Agonistic IgM Antibodies Targeting Immunostimulatory TNFRSF Members GITR and OX40 Enhance Immune Responses beyond that of IgGs

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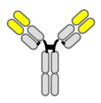
Introduction

- Tumor necrosis factor receptor (TNFR) superfamily T cell immunostimulatory receptors OX40 and GITR require trimerization to induce agonistic signaling to enhance immune responses.
- However, anti-OX40 and anti-GITR therapeutic IgG antibodies evaluated thus far have demonstrated limited anti-tumor activity in the clinic, perhaps due to inefficient multimerization through FcγR engagement in the tumor microenvironment.
- To enhance efficient multimerization of these TNFRSF agonists, we have generated anti-OX40 and anti-GITR IgM antibodies and have evaluated their *in vitro* functional properties in comparison with their corresponding IgGs.

IgM: Next generation antibody-based therapies

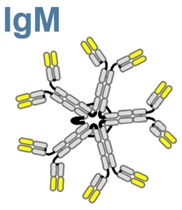
IgG



Conventional therapeutic antibodies are IgG

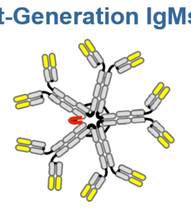
Limitations:
- Only 2 binding sites
- Difficult targets
- Rare targets
- Cross-linking receptors

IgM



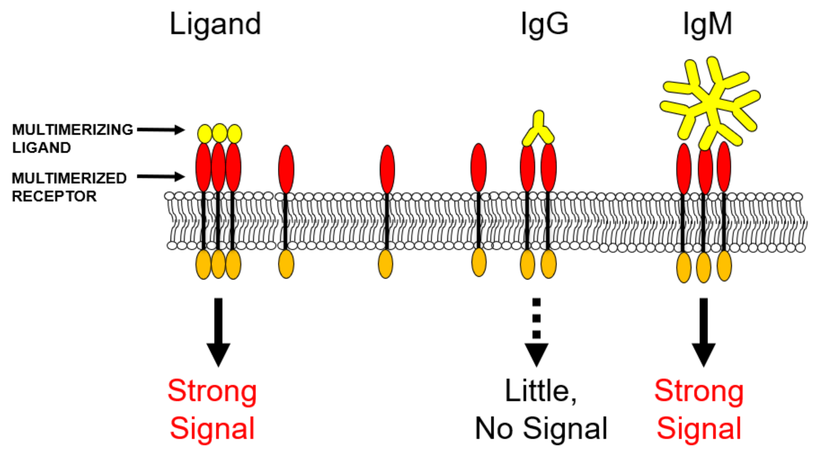
Advantages:
- 10 binding sites
- Binds difficult targets
- Binds rare targets
- Cross-links agonist receptors

Next-Generation IgMs

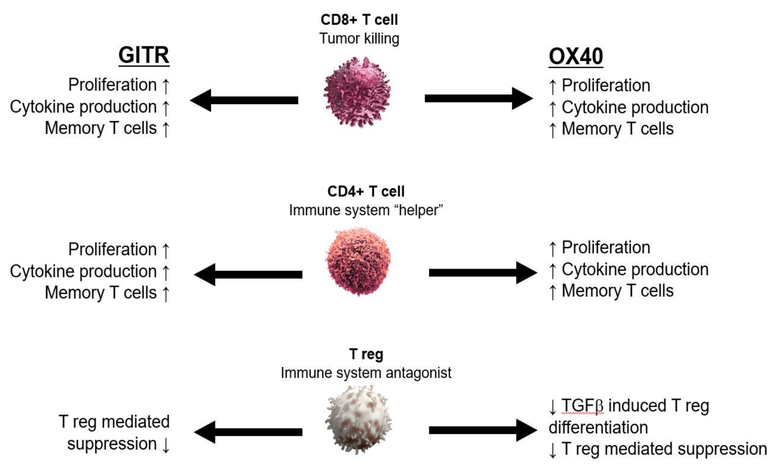


Breakthroughs:
- Improved:
  - Affinity & specificity
  - Half-life
- Bispecific format with:
  - Higher avidity
  - Better safety

IgMs enhance TNFRSF signaling by efficiently clustering receptors



Immune implications of agonizing GITR and OX40



IgM antibodies are more potent OX40 binders compared to IgG antibodies

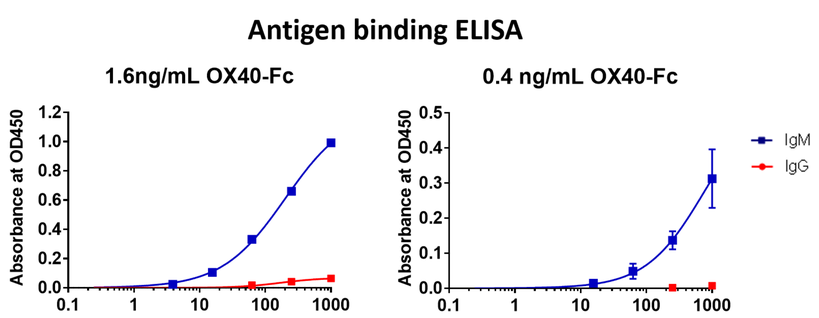


Fig 1. Recombinant human OX40 fused to an Fc was coated on a plate at 1.6 and 0.4 ng/mL and antibody binding was measured by ELISA. Anti-OX40 IgM bound with greater EC50s and to higher maximum levels through enhanced avidity than the IgG counterpart.

IgM antibodies are more potent activators of NFκB signaling than IgG in an OX40 reporter line

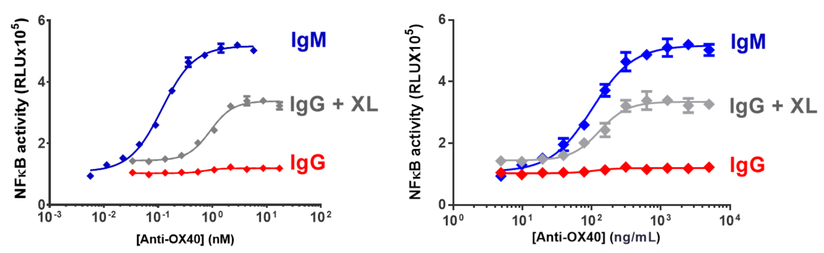


Fig 2. U2OS cells expressing full length human OX40 with an NFκB-driven luciferase reporter were incubated with different concentrations of either OX40-IgM or IgG in the presence or absence of crosslinking antibody (XL). Luciferase measurements were obtained 16 hrs after addition of the antibodies.

OX40 IgM enhances IFNγ secretion in activated CD4+ T cells above IgG

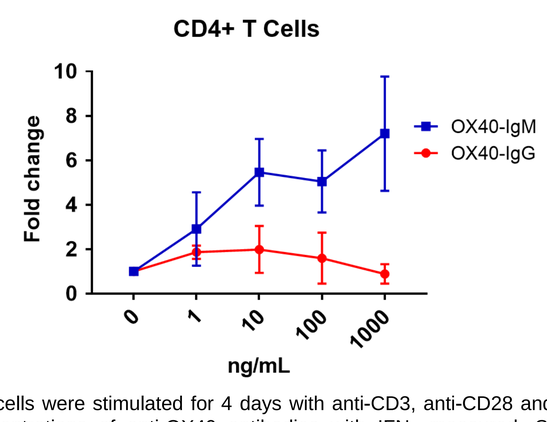


Fig 3. CD4+ T cells were stimulated for 4 days with anti-CD3, anti-CD28 and treated with increasing concentrations of anti-OX40 antibodies with IFNγ measured. Shown is fold change and SEM compared to control in 3 donors.

IgM antibodies are more potent GITR binders compared to IgG antibodies

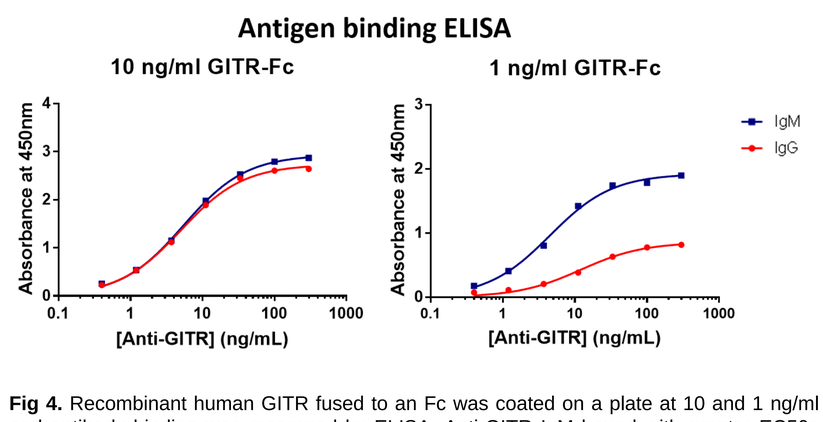


Fig 4. Recombinant human GITR fused to an Fc was coated on a plate at 10 and 1 ng/ml and antibody binding was measured by ELISA. Anti-GITR IgM bound with greater EC50s and to higher maximum levels through enhanced avidity than the IgG counterpart.

IgM antibodies are more potent activators of NFκB signaling than IgG in a GITR reporter line

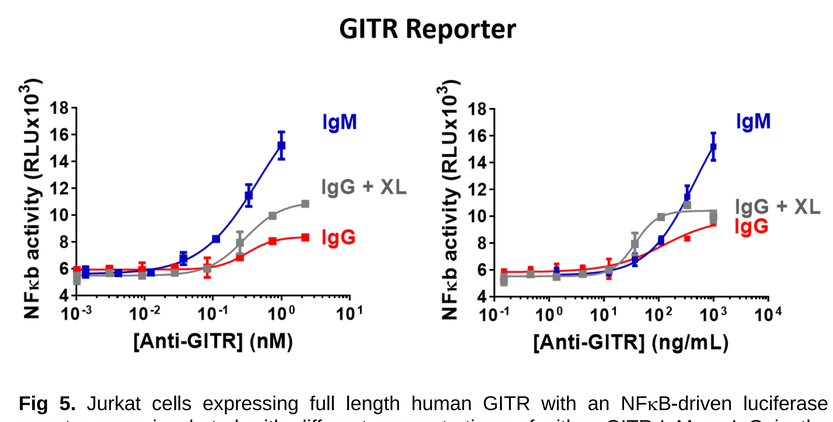


Fig 5. Jurkat cells expressing full length human GITR with an NFκB-driven luciferase reporter were incubated with different concentrations of either GITR-IgM or IgG in the presence or absence of crosslinking antibody (XL). Luciferase measurements were obtained 6 hrs after addition of the antibodies.

GITR IgM enhances inflammatory cytokine secretion in PBMCs over IgG

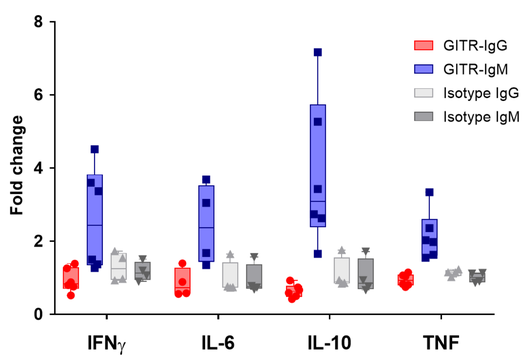


Fig 6. Human T cells in PBMCs were activated with anti-CD3 in the presence of 100 ng/ml antibody and cytokine secretion was evaluated after 5 days. Each point represents an individual donor with median, interquartile range, and minimum and maximum levels observed.

GITR IgM antibodies inhibit T-reg suppressive effects on CD4+ T cells

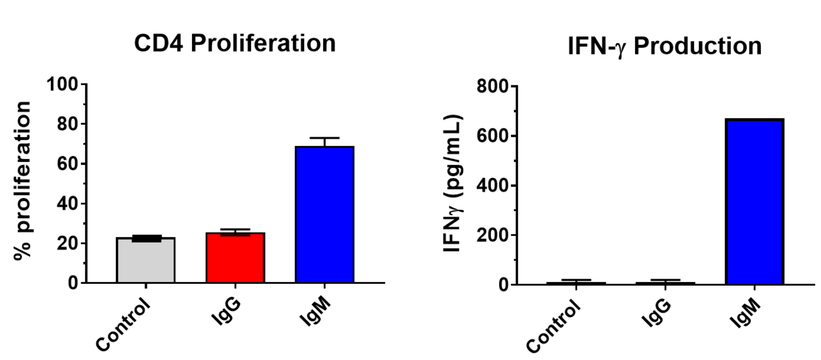


Fig 7. In vitro differentiated T-regs were incubated with CD4+ T cells in the ratio of 1:4 in the presence of anti-CD3 antibody. Anti-GITR IgM or IgG at 40 ng/ml was incubated with the co-culture and effects on proliferation (Cell Trace Violet dilution) or IFNγ secretion into the media was evaluated after 4 days. Representative data from 5 repeats.

Summary and Conclusions

- OX40 and GITR IgM antibodies:
  - Bind with high avidity to low levels of antigen
  - Signal more efficiently than IgGs in reporter assays
  - Enhance T effector cell inflammatory cytokine secretion *in vitro*
  - Inhibit regulatory T cell suppressive effects *in vitro*
- IgM antibodies targeting OX40 and GITR are promising new approaches to enhance anti-tumor immunostimulatory effects

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