3204

Presented at the
American Association for Cancer Research Annual Meeting 2016
New Orleans, LA, USA • April 16–20, 2016

INCAGN01949: A Novel Anti-OX40 Agonist Antibody With the Potential to Enhance Tumor Specific T-cell Responsiveness, While Selectively Depleting Intratumoral Regulatory T Cells

Ana Gonzalez,[1] Mariana Manrique,[1] Ekaterina Breous,[1,2] David Savitsky,[1] Jeremy Waight,[1] Randi Gombos,[1] Yuqi Liu,[1] Shiwen Lin,[1] Taha Merghoub,[3] Daniel Hirschhorn-Cymerman,[3] Gerd Ritter,[4] Jedd Wolchok,[3,4] Reid Huber,[5] Peggy Scherle,[5] Gregory Hollis,[5] Marc van Dijk,[1,2] Robert Stein,[1] and Nicholas S. Wilson[1]

[1]Agenus Inc., Lexington MA; [2]4-Antibody AG, Basel, Switzerland; [3]Memorial Sloan Kettering Cancer Center, New York, NY; [4]The Ludwig Institute for Cancer Research, New York, NY; [5]Incyte Corporation, Wilmington, DE

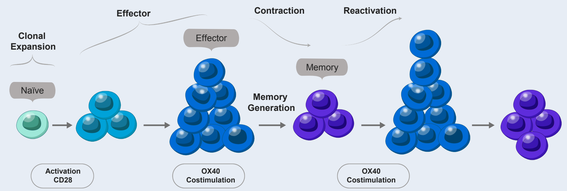
Abstract

OX40 (CD134, TNFRSF4) is a T cell costimulatory receptor that can potentiate T cell receptor (TCR) signaling during CD4⁺ and CD8⁺ T cell priming, effector cell differentiation and memory T cell recall responses. In preclinical mouse tumor models, surrogate anti-OX40 agonist antibodies have shown remarkable single agent anti-tumor efficacy, as well as the ability to combine effectively with other immunomodulatory antibodies and immune education strategies, such as therapeutic cancer vaccines. Agonistic antibodies targeting OX40 are predicted to counteract the immunosuppressive tumor microenvironment and promote tumor-specific T cell immunity via two primary mechanisms: 1) binding and activating OX40 signaling in tumor-specific effector and memory T cells, thereby enhancing their responsiveness to tumor-associated antigens, and 2) co-engaging Fcγ receptors expressed by tumor-associated effector cells, and facilitating the selective depletion of intratumoral regulatory T cells.

INCAGN01949 is a fully human IgG1 monoclonal antibody identified using the Retrocyte Display™ platform and is being developed for the treatment of advanced malignancies. INCAGN01949 specifically binds to human and cynomolgus monkey OX40 with similar affinity. INCAGN01949 has been optimized to mediate receptor forward signaling under suboptimal TCR stimulatory conditions, leading to enhanced production of TNFα and IFNγ and decreased production of IL-10. INCAGN01949 achieves this functionality by virtue of its ability to facilitate OX40 clustering and downstream activation of the NFκB pathway in T cells across a broad range of antibody concentrations. Consistent with mouse preclinical tumor models, OX40 was found to be selectively overexpressed by intratumoral regulatory T cells in a range of human tumor types. Commensurate with its human IgG1 Fc region, INCAGN01949 was shown to effectively co-engage activating Fcγ receptors on immune effector cells, including natural killer cells and macrophages, to mediate ADCC and/or ADCP activities. Taken together, the biophysical and functional attributes of INCAGN01949 make it ideally suited for clinical development, both as a single agent and in combination with other immunomodulatory antibodies or immune education strategies.

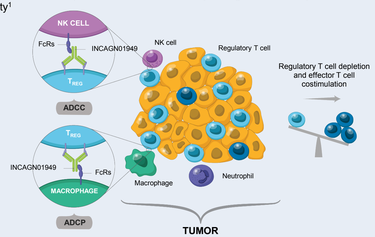
Mechanism 1: OX40 Forward Signaling in T Cells

Paradigm: OX40 signaling in the context of TCR activation enhances effector T cell activation, cytokine production and survival, promoting memory T cell differentiation and reactivation

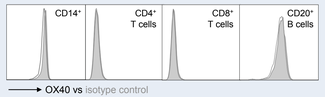


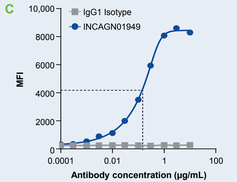
Mechanism 2: Intratumoral Depletion of Treg Cells

Paradigm: Anti-OX40 antibodies promote anti-tumor immunity through selective depletion of intratumoral regulatory T cell (Treg) activity[1]



INCAGN01949 Demonstrates Affinity for OX40 and Recognizes Primary Activated T Cells



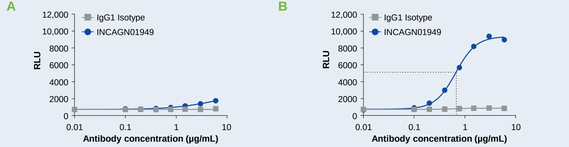


D

Human OX40	K_a (1/Ms)	1.09×10^{6}
	K_d (1/s)	1.26×10^{-4}
	K_D (nM)	0.11

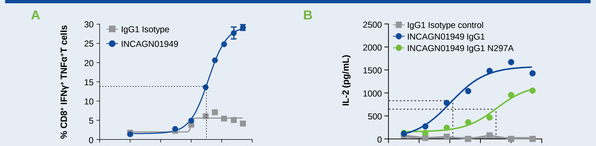
A. INCAGN01949 does not bind to quiescent immune cells (flow cytometry).
B. INCAGN01949 binds to stimulated (anti-CD3 antibody) T cells.
C. Dose titration of INCAGN01949 binding to activated (anti-CD3 antibody) T cells.
D. Affinity of INCAGN01949 by Bio-layer Interferometry.

INCAGN01949 Mediates Dose-Dependent Signal Transduction in T Cells



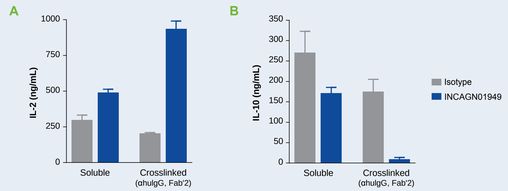
A. NFκB activation (relative light units [RLU]) by soluble INCAGN01949 or an isotype control antibody in OX40 reporter cells.
B. NFκB activation by cross-linked INCAGN01949 or an isotype control antibody in OX40 reporter cells.

INCAGN01949 Enhances Primary T Cell Function Across a Broad Concentration Range



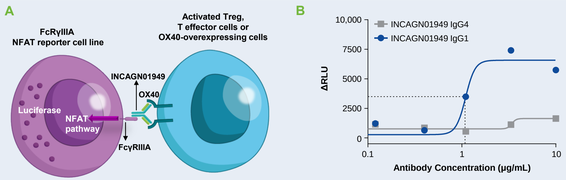
A. Intracellular cytokine (IFNγ and TNFα) readout using flow cytometry post-anti-CD3 antibody stimulation and plate bound (complexed) INCAGN01949 or a matched isotype control stimulation.
B. IL-2 secretion by human primary T cells in the presence of superantigen and INCAGN01949 IgG1, INCAGN01949 aglycosylated IgG1 (N297A), or isotype control.

INCAGN01949 Enhances IL-2 and Reduces IL-10 in Primary Treg:Teff Co-culture Assays



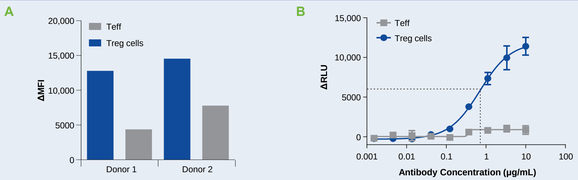
Human Treg cells (CD4⁺ $CD25^{high}$, $CD127^{-}$) were activated with anti-CD2/anti-CD3/anti-CD28 beads for 48 h. Human T cells were isolated (MACs sorted by negative selection) and co-cultured with activated Treg cells (1 Teff:3 Treg) for 4 days in the presence of anti-CD2/anti-CD3/anti-CD28 beads, soluble or crosslinked (with a Fab'2 anti-human Fc) INCAGN01949 or an IgG1 isotype control. Culture supernatants were analyzed for (A) IL-2 and (B) IL-10 levels.

INCAGN01949-Dependent Signal Transduction in FcγRIIIA Reporter Cell Assay Requires IgG1 Isotype



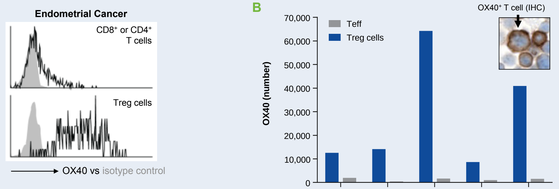
A. Schematic for the FcγRIIIA reporter based cell assay.
B. A titration of INCAGN01949 on an IgG1 or IgG4 backbone were added to co-cultures of FcγRIIIA-expressing reporter cells and OX40⁺ recombinant cells. The delta response light units ([ΔRLU] isotype control values deducted) is shown.

INCAGN01949 Preferentially Binds to Treg Cells and Induces FcγRIIIA Receptor–Dependent Signaling



A. Activated human Treg cells versus T effector cell (Teff) OX40 expression by flow cytometry in 2 different donors. Delta mean fluorescence intensity (ΔMFI) was calculated by deducting the MFI IgG1 isotype values.
B. A titration of INCAGN01949 was added to co-cultures of FcγRIIIA-expressing reporter cells, activated human Treg cells or T effector cells. The ΔRLU is shown.

OX40 is Selectively Expressed by Intratumoral Regulatory T Cells From Multiple Tumor Types



C

Indication	Samples (n)	CD4⁺ T cells	Treg cells
NSCLC	4	+/–	++++
Endometrial	2	+/–	++++
Colorectal	2	–	+
Breast	2	–	++
Ovarian	1	–	+++
Renal	1	–	+

Note: – = negative/no expression, + = weak expression, ++ = moderate expression, +++ = moderate-to-high expression, ++++ = high expression.
CRC, colorectal carcinoma; NSCLC, non-small cell lung carcinoma; RCC, renal cell carcinoma.

A. Representative histograms of OX40 expression from tumor-associated T effector cells (Teff) or Treg cells.
B. OX40 expression across various tumor types.
C. Tabulated summary of OX40 expression by tumor-associated T effector cells and Treg cells.

Conclusions

- **INCAGN01949 IgG1 binds to human OX40 with an estimated affinity (K_D) of 0.11 nM and recognizes OX40 on activated human T cells in a dose dependent manner**
- **INCAGN01949 IgG1 functions as an OX40 agonist antibody in human T cells, activating NFκB signaling and providing T cell costimulation in the context of suboptimal TCR activation**
- **INCAGN01949 IgG1 prevents in vitro Treg cell suppression of T effector cells by inhibiting IL-10 production and results in enhanced IL-2 secretion**
- **INCAGN01949 preferentially labeled activated human primary Tregs as compared to T effector cells, co-engaging FcγRIIIA and mediating its activation in reporter cell assays**
- **Samples from multiple tumor types (ovarian cancer, colorectal carcinoma, endometrial carcinoma, renal cell carcinoma, and non-small cell lung carcinoma) demonstrate an enrichment in $OX40^{high}$ intratumoral regulatory T cells**
- **INCAGN01949 IgG1 functions as effective agonist of the OX40 pathway, and may have the potential in patients to enhance T cell responsiveness to weakly immunogenic tumor-associated antigens, while attenuating the immune suppressive function of intratumoral populations of regulatory T cells. Taken together, these preclinical data support the clinical development of INCAGN01949**

References

1. Smyth MJ, et al. *Immunol Cell Biol*. 2014;92:473–474.
2. Martinet L, and Smyth M. *Nat Rev Immunol*. 2014;15:243–254.
3. Godfrey WR, et al. *J Exp Med*. 1994;180:757–762.
4. Bulliard Y, et al. *J Exp Med*. 2013;210:1685–1693.
5. Weinberg ID, et al. *Immunol Rev*. 2011;244:218–231.
6. Croft M. *Ann Rev Imm*. 2010;28:57–78.
7. Voo KS, et al. *J Immunol*. 2013;191:3641–3650.
8. Piconese S, et al. *J Exp Med*. 2008;205:825–839.
9. Bulliard Y, et al. *Immunol Cell Biol*. 2013;92:475–480.
10. Nimmerjahn F and Ravetch JV. *Curr Opin Immunol*. 2007;19:239–245.

Author Disclosures

Ana Gonzalez, Mariana Manrique, Ekaterina Breous, David Savitsky, Jeremy Waight, Randi Gombos, Yuqi Liu, Shiwen Lin, Robert Stein, Marc van Dijk, Nicholas S. Wilson: *Agenus Inc:* Employment and Stock Ownership. **Reid Huber, Peggy Scherle, Gregory Hollis:** *Incyte Corporation:* Employment and Stock Ownership. **Taha Merghoub, Daniel Hirschhorn-Cymerman, Gerd Ritter, Jedd Wolchok:** Nothing to disclose.

Acknowledgements

The authors would like to thank Rebecca Woelfle for her assistance in preparing poster material and Joseph Connolly and Zhenyu Li for their help characterizing and producing INCAGN01949.
Layout and printing support was provided by Evidence Scientific Solutions, Philadelphia, PA, funded by Incyte Corporation.

Scan code to download a copy of this poster or visit: http://bit.ly/1SbFlqa