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, Funiko Axelrod, Ming-Hong Xie, Jorge Monteeon, Andrew Lam, May Ji, Yuwang Liu, John Lewicki, Tim Hoey, Austin Gurney, and Angie Inkyung Park

OncoMed Pharmaceuticals, Inc., Redwood City, CA

TIGIT (T cell immunoreceptor with Ig and ITIM domains) has been recently described as an inhibitory receptor which blocks CD28-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 combined activity with immune checkpoint inhibitors anti-PD1 and anti-PDL1 in inhibiting tumor growth, promoting complete tumor regression 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 and anti-PD1 or anti-TIGIT and anti-PDL1 combination treatments were protected from subsequent re-challenges with increasing numbers of tumors, suggesting the existence of immunologic memory. IL2 and tumor-specific P815 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 establish that co-targeting of TIGIT and PD1 or PD1L may be an attractive and durable cancer therapy by increasing T cell-mediated anti-tumor immune responses and promoting long-term and durable immunological memory.

In Vivo Studies: The murine colon carcinoma (CT26, ATCC CRL-2638) was obtained from American Type Culture Collection. Single cell suspensions of CT26WT or MC38 tumor cells were injected subcutaneously into the flanks of 7-9 week old NOD/SCID or C57BL/6 mice, respectively. One week after tumor implantation, mice were randomized by tumor volume and injected (i.p. with anti-TIGIT and anti-PDL1 and anti-PD1 antibodies twice a week for 3 weeks (i.d)). Isotype antibodies were used for control. Tumor volumes were monitored by measuring two biassing diameter each week of the same caliper. Tumor volumes were calculated using the formula: V=0.5(ab2), 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 identified in the periphery by using CHEF dextramer from immune mice. T cell cytotoxicity assay: Ten million splenocytes were cultured with a tumor-specific CD8+ T cell peptide for 7 days, washed and counted. Those effector T cells were co-cultured with calcium AM labeled CT26-WT target tumor cells (SET 2.1 & 2.2) for four hours in quadruplicate wells. We used 96 well plate and the supernatants were collected and measured for the release of calcine from tumor cells.

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

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

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

Materials and Methods

In Vivo Studies: The murine colon carcinoma (CT26, ATCC CRL-2638) was obtained from American Type Culture Collection. Single cell suspensions of CT26WT or MC38 tumor cells were injected subcutaneously into the flanks of 7-9 week old NOD/SCID or C57BL/6 mice, respectively. One week after tumor implantation, mice were randomized by tumor volume and injected (i.p. with anti-TIGIT and anti-PDL1 and anti-PD1 antibodies twice a week for 3 weeks (i.d)). Isotype antibodies were used for control. Tumor volumes were monitored by measuring two biassing diameter each week of the same caliper. Tumor volumes were calculated using the formula: V=0.5(ab2), 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 identified in the periphery by using CHEF dextramer from immune mice. T cell cytotoxicity assay: Ten million splenocytes were cultured with a tumor-specific CD8+ T cell peptide for 7 days, washed and counted. Those effector T cells were co-cultured with calcium AM labeled CT26-WT target tumor cells (SET 2.1 & 2.2) for four hours in quadruplicate wells. We used 96 well plate and the supernatants were collected and measured for the release of calcine from tumor cells.

Results and Conclusions

Antigen-specific CD8+ T cell responses can be enhanced by TIGIT engagement. In vitro studies, TIGIT engagement was sufficient to potentiate tumor antigen-specific CD8+ T cell responses. In vivo experiments, TIGIT engagement increased T cell cytotoxicity against parental tumor cells.

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.
- Combination of anti-TIGIT and anti-PDL1-1 to potentiate T cell responses against parental tumor.
- 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.