Development of FPA154, a novel tetravalent anti-GITR antibody, for the treatment of solid tumors

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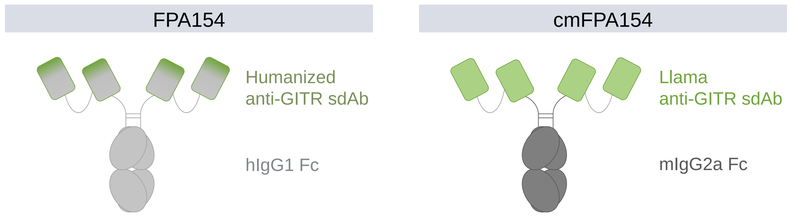
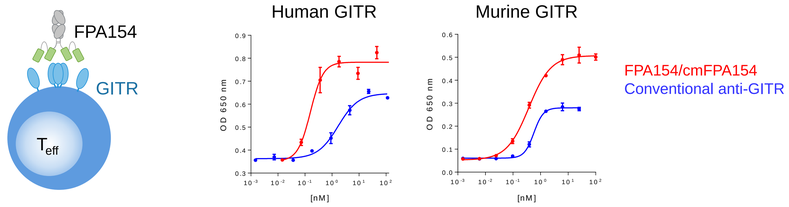
Introduction

Glucocorticoid-induced TNFR-related (GITR, TNFRSF18) is a member of the TNFR superfamily with pleiotropic T cell modulatory activity. Studies performed in mice suggest that GITR antagonizes the suppressive capacity of T_{reg}, whereas it exerts stimulatory activity on conventional effector T cells (both $CD4^+$ and $CD8^+$). Preclinical studies have indicated that GITR-targeting agents inhibit tumor growth via two mechanisms: depletion and possibly suppression of T_{reg} and direct agonism of effector T cells.

We are developing a novel anti-GITR antibody with enhanced agonist activity for the treatment of solid tumors. Our candidate molecule, FPA154, is constructed with humanized llama-derived single-domain antibodies (sdAbs) in a tetravalent format. with an effector-competent human IgG1 Fc domain. A mouse-reactive surrogate molecule (cmFPA154) was constructed for preclinical studies, comprising the parental llama sdAb and a murine IgG2a Fc domain.

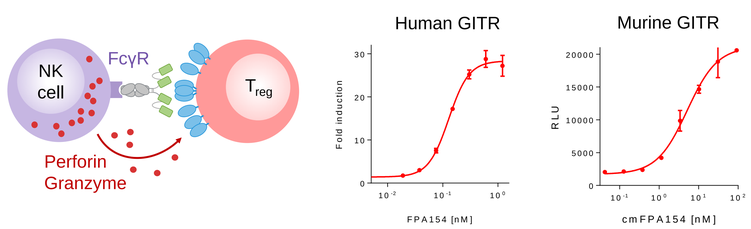


FPA154 exerts superior GITR agonist activity



The tetravalent format induces agonist activity that is superior to a conventional bivalent antibody. Activation of NF-κB activity was assessed in HEK293 reporter cell lines engineered to express GITR (full-length human or a fusion of the murine extracellular domain with the human intracellular domain). FPA154 or cmFPA154 were compared to conventional, bivalent antibodies to human or murine GITR, respectively.

FPA154 exerts ADCC activity against $GITR^{HI}$ cells

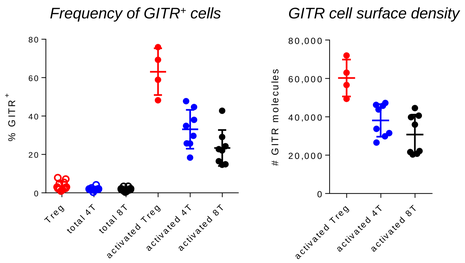


FPA154 and cmFPA154 recruit antibody-dependent cellular cytotoxicity (ADCC) activity against cells that express high levels of GITR, such as activated and intratumoral T_{reg}. ADCC activity was assessed with Jurkat reporter cell lines expressing human or murine FcγRIII. CHO cells engineered to express high levels of human GITR or *in vitro*-activated murine T_{reg} were used as target cells.

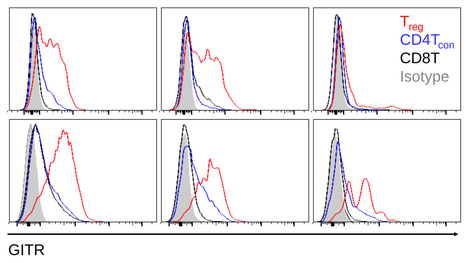
FPA154 binding to primary T cells correlates with GITR density

GITR is not significantly expressed on resting human T cells *ex vivo* but it is upregulated following *in vitro* activation. Upon activation with anti-CD3/anti-CD28, a greater fraction of T_{reg} upregulate GITR and express more GITR molecules than conventional T cells. The higher expression of GITR on Treg versus effector T cells is also observed in tumor infiltrating lymphocytes (TIL) from human tumor samples. Binding of FPA154 and cmFPA154 to primary T cells correlates with the cell surface density of GITR expression. Thus T_{reg} are preferentially targeted for depletion via FcγR-expressing effector cells.

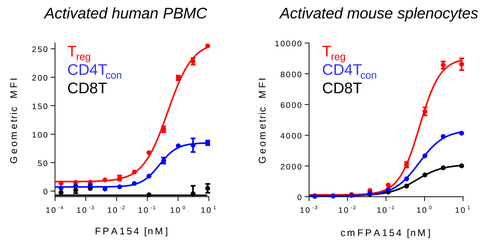
GITR expression on healthy human PBMC



GITR expression on human TIL (kidney, lung)



FPA154/cmFPA154 binding to primary T cells

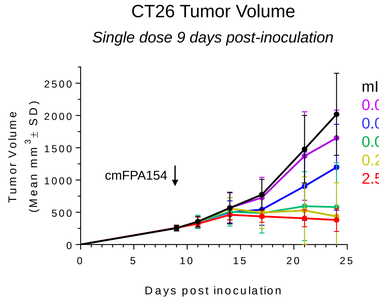


cmFPA154 induces tumor T cell infiltration and inhibits tumor growth

A single dose of cmFPA154 potently inhibits tumor growth in the CT26 and MC38 models. Treatment is capable of inducing complete tumor rejection at doses as low as 0.08 mg/kg. Tumor-infiltrating CD8 T cells (CD8TIL) are increased following cmFPA154 treatment in a dose-dependent fashion.

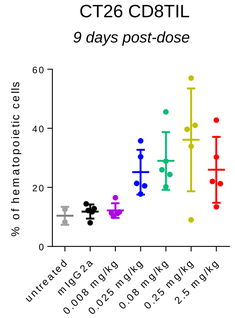
CT26 Tumor Volume

Single dose 9 days post-inoculation



CT26 CD8TIL

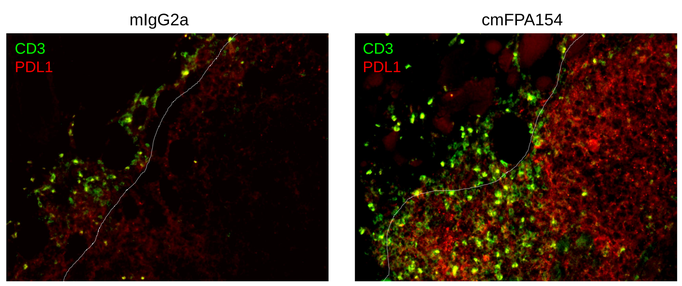
9 days post-dose



cmFPA154 treatment induces the infiltration of T cells into MC38 tumors at 3 days following a single dose at 2.5 mg/kg, as determined by immunofluorescent microscopy. T cell infiltration is accompanied by robust expression of PDL1 in the tumor microenvironment.

MC38 Tumors

3 days post-dose

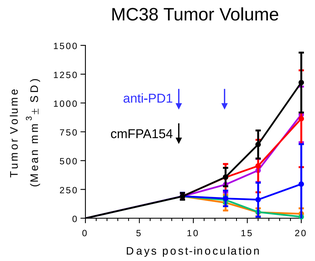


Dotted white line = tumor margin

cmFPA154 and anti-PD1 combination has potent anti-tumor efficacy

In a treatment regimen wherein monotherapies are not fully active, cmFPA154 potently combines with anti-PD1 to completely reject MC38 tumors. On day 9 after MC38 tumor inoculation, mice were administered one dose of cmFPA154 (0.5 mg/kg) and of anti-PD1 (RMP1-14, mIgG2a-silent; 100 µg); a second dose of anti-PD1 was given on day 13. Although less efficacious, a version of cmFPA154 that lacks Fc effector function (cmFPA154-silent; administered at 2.5 mg/kg) is also capable of inducing complete tumor rejection in combination with anti-PD1 in 60% of animals, suggesting that a significant component of the efficacy of cmFPA154 is attributable to its multivalent binding capacity.

MC38 Tumor Volume



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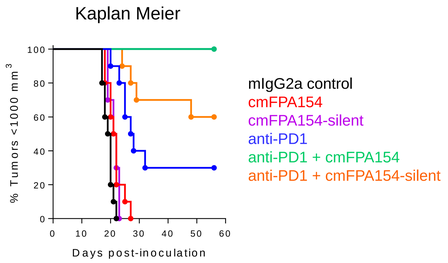

Conclusions

Tetravalent anti-GITR antibodies FPA154 and cmFPA154 demonstrate GITR agonist activity that is superior to conventional antibodies.

FPA154 and cmFPA154 can recruit ADCC activity to cells expressing high levels of GITR, such as activated or intratumoral T_{reg}.

Tumor control induced *in vivo* by cmFPA154 is accompanied by robust T cell infiltration into the tumor.

cmFPA154 demonstrates potent anti-tumor activity in combination with PD1 blockade, even in the absence of Fc effector function.

In summary, multivalent GITR agonist antibodies have potent anti-tumor activity and are a promising modality for the treatment of cancer.