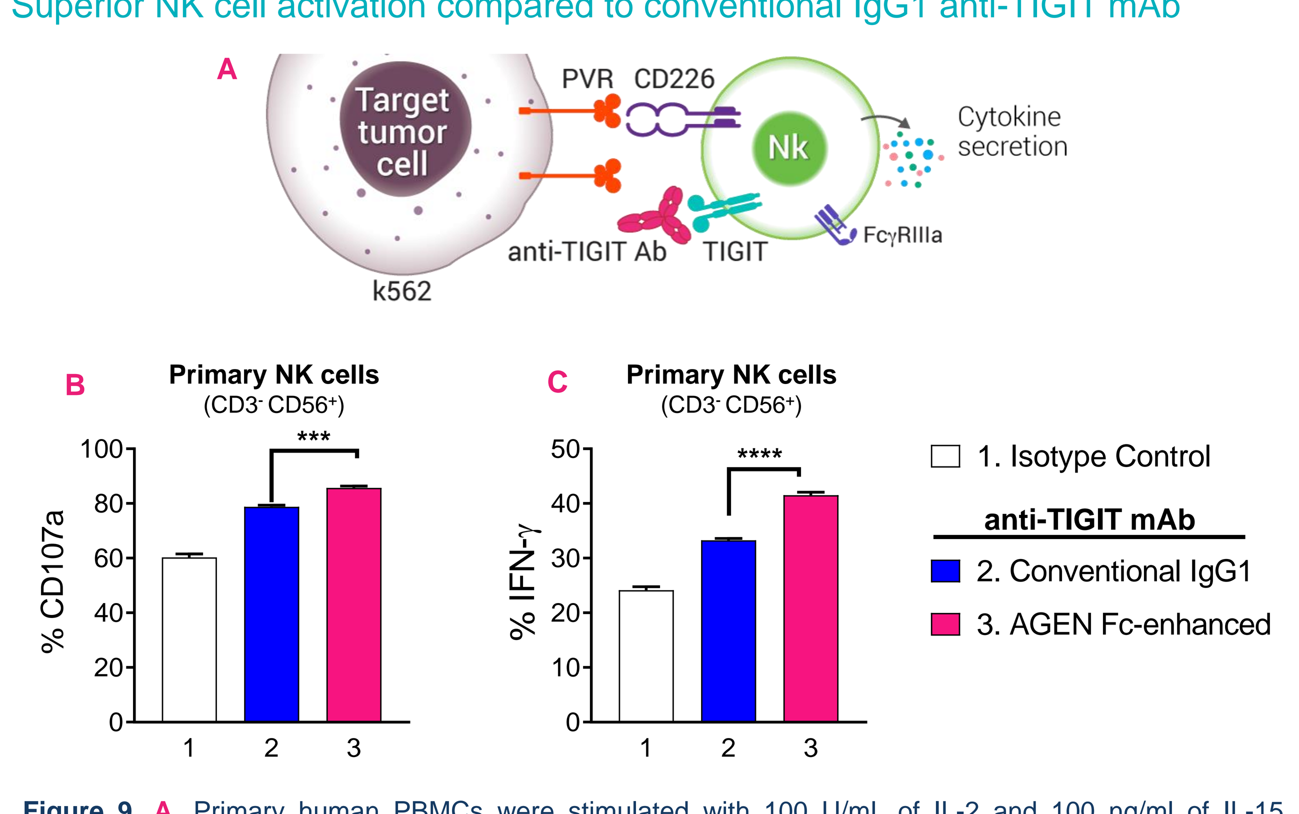


Figure 9. **A.** Primary human PBMCs were stimulated with 100 U/mL of IL-2 and 100 ng/ml of IL-15 overnight and subsequently co-cultured with K562 tumor cells at an effector to target ratio of 10:1 for 5 hours in the presence of 10 µg/ml of AGEN Fc-enhanced anti-TIGIT, conventional IgG1 anti-TIGIT or isotype control antibodies. Cell were treated with Brefeldin A and monensin to enable intracellular detection of cytokines. After 5 hours, the effector function of NK cells (CD3⁻ CD56⁺) were assessed by flow cytometry and expressed as a percentage of **B.** CD107a-expressing or **C.** IFN-γ-expressing NK cells.

AGEN Fc-enhanced anti-TIGIT mAb demonstrates superior combination potential with anti-PD-1 therapy

Superior functional activity compared to conventional IgG1 anti-TIGIT mAb

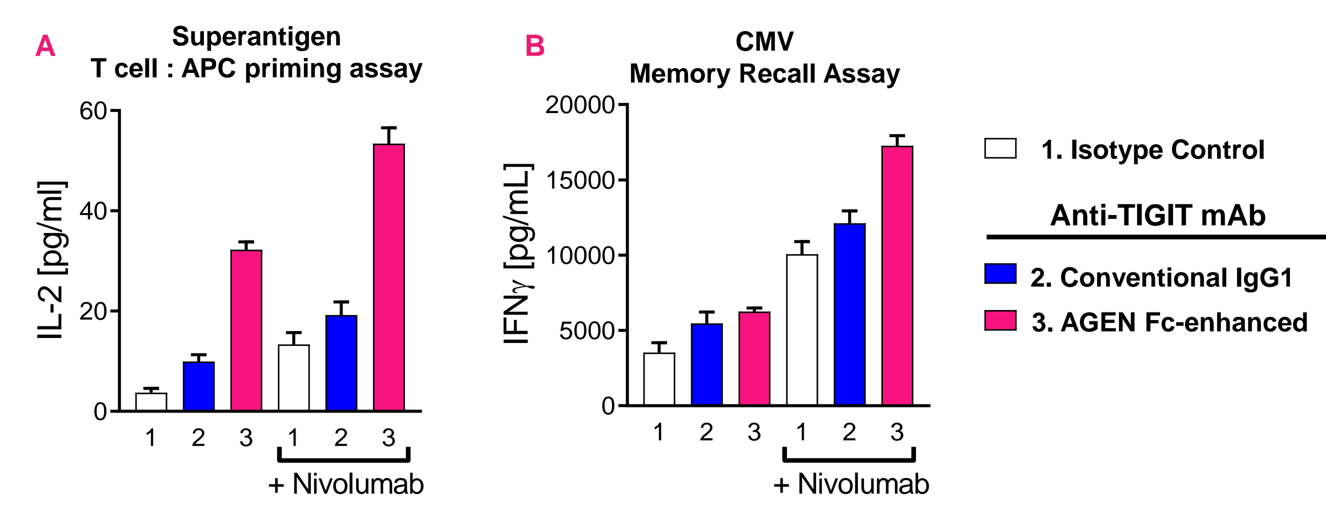


Figure 10. **A.** Healthy donor PBMCs stimulated with a sub-optimal concentration of SEA antigen and treated with 10 µg/mL of AGEN Fc-enhanced anti-TIGIT, conventional IgG1 anti-TIGIT, or isotype control antibodies alone or in combination with 10 µg/mL of anti-PD-1 (Nivolumab). Cytokine secretion was analyzed by AlphaLISA from supernatants collected after 4 days. **B.** CMV-reactive healthy donor PBMCs were stimulated with a sub-optimal concentration of CMV whole antigens and treated with 10µg/mL of AGEN Fc-enhanced anti-TIGIT, conventional IgG1 anti-TIGIT or isotype control antibodies alone or in combination with 10 µg/mL of anti-PD-1 (Nivolumab). Cytokine secretion was analyzed by AlphaLISA from supernatants collected after 4 days.

AGEN Fc-enhanced TIGIT mAb is designed to target a broader patient population than conventional TIGIT mAbs

Improved binding to low and high affinity FcγRIIIA compared to IgG1 variants

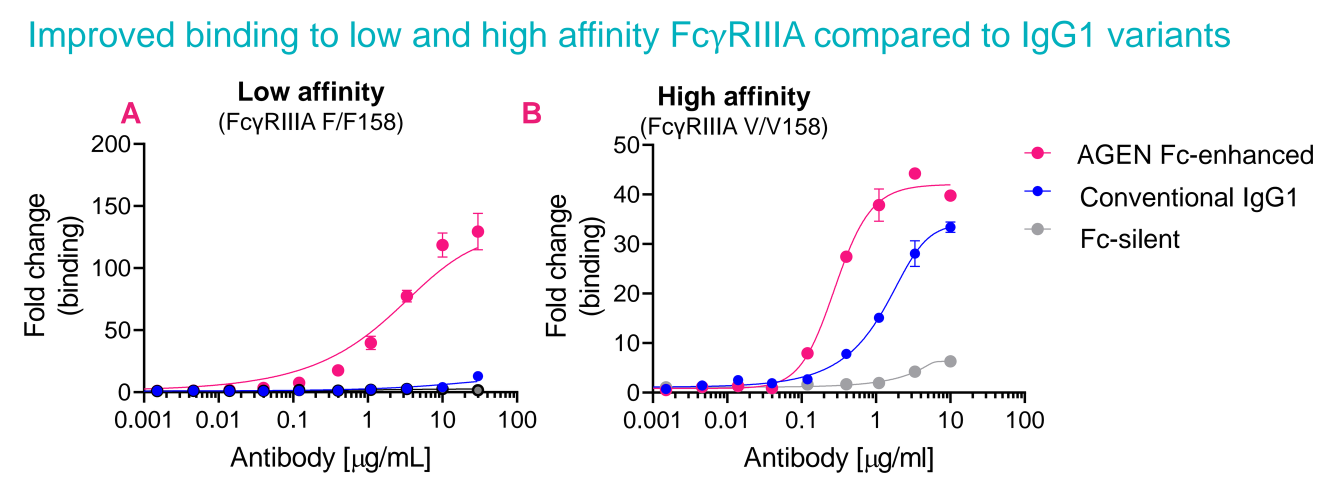


Figure 11. Binding profiles of antibody Fc variants to CHO cells stably expressing **A.** FcγRIIIA-F/F158 (low affinity) or **B.** FcγRIIIA-V/V158 (high affinity). Binding was assessed by flow cytometry and mean fluorescence intensity normalized according to standard methods.

Fc-enhanced anti-TIGIT outperforms an IgG1 analog to improve T cell responsiveness across all FcγRIIIA genotypes

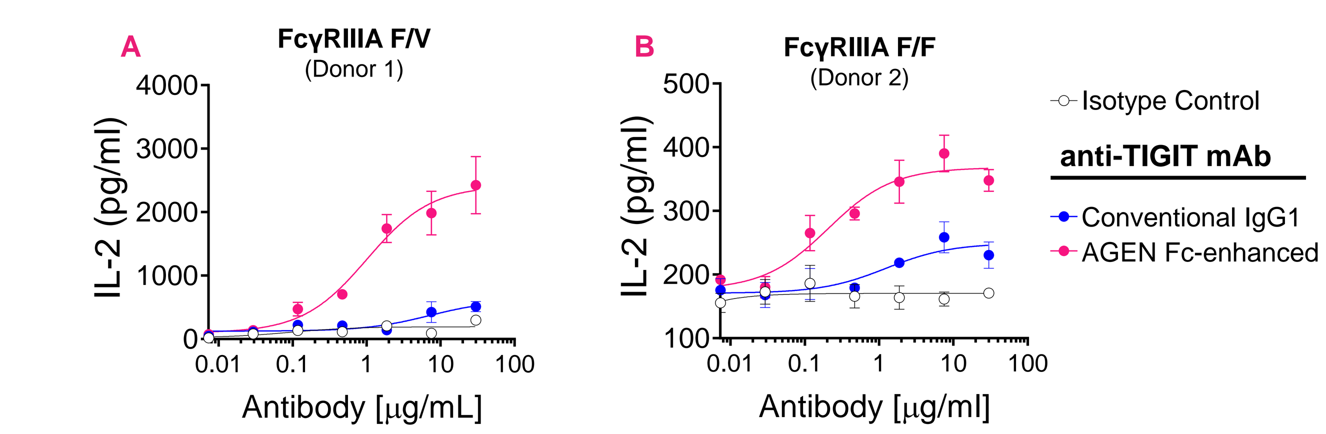


Figure 12. **A.** FcγRIIIA heterozygous (F/V) and **B.** low affinity homozygous (F/F) healthy donor PBMCs were stimulated with a sub-optimal concentration of SEA antigen and treated with increasing concentrations of AGEN Fc-enhanced anti-TIGIT, conventional IgG1 anti-TIGIT or isotype control antibodies ranging from 30-0.007 µg/mL. Cytokine secretion was analyzed by AlphaLISA from supernatants collected after 4 days.

Conclusions:

1. Our data describe a novel FcγR-dependent mechanism of action that enhances the therapeutic activity of anti-TIGIT mAbs in preclinical studies.
2. FcγR co-engagement is critical for the activity of anti-TIGIT mAbs.
3. AGEN Fc-enhanced anti-TIGIT antibody shows monotherapy and superior combination potential compared to conventional anti-TIGIT mAbs.
4. AGEN Fc-enhanced anti-TIGIT antibody is expected to extend therapeutic benefit to an additional 40% of patients who express the low affinity FcyRIIIA and are less likely to respond optimally to conventional TIGIT antibodies.

References:

1. Waight et al. Cancer Cell 2018
2. Johnston et al., Cancer Cell 2014
3. Mahaweni et al., Scientific Reports 2018

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