FPT155, a novel therapeutic CD80-Fc fusion protein, with potent anti-tumor activity in preclinical models

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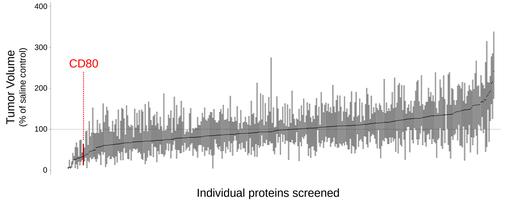
Introduction

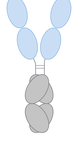
T cell regulation involves the integration of multiple signaling pathways: signaling via the TCR complex and through co-signaling receptors, both co-stimulatory and co-inhibitory. CD80 is a transmembrane protein and a well-characterized co-signaling ligand. It is expressed on professional antigen presenting cells (APC) such as dendritic cells and activated macrophages. Following TCR recognition of cognate peptide-major histocompatibility complex (MHC), CD80 acts as a co-stimulatory ligand via interactions with its receptor, CD28, expressed on T cells. In addition to signaling via CD28, CD80 also interacts with co-inhibitory molecules CTLA4 and PDL1. CD80 interactions with CTLA4 are central for dampening the T cell response once activated T cell responses are no longer needed, while the biological significance of the CD80 interaction with PDL1 is not as well understood. Together, the co-stimulatory and co-inhibitory ligands ensure both tolerance to self-antigens and the ability to mount an appropriate immune response to non-self antigens.

Screens using Five Prime's Rapid *in vivo* Protein Production System (RIPPS®) identified a soluble version of CD80 as one of the most potent inhibitors of CT26 tumor growth among a library of 440 extracellular proteins evaluated. The activity of soluble CD80 was comparable or superior to that of other T cell agonists such as GITRL, OX40L, and 4-1BBL.

RIPPS® Screen of Soluble Extracellular Proteins in the CT26 Tumor Model



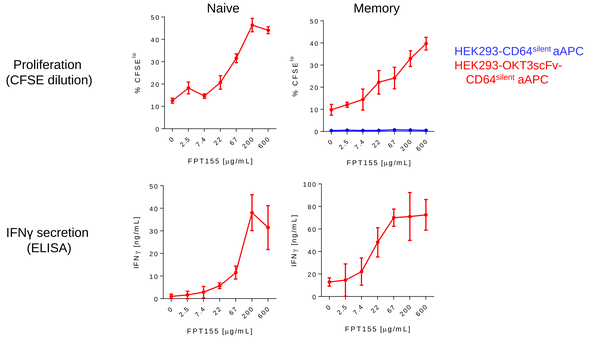
We are developing FPT155, a soluble CD80 fusion protein, for the treatment of solid tumors. FPT155 is a recombinant fusion protein composed of the extracellular domain (ECD) of human CD80 fused with human IgG1 wild-type Fc domain. FPT155 is designed to act as a potent stimulator of anti-tumor immunity and efficiently co-stimulates primary human T cells in the presence of antigenic stimulation. A murine surrogate molecule (mFPT155) was constructed for preclinical studies, comprising the ECD of murine CD80 fused with mouse IgG2a wild-type Fc domain. mFPT155 demonstrates potent anti-tumor activity *in vivo* and induces a favorable microenvironment for an effective anti-tumor immune response. We are currently performing IND-enabling studies and plan to initiate a clinical study with FPT155 in 2018.

FPT155	mFPT155
	
Human CD80 ECD	Murine CD80 ECD
Human IgG1 Fc	Mouse IgG2a Fc

FPT155 stimulates primary T cells in a TCR-dependent fashion

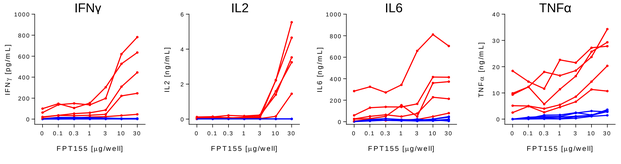
FPT155 potently co-stimulates naïve and memory CD4$^+$ T cells to proliferate and secrete IFNγ in a dose-dependent fashion in the presence of "artificial APC" (aAPC) that co-express anti-CD3 scFv and a non-signaling CD64. Importantly, memory CD4$^+$ T cells are not stimulated by FPT155 with aAPC that do not express the OKT3 scFv, demonstrating that FPT155 must be co-presented with TCR stimulus to activate T cells.

Human CD4$^+$ T Cell Stimulation *in vitro*



To confirm that FPT155 does not have superagonistic, TCR-independent activity, we evaluated it in the Stebbings "wet-coating" assay, in which the test article is presented to PBMC in a highly-crosslinked, immobilized form. This assay format predicts the cytokine release syndrome (CRS) induced in patients by the anti-CD28 superagonist antibody TGN1412. By contrast, FPT155 does not induce the spontaneous release of CRS-associated cytokines, even at high concentrations, unless it is co-immobilized with anti-CD3 (5 donors shown). We thus conclude that FPT155 is not a CD28 superagonist and does not pose the same clinical risk as TGN1412.

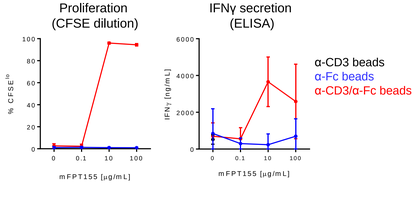
PBMC Cytokine Release *in vitro*



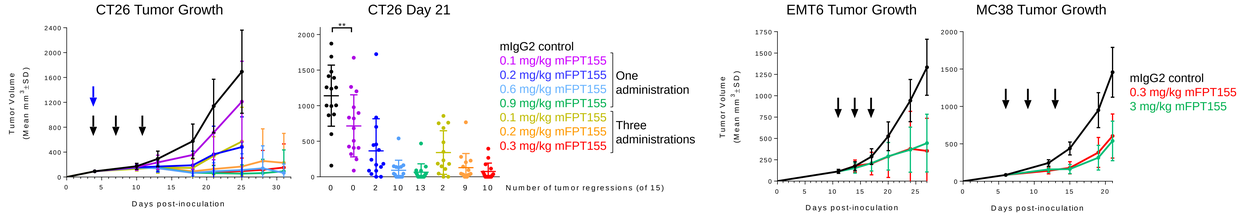
mFPT155 co-stimulates murine T cells

mFPT155 co-stimulates murine CD4$^+$ T cells in a bead-based assay format. As observed for FPT155, this activity requires co-ligation with anti-CD3 via co-immobilized anti-Fc capture: mFPT155 does not stimulate T cell activation when the beads are coated with anti-CD3 alone or when no anti-CD3 is present.

Murine CD4$^+$ T Cell Stimulation *in vitro*



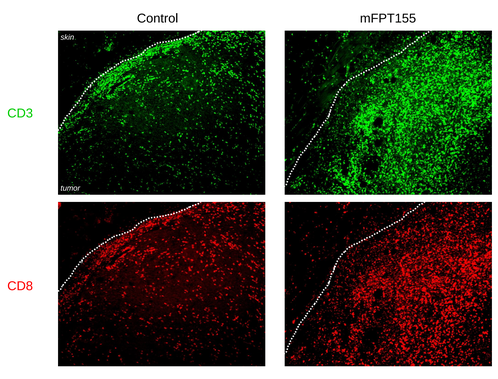
mFPT155 has potent single-agent anti-tumor efficacy



mFPT155 exerts dose-dependent inhibition of CT26 tumor growth following one or three administrations starting at an average tumor volume of 80-100 mm^3. Tumor growth is significantly inhibited even following a single administration at 0.1 mg/kg, and we observe complete tumor regressions with a single administration of 0.2 mg/kg mFPT155. mFPT155 also controls the growth of EMT6 and MC38 tumors following three administrations.

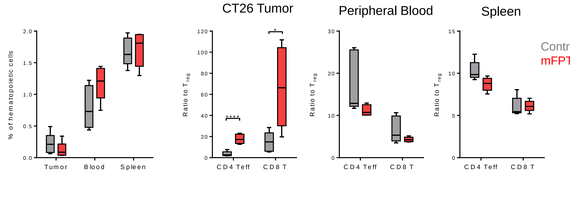
Pharmacodynamic responses

T Cell Infiltration into CT26 Tumors



Following two administrations of mFPT155 at 0.3 mg/kg, we observe robust infiltration of T cells into the tumor mass. This effect is specific to effector T cell subsets, resulting in an upward shift in the ratio of these cell types to intratumoral T_{reg}. This effect is also specific to the tumor microenvironment and is not observed in normal lymphoid compartments such as peripheral blood or spleen.

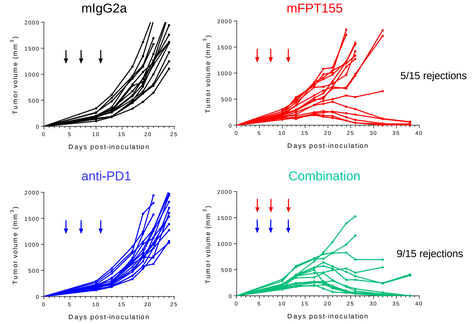
Frequency of T_{reg} | Ratio of Effector T Cells to T_{reg}



* $p < 0.05$, ** $p < 0.005$, **** $p < 0.0001$

mFPT155 has synergistic combination activity with anti-PD1

CT26 Tumor Growth



mFPT155 exerts partial anti-tumor activity in the CT26 model when administered at a large starting tumor volume (3 doses of 0.3 mg/kg at starting at 200 mm^3). However combination with anti-PD1 (RMP1-14, mouse IgG2a-silent, 3 administrations of 5 mg/kg) synergistically enhances tumor growth control ($p < 0.03$ compared to monotherapy). The combination regimen also increases the number of complete tumor rejections to 9 of 15, compared to 5 of 15 with mFPT155 alone.

Conclusions

FPT155 promotes T cell responses in the presence of antigenic stimulation but is not a CD28 superagonist.

Tumor control induced *in vivo* by mFPT155 is accompanied by robust effector T cell infiltration into the tumor and an increased effector T cell to T_{reg} ratio.

mFPT155 demonstrates potent anti-tumor activity in combination with PD1 pathway blockade.

In summary, FPT155, a CD80-Fc fusion protein, has potent anti-tumor activity in preclinical models and is a promising modality for the treatment of cancer.