Anti-TIGIT induces T cell-mediated anti-tumor immune responses and combines with immune checkpoint inhibitors to enhance strong and long term anti-tumor immunity

Minu K. Srivastava, Rui Yun, Hyun-Bae Jie, Erin Mayes, Janice Yu, Fumiko Axelrod, Ming-Hong Xie, Jorge Monteon, Andrew Lam, May Ji, Yuwang Liu, John Lewicki, Tim Hoey, Austin Gurney, and Angie Inkyung Park

OncoMed Pharmaceuticals, Inc., Redwood City, CA

ABSTRACT

TIGIT (T cell immunoreceptor with Ig and ITIM domains) has been recently described as an inhibitory receptor which blocks CD8 T cell-mediated anti-tumor immune responses. We have generated an anti-mouse TIGIT antibody (313R12) to evaluate drug efficacy and mechanism of action in pre-clinical tumor models. Anti-TIGIT as a single agent promoted an anti-tumor immune response in multiple syngeneic mouse tumor models. Anti-TIGIT enhanced tumor specific T cell responses, particularly of the Th1 type and reduced Th2 type responses and also increased the function of cytotoxic T cells. Furthermore, anti-TIGIT displayed combination activity with immune checkpoint inhibitors anti-PD1 and anti-PDL1 in inhibiting tumor growth, promoting complete tumor rejection and significantly increasing mouse survival in the murine CT26 colon carcinoma model as compared to controls and single agents alone. Mice "cured" with anti-TIGIT/anti-PDL1 or anti-TIGIT/anti-PD1 combination treatments were protected from subsequent re-challenges with increasing numbers of tumor cells, suggesting the existence of immunologic memory. IL2 and tumor-specific IFN-γ production by splenic T cells were increased in the rescued mice in combination treatment compared to controls. Additionally, both effector and memory CD8+ T cell frequencies were increased within the total CD8+ T cell population in the rescued mice. We also demonstrated an increase in tumor-specific CD8 T cells in the periphery in anti-TIGIT/anti-PDL1 combination treatment compared to controls. Therefore, these results show that co-targeting of TIGIT and PD1 or PDL1 may be an effective and durable cancer therapy by increasing T cell-mediated anti-tumor immune responses and promoting long-term immunological memory.

MATERIALS AND METHODS

In Vivo Studies: The murine colon carcinoma (CT26-WT, ATCC CRL-2638) was obtained from American Type Culture Collection. Single cell suspensions of CT26.WT or MC38 tumor cells were injected subcutaneously into the flanks of 7-8 week old BALB/c or C57BL/6N mice, respectively. One week following tumor implantation, mice were randomized by tumor volume and injected *i.p.* with anti-TIGIT and anti-PDL1/anti-PD1 antibodies twice a week for 3 weeks (6 doses). Isotype antibodies were used for control. Tumor volumes were monitored by measuring two bisecting diameters of each tumor with electronic calipers. Tumor volumes were calculated using the formula: V=0.5ab2, with a as the larger diameter and b as the smaller diameter.

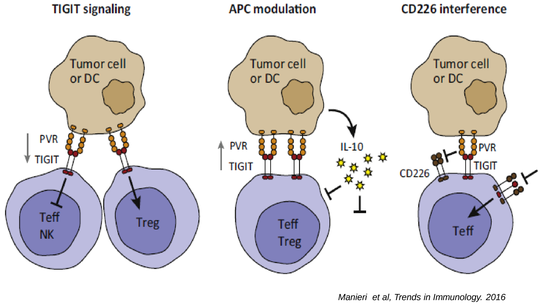
ELISPOT: Splenocytes were cultured in the presence or absence of tumor specific CD8 T cell peptide in T cell media for 48 hrs. followed by the ELISPOT assay as described by manufacturer's instruction.

Flow Cytometry: Single cell suspensions of splenocytes or tumor digests were pretreated with Fc block and stained with indicated antibodies and their isotype controls followed by fixing. Cells were analyzed by flow cytometry (FACS Canto II) and data was processed using Diva software. Tumor specific T cells were analyzed in the periphery by using AH1 dextramer from Immudex.

T cell cytotoxicity assay: Ten million splenocytes were cultured with a tumor-specific CD8+ T cell peptide for 7 days, washed and counted. These effector T cells were co-cultured with calcein AM labeled CT26.WT target tumor cells (E:T of 25:1 & 50:1) for four hours in triplicate wells in a 96-well plate and the supernatants were collected and measured for the release of calcein from tumor cells.

RESULTS AND CONCLUSIONS

Modes of action of TIGIT



Manieri et al, Trends in Immunology. 2016

Fig 1: TIGIT can block T cell lymphocytes through multiple mechanisms of action. APC, antigen-presenting cell, PVR, poliovirus receptor, Tigit, T cell immunoreceptor and ITIM domain; Teff, effector T cell; Treg, regulatory T cell.

Anti-TIGIT reduces tumor growth in CT26.WT and MC38 murine CRC models

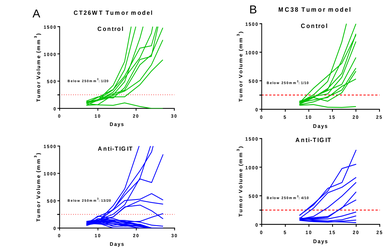


Fig 2. Anti-TIGIT reduces tumor growth in CT26.WT & MC38 mouse tumor models. BALB/c or C57BL/6N mice were inoculated with $3x10^4$ CT26.WT cells (A) or $4x10^5$ MC38 cells (B), and at day 7 mice were treated with isotype control or anti-TIGIT antibody. Spaghetti plots show individual mice tumor growth over time.

Anti-TIGIT increased tumor specific CD8+ T cell-mediated IFN-γ production and T cell cytotoxicity against parental tumor cells

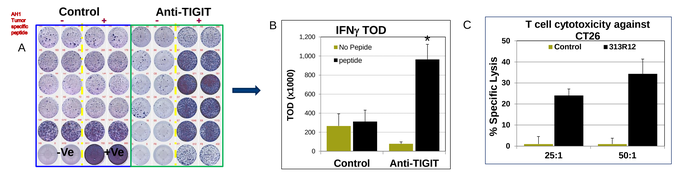


Fig 3. CT26.WT tumor bearing BALB/c mice were dosed with control or anti-TIGIT antibody. At the end of experiment, spleen cells from each group were isolated and tested for functions. (A-B) IFN-γ ELISPOT. (C) Tumor-specific T cell cytotoxicity

Combination of anti-TIGIT and anti-PD1 inhibits tumor growth and increases survival in CT26.WT tumor model

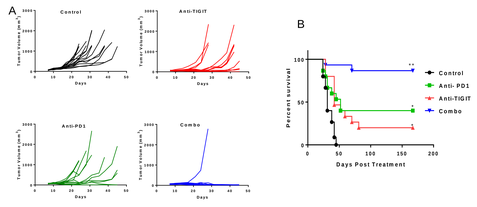
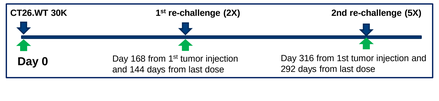


Fig 4. The combination of anti-TIGIT and anti-PD1 resulted in significant tumor growth inhibition and tumor regression. (A) CT26.WT tumor bearing BALB/c mice were dosed with control, anti-PDL1, anti-TIGIT, or combination of both antibodies twice a week for 3 weeks. (A) Tumor growth curves (n=15 mice per group). (B) Survival curve. ** indicate $P < 0.0001$.* indicate $P < 0.05$ against the control group.

Anti-TIGIT + anti-PD1 facilitates long-term immune memory against parental tumor cells



Tumor Free Mice (Re-challenge study)			
Treatment	Initial 30k	1st re-challenge **60k**	2nd re-challenge **150k**
Control		0% (0/10)	0% (0/10)
Anti-TIGIT	20% (3/15)	100% (2/2)	100% (2/2)
Anti-PD1	40% (6/15)	100% (6/6)	100% (6/6)
Combo	**86% (13/15)**	**100% (13/13)**	**100% (12/12)**

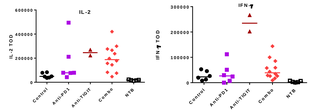


Fig 5 : The combination of anti-TIGIT and anti-PD1 resulted in a long-term protection against the original tumor. Mice "cured" of CT26.WT colon cancer were re-challenged in the opposite flank of the original injection site with the indicated number of tumor cells (1st: 60K, 2nd: 150K CT26.WT cells). Naïve mice were also inoculated with the same number of cells for controls. Spleens of the "cured" mice after 2nd re-challenge were analyzed for IFNγ and IL2 by ELISPOT. NTB: Non tumor bearing mice.

Combination of anti-TIGIT and anti-PDL1 inhibits tumor growth and increases survival in CT26.WT tumor model

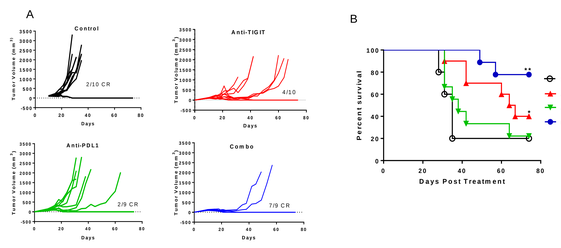
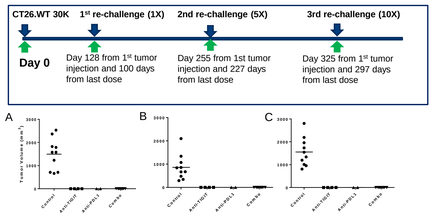


Fig 6. The combination of anti-TIGIT and anti-PDL1 resulted in significant tumor growth inhibition and tumor regression. CT26.WT tumor bearing BALB/c mice were treated *i.p.* with control, anti-PDL1, anti-TIGIT, or combination twice a week for 3 weeks. (A) Individual mice tumor growth curves (n=9 mice per group). (B) Survival curve. ** indicate SEM $P < 0.009$.* indicate SEM $P < 0.05$ against the control group.

Anti-TIGIT + anti-PDL1 facilitates long-term immune memory against parental tumor cells



Tumor Free Mice (Re-challenge study)				
Treatment	Initial 30k	1st re challenge **30k**	2nd re challenge **150k**	3rd re challenge **300k**
Control		0% (0/10)	0% (0/10)	0% (0/10)
Anti-TIGIT	65% (13/20)	84% (11/13)	100% (11/11)	100% (11/11)
Anti-PDL1	20% (2/10)	100% (2/2)	100% (2/2)	100% (2/2)
Combo	**70% (7/10)**	**100% (7/7)**	**100% (7/7)**	**100% (7/7)**

Fig 7 : The combination of anti-TIGIT and anti-PDL1 resulted in long-term protection against the original tumor. Mice "cured" of CT26.WT tumors were subjected to a series of tumor re-challenges. 1st re-challenge using 30K tumor cells, (B) 2nd re-challenge using 150K cells (C)3rd re-challenge using 300K cells. Naïve mice were also inoculated with the same number of cells for controls.

Combination of anti-TIGIT and anti-PDL1 increases strong anti-tumor immune responses in re-challenged mice

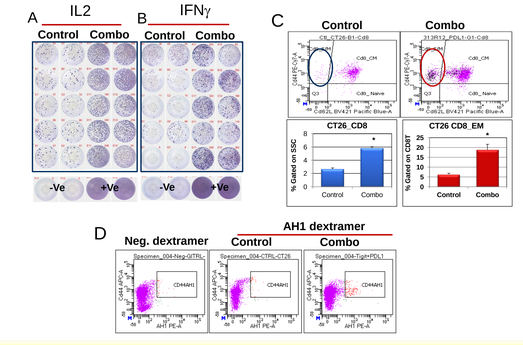


Fig 8 : Spleens of the "cured" mice after 3rd re-challenge from Fig. 7C were analyzed for IL2 (A) and IFNγ (B) production by ELISPOT and T cell memory population (C) and tumor-specific (AH1+) T cells (D).

SUMMARY

- Anti-TIGIT facilitates potent anti-tumor immune response in multiple mouse tumor models.
- Anti-TIGIT potentiates tumor antigen-specific T-cell IFN-γ production and increases T cell cytotoxicity against parental tumor.
- Anti-TIGIT combines with anti-PDL1/anti-PD1 to enhance efficacy as compared to single agents in CT26.WT tumor models.
- Combination of anti-TIGIT and anti-PDL1 increases tumor regression, increases tumor-specific T cells and generates long-term immune memory against the originally implanted tumor cells.