Antibody against T cell Immunoreceptor with Ig and ITIM domains (TIGIT) Induces anti-Tumor Immune Response and Generates Long-Term Immune Memory

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ABSTRACT

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 tail, and is highly expressed on regulatory T cells and activated CD4+ T, CD8+ T, and NK cells. TIGIT competes with CD226, which contains an immunoreceptor tyrosine-based activation motif (ITAM) within its cytoplasmic tail for ligands poliovirus receptor (PVR) and poliovirus receptor-related 2 (PVRL2), with higher affinity to PVR. The ligands are expressed on the surface of antigen presenting cells and at high levels on most tumors. Therefore, when TIGIT is present, the ligands preferentially 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 and 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 and kidney tumors in immune competent mice. In some cases, anti-TIGIT antibody 313R12 caused complete tumor regression and a potent anti-tumor immune memory 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 response and increase cytotoxic T lymphocyte (CTL) activity. By *in vivo* depletion of T cell populations, we have shown that CD8 T cell depletion completely abrogated the anti-TIGIT therapeutic effect, whereas CD4 T cell depletion led to partial reversal of efficacy of anti-TIGIT. Therefore, both CD4+ and CD8+ T cells are critical for anti-TIGIT-mediated 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.

MATERIALS AND METHODS

FACS binding assay: Human, cynomolgus monkey, or mouse TIGIT ECD fused to CD4TM-GFP was transiently expressed in 293T cells, and incubated with 1 ug/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 instrument. 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 diagonal in the upper right quadrant of the plot reflects specific binding of the antibody tested.

Western Blot: To evaluate TIGIT phosphorylation in response to PVR, TIGIT expressing Jurkat cells were serum-starved for 2 hours at 37°C, then pre-treated with 20 µg/mL of anti-TIGIT mAb (313R12, 313R19, or 313M32) for 15 minutes at room temperature, and then mixed with parental (PVR null) or PVR expressing cells at a ratio of 5:1 in the presence of 10 mM sodium orthovanadate. Cells were incubated at 37°C for 5 minutes, then lysates were prepared and immunoprecipitated overnight with anti-FLAG magnetic beads or anti-TIGIT antibody followed by ProteinA/G magnetic beads. Immunoprecipitates were evaluated by immunoblotting with a HRP-linked anti-phosphotyrosine antibody. Total TIGIT was detected with an anti-FLAG-HRP or anti-TIGIT antibody .

Cytokine Production in PBMCs: T cells isolated from peripheral blood leukopaks were activated *ex vivo* for 7 days with 2 ug/ml PHA and 4 ng/ml IL-2. T cells were then rested and reactivated with anti-CD3 antibody. PVR was added either alone or along with 313R19 at 10 ug/ml and 25 ug/ml or OMP-313M32 at 10 ug/ml and 25 ug/ml. Production of IL-2 and IFNγ was measured by ELISA and results expressed relative to the anti-CD3-induced levels.

In Vivo Studies: The murine colon carcinoma (CT26-WT, ATCC CRL-2638) and the murine renal cortical adenocarcinoma (Renca, ATCC CRL-2947) 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 week old Balb/c mice. One or two weeks following tumor inoculation, mice were randomized and treated with isotype control or anti-TIGIT antibody 313R12. Anti-CD4 (clone: GK1.5), anti-CD8 (clone: YTS169.4), anti-GM1 antibodies were used to deplete $CD4^+$, $CD8^+$ T cells and NK/Meyloid cells, respectively. For in vivo study using humanized NSG mice, OMP-M9 melanoma cells were implanted and treated with Control IgG, OMP-313M32, or anti-PD1 (Keytruda).

ELISPOT: Splenocytes were cultured in the presence and absence of tumor specific CD8 T-cell peptide (1µg/ml final concentration of AH-1 peptide for CT26 model) in T cell media for 48 hrs followed by the ELISPOT assay as described in manufacturer's instructions.

T cell Cytotoxicity Assay: Ten million splenocytes were cultured with tumor specific CD8 T-cell peptide AH1 for 7 days, washed and counted. These effector T cells were co-cultured with calcein AM labeled CT26 tumor cell targets as indicated for four hours in triplicate wells in a 96-well plate and the supernatants were collected and measured for the release of calcein from the tumor cells. Specific lysis is calculated using the formula: Specific lysis (%)=(experiment release-target spontaneous)/(maximum release-target spontaneous)X100.

Flow Cytometry: Tumors from control and anti-TIGIT antibody treated mice were processed to single cell suspension and stained with anti-Cd45, anti-Cd4, anti-Cd8, and anti-TIGIT antibodies. Live immune cells were used for gating.

Treg Suppression Assay CD4+CD25+ regulatory T-cells from each treatment group and naïve T cells from naïve mice were isolated. Naïve T cells were labeled with VTD dye and stimulated with anti-CD3 and anti-CD28 antibody in the presence of Tregs.

RESULTS AND CONCLUSIONS

Characterization of anti-TIGIT antibodies

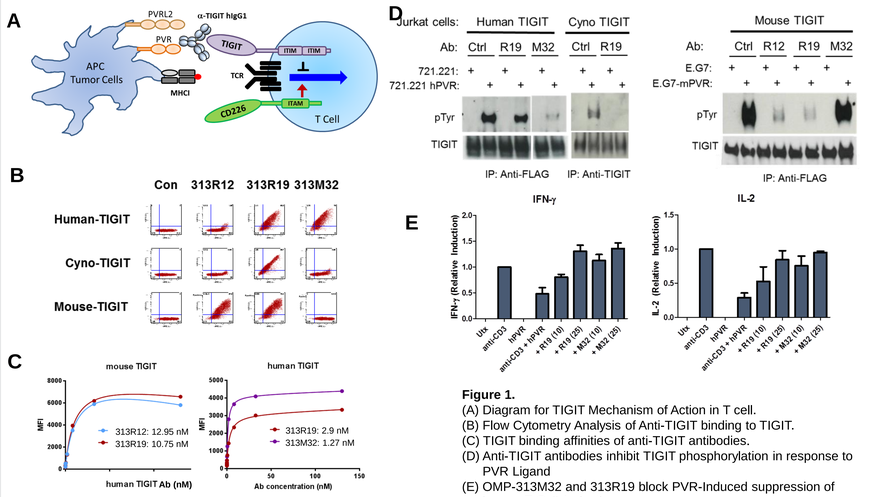

Figure 1.
(A) Diagram for TIGIT Mechanism of Action in T cell.
(B) Flow Cytometry Analysis of Anti-TIGIT binding to TIGIT.
(C) TIGIT binding affinities of anti-TIGIT antibodies.
(D) Anti-TIGIT antibodies inhibit TIGIT phosphorylation in response to PVR Ligand
(E) OMP-313M32 and 313R19 block PVR-Induced suppression of human T-Cell Activation

Anti-TIGIT antibody significantly reduces tumor growth and generates long-term immune memory cells

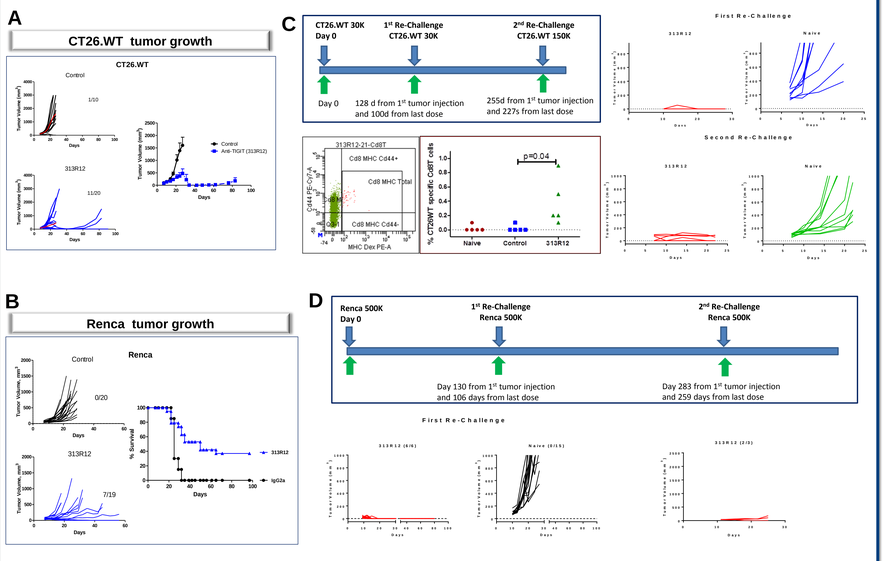

Figure 2. Anti-TIGIT antibody significantly reduces tumor growth in CT26.WT murine colorectal carcinoma (A) and Renca renal cell carcinoma (B). Mice with complete tumor regression were re-challenged with fresh tumor cells in the absence of further treatment (C and D)

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

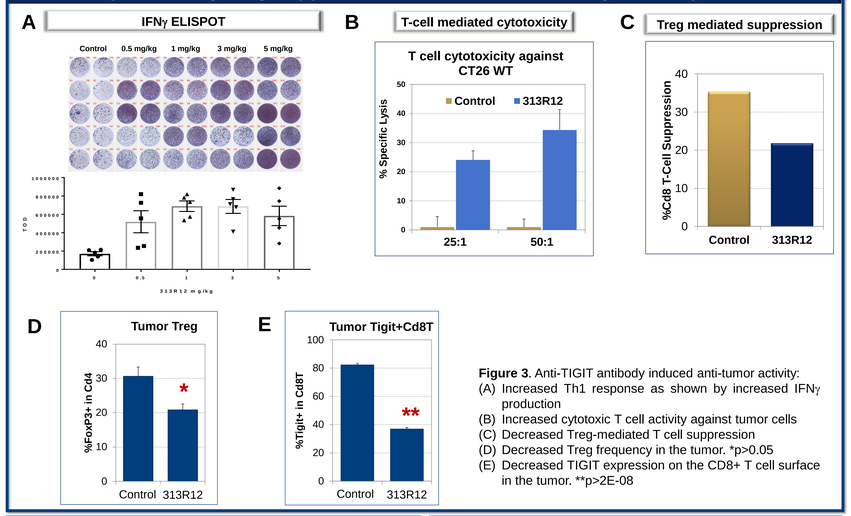

Figure 3. Anti-TIGIT antibody induced anti-tumor activity:
(A) Increased Th1 response as shown by increased IFNγ production
(B) Increased cytotoxic T cell activity against tumor cells
(C) Decreased Treg-mediated T cell suppression
(D) Decreased Treg frequency in the tumor. *p>0.05
(E) Decreased TIGIT expression on the CD8+ T cell surface in the tumor. **p>2E-08

Anti-TIGIT requires Cd8+ T cells for anti-tumor efficacy

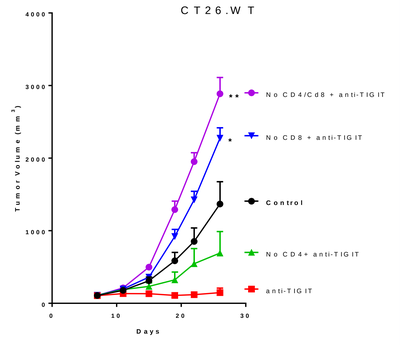

Figure 4. Anti-TIGIT antibody mediates robust anti-tumor immunity in a T cell dependent manner. Anti-tumor activity of 313R12 was lost when Cd8 T cells or both Cd4 and Cd8 T cells are depleted, and tumors grew larger than the control group even in the presence of 313R12. Tumor volume measures at day 26. *p>0.05, **p>0.005 vs. control

Clinical Candidate OMP-313M32 is active against human melanoma PDX in humanized NSG mice

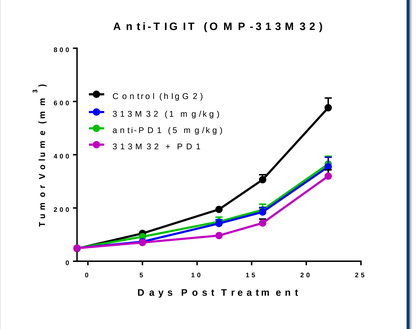

Figure 5. Clinical candidate OMP-313M32 anti-TIGIT antibody inhibits growth of patient-derived melanoma in NSG-humanized mice.

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.