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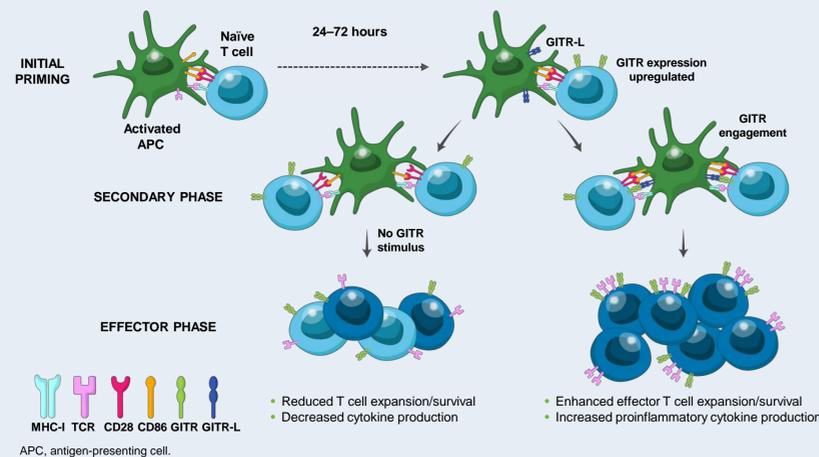
Abstract

Glucocorticoid-induced TNFR family related protein (GITR, CD357 or TNFRSF18) is a member of the tumor necrosis factor receptor superfamily (TNFRSF). Like other T cell co-stimulatory TNFR family members, GITR utilizes multiple oligomerization states to regulate the initiation of downstream signaling during T cell activation by antigen presenting cells (APCs). The formation of receptor superclusters, comprised of two or more trimeric molecules, has been defined for multiple TNFRs as a means of regulating downstream signal amplification. For co-stimulatory TNFRs, like GITR, CD137 and OX40, signaling outcomes in T cells are primarily mediated via the NFκB pathway that promotes cell survival and effector cell activities in response to suboptimal T cell receptor (TCR) stimulation. It has been hypothesized that the manipulation of the oligomeric states of co-stimulatory TNFRs using antibodies may have therapeutic utility in enhancing the activity of tumor-reactive T cells, either as single agents or in combination with other immunomodulatory or immune education strategies.

Here we describe a structure-based analysis of two functionally distinct classes of anti-human GITR antibodies that stabilize unique conformational states of the receptor. INCAGN1876, a human IgG1 monoclonal anti-GITR antibody, was found to engage a conformational epitope located within a β-turn of the extracellular domain of GITR. This antibody binding site modified the equilibrium of GITR monomer, dimer and trimers to promote receptor oligomerization, resulting in downstream NFκB signaling. Notably, this mode of INCAGN1876 receptor engagement enabled it to effectively activate the GITR pathway in recently primed T cells. By contrast, a second reference anti-GITR antibody required concomitant TCR co-engagement in order to modulate the GITR pathway. High content confocal analysis was used to evaluate the kinetics of GITR clustering by both classes of anti-GITR antibody, confirming our T cell functional analysis. The ability of INCAGN1876 to engage and effectively activate GITR on recently primed T cells may enable them to overcome suppressive features of the tumor microenvironment. Notably, INCAGN1876 was shown to promote T cell co-stimulation both as a single agent and in combination with other antibodies targeting PD-1, CTLA-4 and OX40. Finally, we compared the pharmacologic activity of INCAGN1876 to Fc variants of this antibody with diminished binding to the inhibitory Fcγ receptor (FcγR), CD32B. The superiority of an IgG1 antibody in these assays was consistent with the potential to achieve optimal GITR clustering by FcγRs, while maintaining the potential for FcγR-mediated effector cell activity directed toward intratumoral GITR^{high} regulatory T cells. INCAGN1876 is currently under evaluation in Phase 1/2 studies in subjects with advanced metastatic solid tumors (NCT02697591).

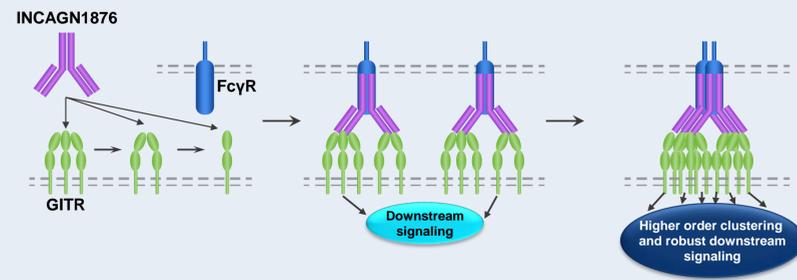
The GITR Pathway Promotes T Cell Co-stimulation

Paradigm: GITR signaling in the context of TCR activation enhances effector T cell activation, cytokine production, and survival (modified from ref. 1).

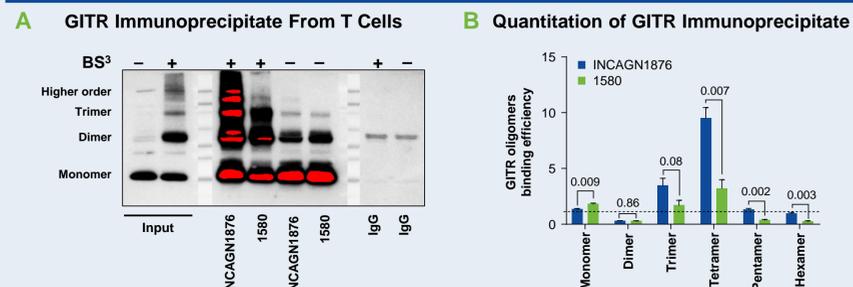


Fcγ Receptors Facilitate GITR Clustering by INCAGN1876

Hypothesis: INCAGN1876 binds to GITR expressed on the surface of recently activated T cells and facilitates GITR clustering via Fcγ receptor (FcγR) interaction mediating higher order receptor clustering and downstream signaling (modified from ref. 3).

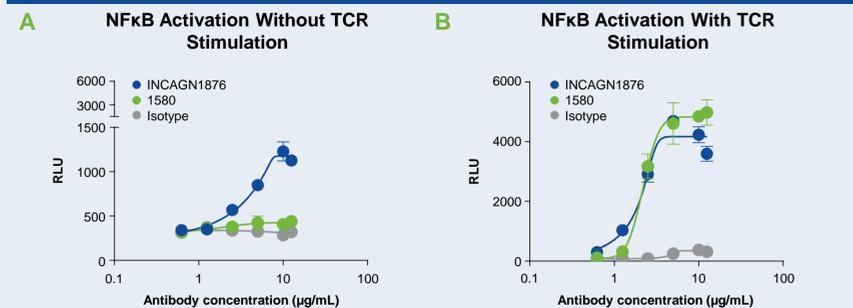


INCAGN1876 Favors Binding to Higher Order GITR Complexes



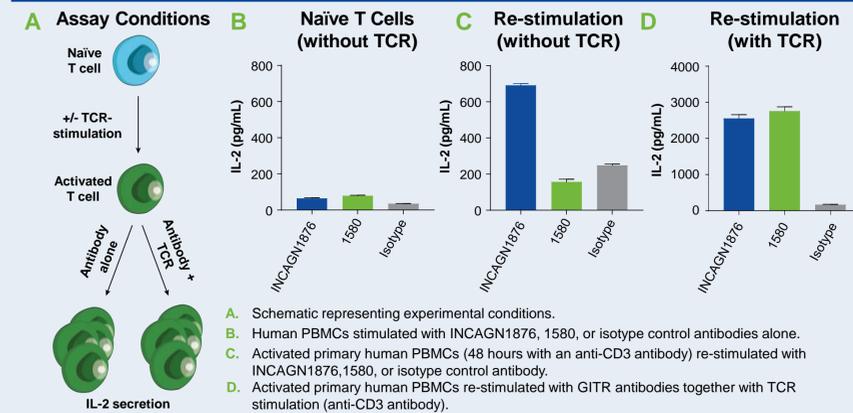
- A.** GITR-expressing T cells (Jurkat) were treated with or without bis(sulfosuccinimidyl)suberate (BS³), lysed, and immunoprecipitated using GITR antibodies (either INCAGN1876 or 1580 [a reference GITR antibody]) versus pull-down with an isotype control antibody. Samples were separated by SDS-PAGE, transferred to nitrocellulose, and blotted for detection of GITR.
- B.** Western blot signal was normalized within a lane and within the cell lysate fraction. Significant *P* values were calculated with the 2-stage step-up method of Benjamini, Krieger, and Yekutieli.⁵ Each column represents *n* = 3 individual experiments and error bars represent standard deviation.⁵

INCAGN1876 Promotes GITR Signaling in Recently Activated T Cells, Including in the Absence of Concomitant TCR Stimulation

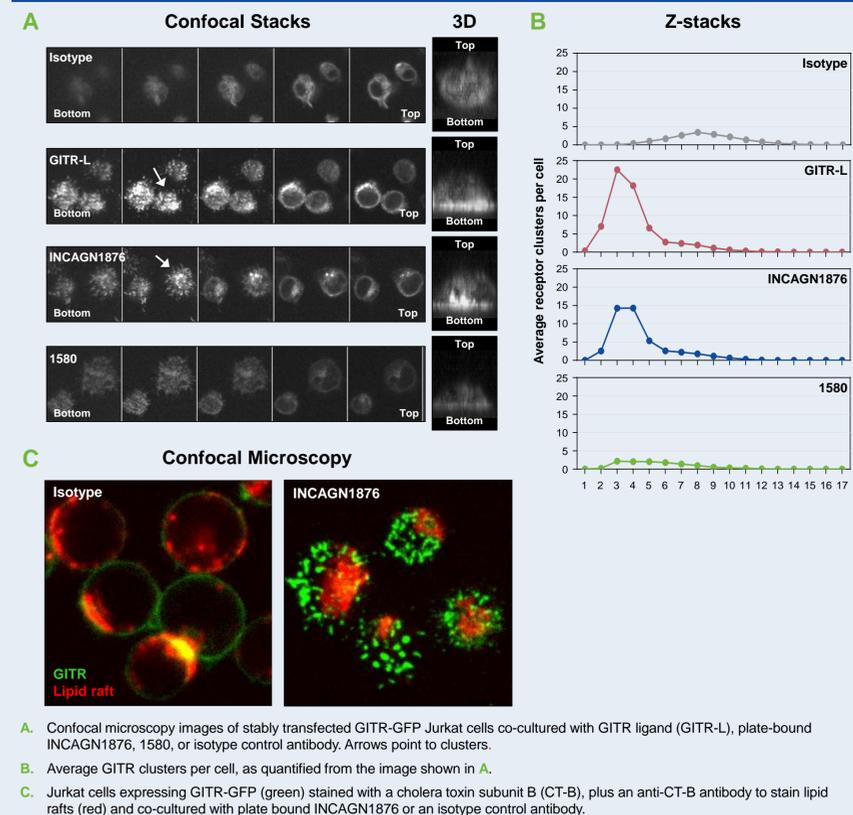


- A.** GITR signaling (NFκB activation) (relative light units [RLU]) mediated by Fc crosslinked INCAGN1876, 1580, or an isotype control antibody in the absence of TCR (anti-CD3 antibody) stimulation, as measured using a reporter assay.
- B.** NFκB activation by cross-linked INCAGN1876, 1580, or an isotype control antibody with concomitant TCR stimulation.

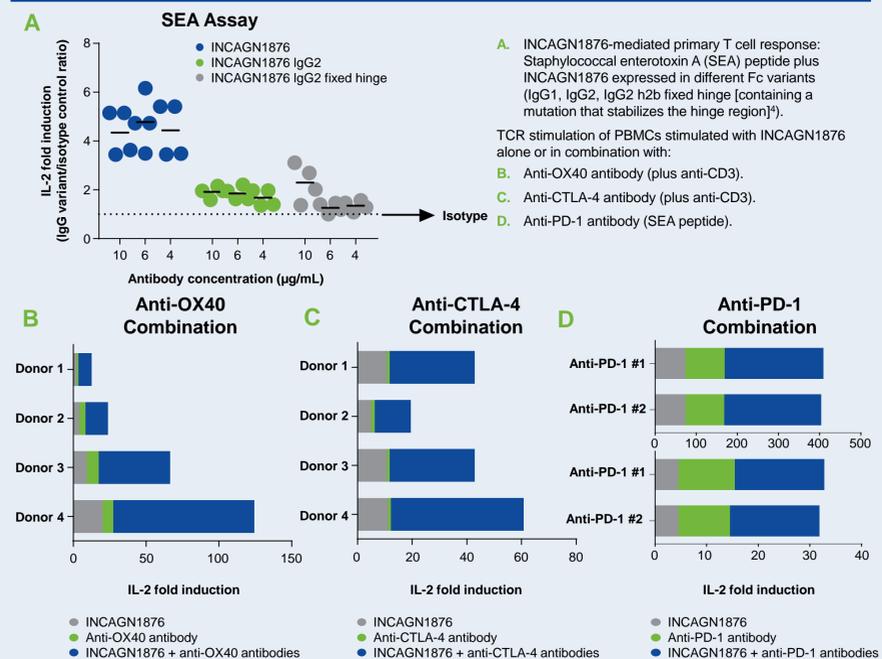
INCAGN1876 Promotes GITR Signaling in Recently Activated T Cells, Including in the Absence of Concomitant TCR Stimulation



INCAGN1876 Mediates GITR Clustering on the Surface of Cells That Correlates With T Cell Activation



INCAGN1876 Demonstrates Increased Activity as an IgG1 Fc and Cooperates With Other Immunomodulatory Antibodies



Summary

- INCAGN1876 preferentially binds to dimers, trimers, and higher order GITR complexes, as compared with reference antibody
- INCAGN1876 promotes GITR forward signaling in recently activated T cells in the presence and absence of concomitant TCR stimulation
- INCAGN1876 efficiently promotes the formation of GITR clusters on the surface of cells that correlates with GITR forward signaling
- INCAGN1876 functions optimally on an IgG1 Fc backbone, and combines with anti-OX40, anti-CTLA-4, or anti-PD-1 antibodies to enhance T cell cytokine production
- INCAGN1876 has at least 3 potential mechanisms of action:
 - Promote tumor-specific T cell priming in the context of APCs alone or in combination with other immunomodulatory antibodies
 - Enhance recently activated tumor-specific T cell function in the absence of APCs
 - Selectively depletes intratumoral populations of activated regulatory T cells

References

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Author Disclosures

Ana Gonzalez, Ekaterina Breous, Mariana Manrique, Lukasz Swiech, Thomas Horn, Jeremy Waight, Yuqi Liu, Shiwen Lin, Olivier Léger, Dennis Underwood, Volker Seibert, Marc van Dijk, Jennifer Buell, Robert Stein, Nicholas S Wilson: Agenu Inc.; Employment/consultancy and Stock Ownership. Kevin Heller, Kimberli Brill, Reid Huber, Peggy Scherle, Gregory Hollis: Incyte Corporation; Employment and Stock Ownership. Taha Merghoub, David Schaefer, Roberta Zappasodi, Gerd Ritter: Nothing to disclose.

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