Novel tetravalent anti-GITR antibody is a potent anti-tumor agent *in vivo*

#175

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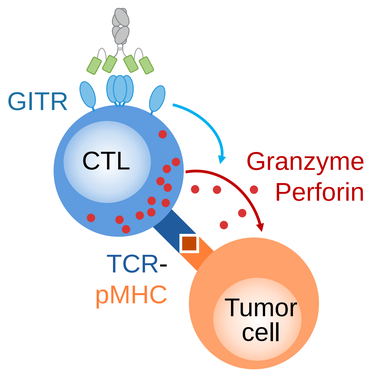
Introduction

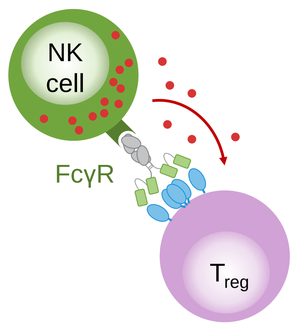
Glucocorticoid-induced TNFR-related (GITR, TNFRSF18) is a member of the TNFR superfamily with pleiotropic T cell modulatory activity. In humans and mice, GITR is expressed at high levels on effector T cells and at higher levels on intratumoral regulatory T cells (T_{reg}). Studies performed in mice suggest 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/or direct agonism of effector T cells.

We have developed a novel anti-GITR antibody with enhanced agonist activity using llama-derived single-domain antibodies (sdAbs) in a tetravalent format. A tetravalent anti-GITR agonist antibody induces NF-κB activation and effector T cell stimulation *in vitro* that is superior to a conventional bivalent antibody and confers agonist activity in the absence of Fc-mediated crosslinking. T_{reg}-depleting activity is obtained with an Fc effector-competent format. Herein we present data with a mouse-reactive surrogate molecule which provides proof of concept that a humanized version of this molecule is a promising modality for the treatment of cancer.

Fc-independent effector T cell agonism

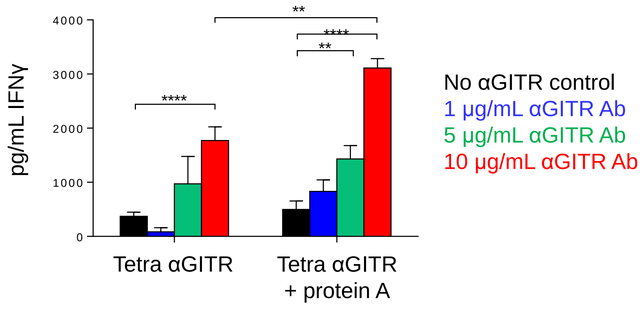


ADCC-mediated T_{reg} depletion



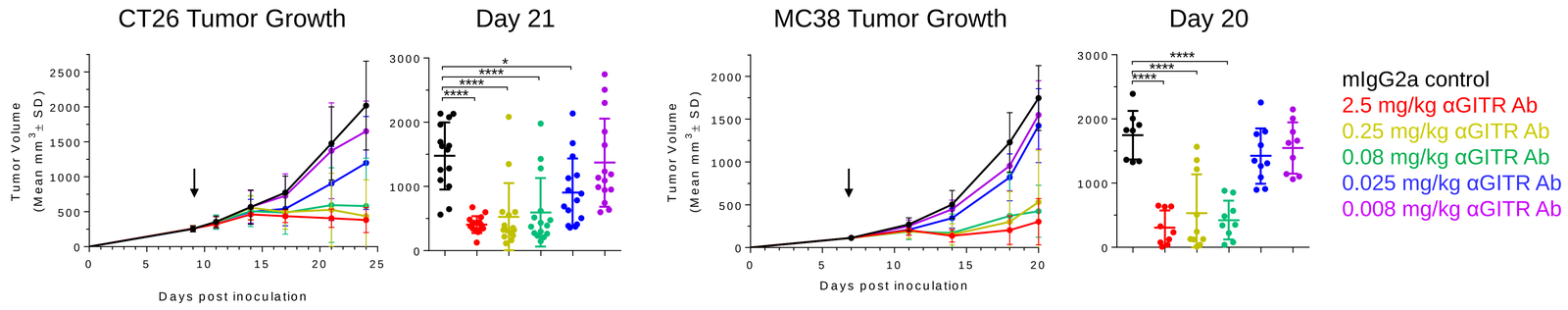
Fc-independent agonist activity

Murine CD8 T cell activation *in vitro*



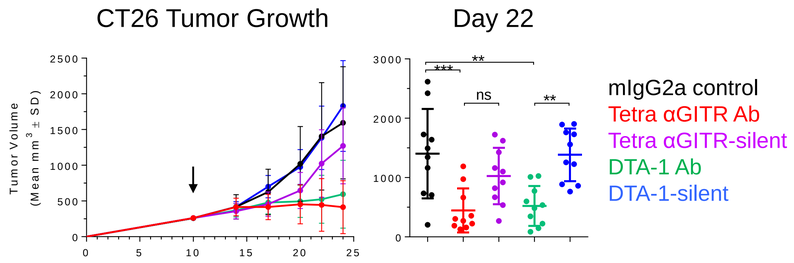
Tetravalent anti-GITR costimulates the production of IFNγ by purified murine CD8 T cells activated *in vitro* with anti-CD3. Dose-dependent activity is observed with soluble tetravalent anti-GITR and is further increased by secondary crosslinking with protein A.

Potent anti-tumor activity following a single dose of tetravalent anti-GITR



A single dose of tetravalent anti-GITR potently inhibits tumor growth control in multiple models including CT26 and MC38. Treatment is capable of inducing complete tumor rejection, and activity is observed at doses as low as 0.08 mg/kg in both models.

Role of Fc effector function *in vivo*

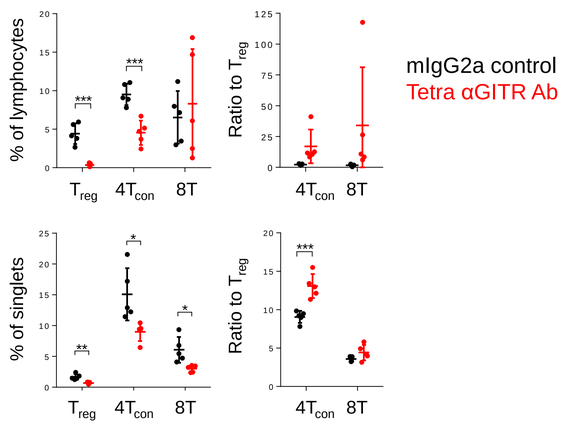


Tetravalent anti-GITR antibody retains some tumor growth inhibition activity in the absence of Fc-mediated crosslinking or effector function, whereas a conventional bivalent antibody (DTA-1) requires Fc function.

Pharmacodynamic responses

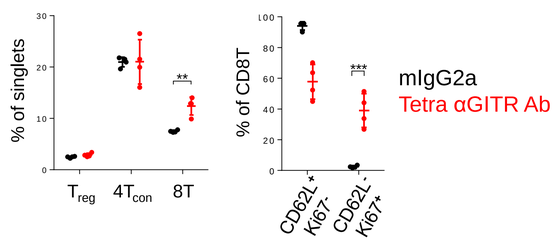
CT26 tumors
3d post-treatment

Peripheral blood
3d post-treatment



Tetravalent anti-GITR antibody treatment reduces the frequency of T cells in the peripheral blood 3 days post-treatment. In the tumor, T_{reg} and conventional CD4 T cells ($4T_{con}$) are decreased, but CD8 T cell (8T) numbers are maintained. This results in a favorable ratio of CD4 and CD8 effector T cells to T_{reg} within the tumor.

Peripheral blood
12d post-treatment

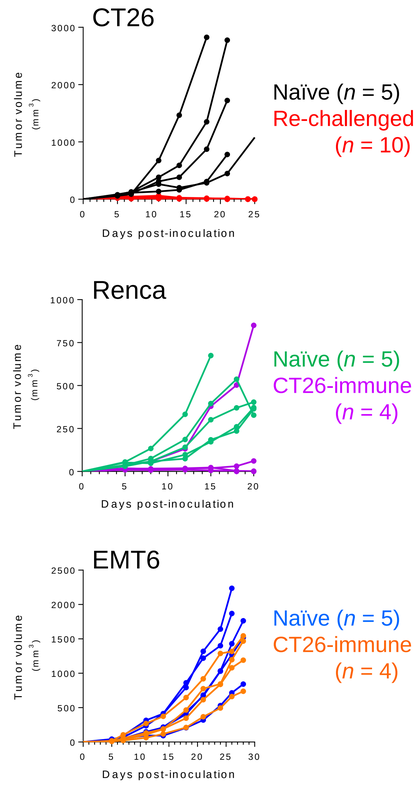


T_{reg} and CD4 T cell frequencies return to baseline in peripheral blood by 12d post-treatment, but CD8 T cells are expanded and demonstrate an activated, $Ki67^+$ phenotype.

Induction of long-term immunity

Mice that eliminated CT26 tumors in response to tetravalent anti-GITR are resistant to re-challenge. Animals also display resistance to Renca tumors, which share a T cell epitope with CT26, whereas they are not protected from antigenically-unrelated EMT6 tumors.

Resistance effects are also observed with antigenically-related and –unrelated syngeneic tumors in C57Bl/6 mice that eliminated MC38.



Conclusions

Tetravalent anti-GITR antibody potently inhibit tumor growth in mouse tumor models.

Treatment reduces T_{reg}, thereby altering the ratio to effector T cells within the tumor to create a favorable environment for an effective anti-tumor immune response.

CD8 T cell activation and proliferation is observed *in vivo*, and treatment confers long-term immunity to tumor re-challenge.

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

Statistical analysis of IFNγ production and pharmacodynamic effects was performed by 2-tailed *t*-test, and analysis of tumor volumes was performed by one-way ANOVA (* $p < 0.01$, ** $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$).