T cell immunoreceptor with Ig and ITIM domains (TIGIT) is a co-inhibitory molecule containing an immunoreceptor tyrosine-based inhibition motif (ITIM) within its cytoplasmic domain, and an immunoreceptor tyrosine-based activation motif (ITAM) in the extracellular domain for ligands of poliovirus receptor (PVR) and poliovirus receptor-related 2 (PVR2), with higher affinity to PVR. The ligands are expressed on the antigen-presenting cells and at high levels on tumors. Therefore, when TIGIT is present, the ligands may engage TIGIT rather than CD226, leading to cell suppression. We have generated antibodies against TIGIT that blocks ligand binding and inhibits TIGIT signaling. The clinical candidate OMP-313M32 binds human TIGIT but not rodent or non-human primate TIGIT. Therefore, a surrogate antibody was generated for pre-clinical assessments in mice. Antibody 313R12 is an anti-mouse TIGIT antibody that can block mouse PVR ligand binding and inhibit TIGIT signaling in a manner similar to the clinical candidate OMP-313M32. 313R12 inhibited the growth of syngeneic colon tumor in immune competent mice. In some cases, anti-TIGIT antibody caused complete tumor regression and a potent anti-tumor immune response as demonstrated by the lack of tumor growth upon re-challenge of mice that remained tumor-free after prior anti-TIGIT treatment. Mechanistically, anti-TIGIT antibody 313R12 was shown to induce a Th1 type response and increase cytotoxic T lymphocyte (CTL) activity. In vivo depletion of T cell populations, we have shown that CD4 T cell depletion completely abrogates the anti-TIGIT therapeutic effect, whereas CD8 T cell depletion led to partial reversal of efficacy of anti-TIGIT. Therefore, both CD4 and CD8 T cells are critical for anti-TIGIT-modulated immune responses. Using mice reconstituted with human hematopoietic stem cells, we also demonstrated that the clinical candidate OMP-313M32 inhibits patient-derived melanoma tumor growth. Taken together, these data demonstrate that anti-TIGIT therapy suppresses tumor growth and generates long-term immunological memory against multiple tumors.

**ABSTRACT**

**RESULTS AND CONCLUSIONS**

**MATERIALS AND METHODS**

**FACS binding assay:** Human, cynomolgus monkey, or mouse TIGIT ECD fused to CD47-GFP was transiently expressed in 293T cells, and incubated with 1 μg/ml anti-TIGIT antibody. After washing, anti-human or anti-mouse secondary antibody, conjugated with APC was used to detect bound antibodies by flow cytometry using a FACSCanto II Immurometer. The data were processed using FlowJo software and plotted as number of APC-positive cells (y axis) as a function of GFP-positive cells (x axis). A diagram indicates the mean fluorescence intensity of the antibody tested.

**Western Blot:** To evaluate TIGIT phosphorylation in response to TIGIT, expressing Jurkat cells were serum-starved for 2 hours at 37°C. Then they were treated with 2 μg/ml of anti-TIGIT MAAb (313R12, 313R18, and 313R20) or ATCC human TIGIT. Cells were then incubated in 37°C for 10 minutes, then lysates were prepared and blotted with antibody. Immune precipitates were evaluated by immunoblotting with a HRP-linked phospho-serine antibody. Total TIGIT was detected with an anti-human TIGIT polyclonal antibody.

**Cytosine Production in RPMCs:** T cells isolated from peripheral blood leukocytes were activated with P815 for 7 days with 2 μg/ml of anti-CD28 and 2 μg/ml of anti-CD40 antibodies. Control RPMCs were prepared and were restimulated with anti-CD3 and anti-CD28 antibody. P815 were added either alone or with the addition of anti-CD3, anti-CD28, and anti-CD8 antibodies along with 313R12 (at 1 μg/ml) or at 25 μg/ml or 313R302 (at 10 μg/ml) and 25 μg/ml. Production of IL-2 and IFN-γ was detected by ELISPOT assay and results expressed relative to the anti-CD3 baseline.

**In Vivo Studies:** The murine colon carcinoma (CT26.WT, ATCC CRL-3683) and the murine renal cortical adenocarcinoma (Renca, ATCC CRL-11055) were obtained from American Type Culture Collection. Single cell suspensions of CT26 or Renca tumor cells were injected subcutaneously into the flanks of 7-8 weeks old BALB/c mice. One or two weeks following tumor cell injection, mice were intraperitoneally treated either with control or anti-TIGIT antibody 313R12, control IgG1, anti-CD4, anti-CD8, and anti-CD3 antibodies were used to deplete CD4, CD8 T cells and NK/NKlymph cells, respectively. In this study using human melanoma cells, the clinical candidate OMP-313M32 was used against PVR. OMP-313M32, and anti-PD1 (Keytruda).

**ELISPOT:** Spontaneous or antigen-induced IFN-γ and IL-2 production was determined in ELISpot assay using mouse anti-IFN-γ and anti-IL-2 antibodies as capture. SPOT forming units were counted in a blinded manner for each group. All ELISPOT was performed on 48 well disposable ELISPOT plates with 105 cells per well. Data were analyzed using GraphPad software.

**T cell Cytoxicity Assay:** Ten million splenocytes were cultured with tumor specific CD8 T cell pellet 10H for 7 days and washed with 1:50 PBS. Each cell group was cultured with two effectors cells. These two effectors cells were co-cultured with canine melanoma cell line CT26. The CT26 tumor cells were incubated in 96-well plates in triplicates and were collected and measured for released of cytokines. The cytokine is calculated using the formula: Cytotoxicity (%) = (1 - (average absorbance of experimental groups/average absorbance of control) x 100). Data were presented as mean ± SEM from at least three independent experiments. **Flow Cytometry:** Tumors from control and anti-TIGIT antibody treated mice were processed to single cell suspension and stained with anti-CD4, anti-CD8, and anti-CD3 antibodies. Live tumor cells were gated by Tricolor/CD11 b fluorescence and analyzed on a FACSCanto II Immurometer.

**Anti-TIGIT antibody meditates robust anti-tumor activity through T and NK cells by inhibiting Treg suppressive function and inducing Th1 response**

**Summary**

- Anti-TIGIT antibodies block PVR ligand binding and inhibit downstream signaling.
- Anti-TIGIT antibody induces a potent anti-tumor immunity by promoting Th1 type immune response and inhibiting Tregs.
- Anti-TIGIT significantly reduces tumor growth in a T cell-dependent manner.
- Anti-TIGIT enables complete eradication of some tumors as a single agent.
- Clinical candidate OMP-313M32 is active against PDX melanoma in humanized mice.