GTLR ligand protein (GITRL-Fc) induces T cell mediated anti-tumor immune response and can combine with anti-PDL1 to enhance anti-tumor immunity and long-term immune memory.

OncoMed Pharmaceuticals, Inc., Redwood City, CA.

**ABSTRACT**

**GITLIR Immunotherapy**

**GIRLFR Agonism:**
- Increase effector T cells
- Production
- Cyclokine release

Reduce T regulatory cell suppressive activity

**MATERIALS AND METHODS**

In Vivo Studies: The murine colon carcinoma (CT26-RT, ATCC-CRL-2632) and murine mammary carcinoma (EMT-4, ATCC-CRL-2755) were obtained from American Type Culture Collection. Single cell suspensions of CT26 or EMT-4 tumor cells were injected subcutaneously into the flanks of athymic mice on day 0/7 of 7-8 week old female Balb/c mice, respectively. Ten days following tumor inoculation, mice with palpable tumors were injected i.p. with GITLIR-Fc (3 doses) and anti-PDL1 for 5 doses for 5 weeks (5 doses). Isotype antibodies were used for control. Tumor volumes were monitored by measuring the lengths and widths of each tumor with electronic calipers and 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 and absence of tumor specific CD8 T cell peptide in 96-well plates followed by the ELISPOT assay as described by manufacturer’s instructions.

Luminex Analysis: Cytokines in plasma were measured using mouse CD8 T cell multiplex kits (EMD Millipore) according to manufacturer’s instructions using drop array technology (Cytinus). The fluorescence intensity of the beads was measured using the Luminex-200 reader.

Treg Suppression Assay: To determine the impact of Tregs on T cell proliferation, proliferative tracking dye (VTD, labeled native splenic T cells were co-cultured with different numbers of isolated splenic Tregs (at 1:1 ratio) or E-Treg (E-Treg mean 1.5 and 1.6) in the presence of anti-CD3 and anti-CD28 beads for 4 hrs for 3 days with proliferation. The mean in dilution of VTD dye was used to calculate the proliferation by FACs. Tregs and naïve T cells were isolated using kits from Miltenyi Biotec.

Flow Cytometry: Single cell suspensions of splenocytes or tumor digests were used for staining with fluorochrome labeled antibodies and their isotype controls. Cells were preincubated with anti-CD16/CD32 for 20 min followed by PE-conjugated antibodies with FACs buffer (FCS-FCS PBS) and fixed with PBS-2% formaldehyde (v/v). Fluorescence was detected by FACs (FACS Canto II). Analysis were performed with the Diva software for FACs. Treg tracking, intracellular FoxP3 staining was stained using Treg staining kit from ebioscience according to manufacturer’s instructions.

**RESULTS AND CONCLUSIONS**

N- and C-terminus of TNF family monomers are in close proximity and can be tethered without use of a linker sequence to create fully functional trimers.

Native form of GITLIR trimer on cell surface

**SUMMARY**

**GIRLFR-Fc treated tumor bearing mice have less suppressive splenic Treg in GITLIR-Fc and anti-PDL1 + anti-TLR10 facilitates long-term immune memory against parental tumor cells**

**GIRLFR-Fc and anti-PDL1 in combination results in significant tumor growth inhibition. Balb/c mice were inoculated with 3X10^6 CT26 cells i.p. in the right flank of the abdominal cavity and the tumor volumes were measured using caliper digital caliper. At day 10, mice with palpable tumors were treated with GITLIR-Fc (3 doses) and anti-PDL1 for 5 doses (5 weeks) (3 doses). Isotype antibodies were used for control. Tumor volumes were measured using the formula: V=0.5ab2, with a as the larger diameter and b as the smaller diameter.

**GIRLFR-Fc reduces tumor growth and increases survival as compared to agonist GITR mAb**

**GIRLFR-Fc in vivo with immune checkpoint inhibitor anti-PDL1**

**GIRLFR-Fc and anti-PDL1 resulted in significant tumor growth inhibition. Balb/c mice were inoculated with 3X10^6 CT26 cells i.p. in the right flank of the abdominal cavity and the tumor volumes were measured using caliper digital caliper. At day 10, mice with palpable tumors were treated with GITLIR-Fc (3 doses) and anti-PDL1 for 5 doses (5 weeks) (3 doses). Isotype antibodies were used for control. Tumor volumes were measured using the formula: V=0.5ab2, with a as the larger diameter and b as the smaller diameter.