

INCAGN1949, an Anti-OX40 Antibody With an Optimal Agonistic Profile and the Ability to Selectively Deplete Intratumoral Regulatory T Cells

Ana Gonzalez,¹ Mariana Manrique,¹ Lukasz Swiech,¹ Thomas Horn,¹ Ekaterina Breous,^{1,2} Jeremy Waight,¹ David Savitsky,¹ Yuqi Liu,¹ Shiwen Lin,¹ Christopher Clarke,¹ Taha Merghoub,³ Daniel Hirschhorn-Cymerman,³ David Schaer,³ Gerd Ritter,⁴ Jennifer Pulini,⁵ Kevin Heller,⁵ Peggy Scherle,⁵ Gregory Hollis,⁵ Reid Huber,⁵ Marc van Dijk,^{1,2} Jennifer Buell,¹ Robert Stein,¹ and Nicholas S. Wilson¹

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

Abstract

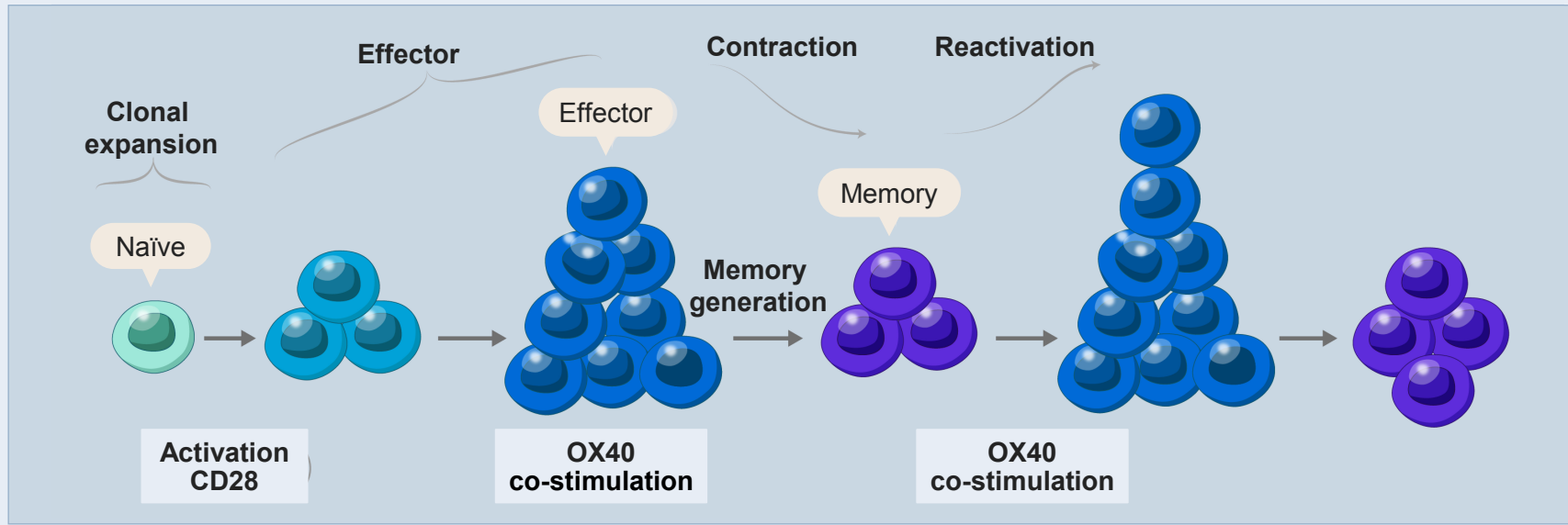
OX40 is a T cell co-stimulatory receptor that can enhance the magnitude and durability of T cell immune responses. Anti-OX40 agonist antibodies have shown significant single agent tumoricidal activity in preclinical models, and can combine effectively with other immunomodulatory antibodies, targeted therapies and vaccines. OX40 agonists are able to counteract the immunosuppressive tumor microenvironment and promote tumor-specific cellular immunity via at least two distinct mechanisms: 1) promoting OX40 forward signaling in tumor-specific T cells; and 2) co-engaging Fc receptors expressed by tumor-associated effector cells, and facilitating the selective elimination of OX40^{high} intratumoral regulatory T cells.

INCAGN1949, an anti-OX40 human IgG1 antibody, was selected based on its ability to optimally enhance T cell responsiveness under conditions of suboptimal T cell receptor stimulation. INCAGN1949 was shown to mediate effective apical OX40 clustering that is translated into effective downstream activation of the NFκB pathway. Notably, INCAGN1949 was shown to maintain a sigmoidal dose response curve across a broad range of antibody concentrations. This suggests a wide therapeutic window and may be advantageous for dosing considerations. By contrast, evaluation of reference OX40 antibodies indicated an inverted U-shaped dose response curve, leading to impaired T cell responses at high concentrations. INCAGN1949 was selected for clinical development based on its optimal agonist profile, further reinforced by its ability to combine with other co-inhibitory and co-stimulatory antibodies to augment T cell responsiveness. Prior to human testing, the pharmacology and tolerability of INCAGN1949 was evaluated in non-human primates (NHPs). Pharmacokinetic (PK) and pharmacodynamic (PD) parameters were evaluated including longitudinal measurements of serum cytokines, immune cell populations, activation state and T cell-mediated immune responses to reporter vaccine antigens. INCAGN1949 exhibited a linear PK profile and was well tolerated at all doses tested, with no maximum tolerated dose established. Co-administration of INCAGN1949 and vaccines in NHPs showed an immune-based PD signature across a broad exposure range. These studies were in line with *in vitro* findings and support a wide PD range for INCAGN1949 in patients. An important secondary mechanism of INCAGN1949 is the ability of its IgG1 Fc region to mediate selective depletion of OX40^{high} intratumoral regulatory T cells. Immunohistochemistry and flow cytometry analyses support the validity of this regulatory T cell depletion mechanism in a range of tumors.

The functional *in vitro* and *in vivo* attributes of INCAGN1949 make it suitable for clinical development. It is currently under evaluation in a Phase 1/2 study in subjects with advanced or metastatic tumors (NCT02923349).

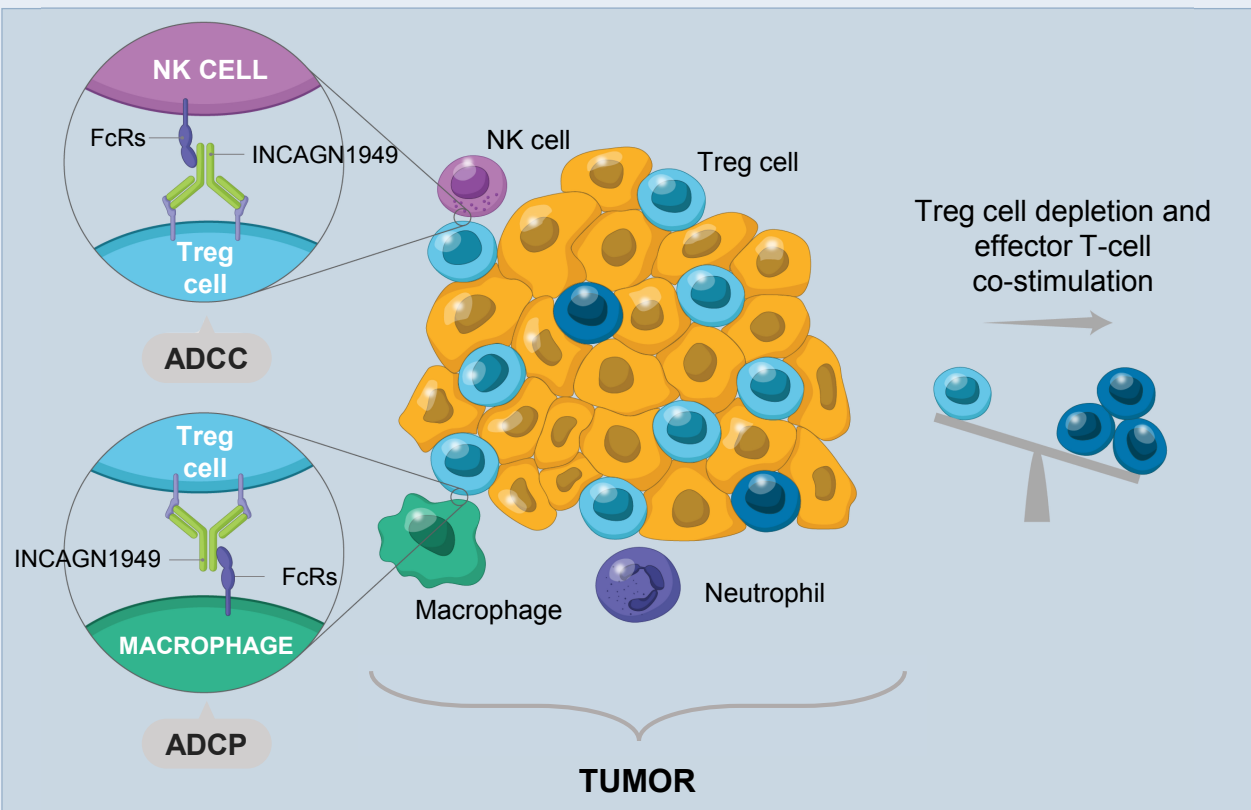
Mechanism 1: OX40 Forward Signaling in Activated T Cells

Mechanism 1: Anti-OX40 antibodies mediate receptor forward signaling in the context of T cell antigen receptor (TCR) activation, enhance effector T cell activation, cytokine production, and survival, as well as promote memory T cell differentiation and reactivation (modified from ref. 1).

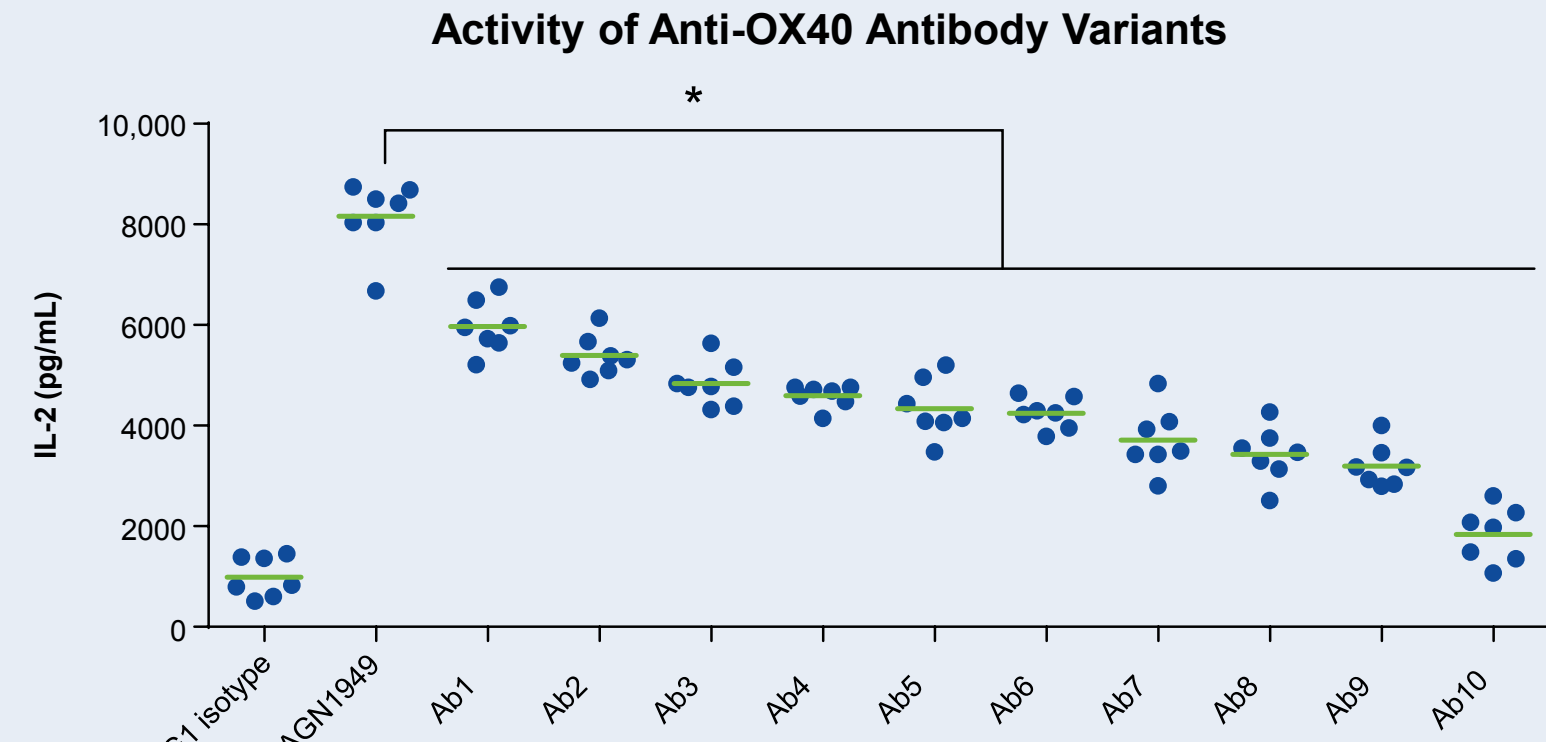


Mechanism 2: Intratumoral Depletion of Treg Cells

Mechanism 2: Anti-OX40 antibodies mediate the selective depletion of intratumoral regulatory T cells (Treg cell), thereby promoting anti-tumor activity (modified from ref. 2).

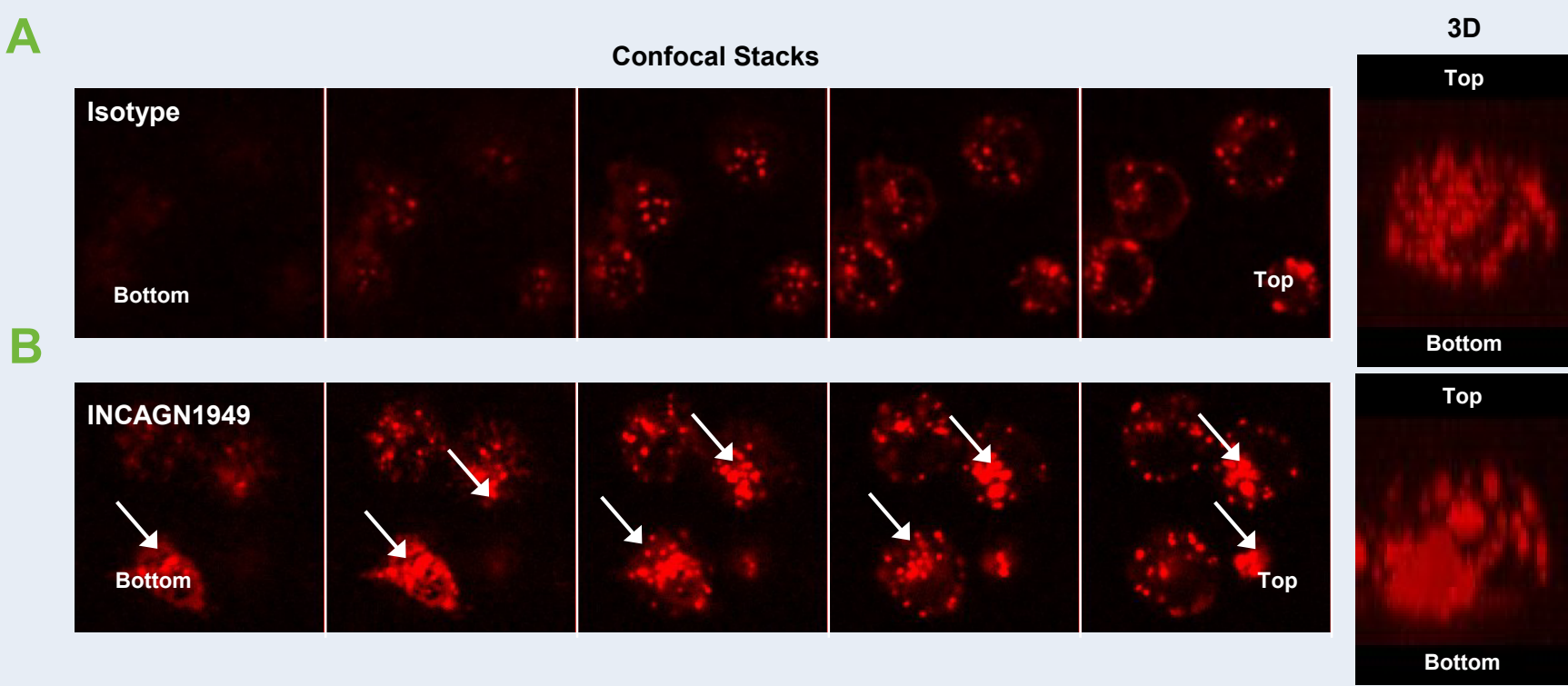


INCAGN1949 Demonstrates Increased Activation of Primary T Cells



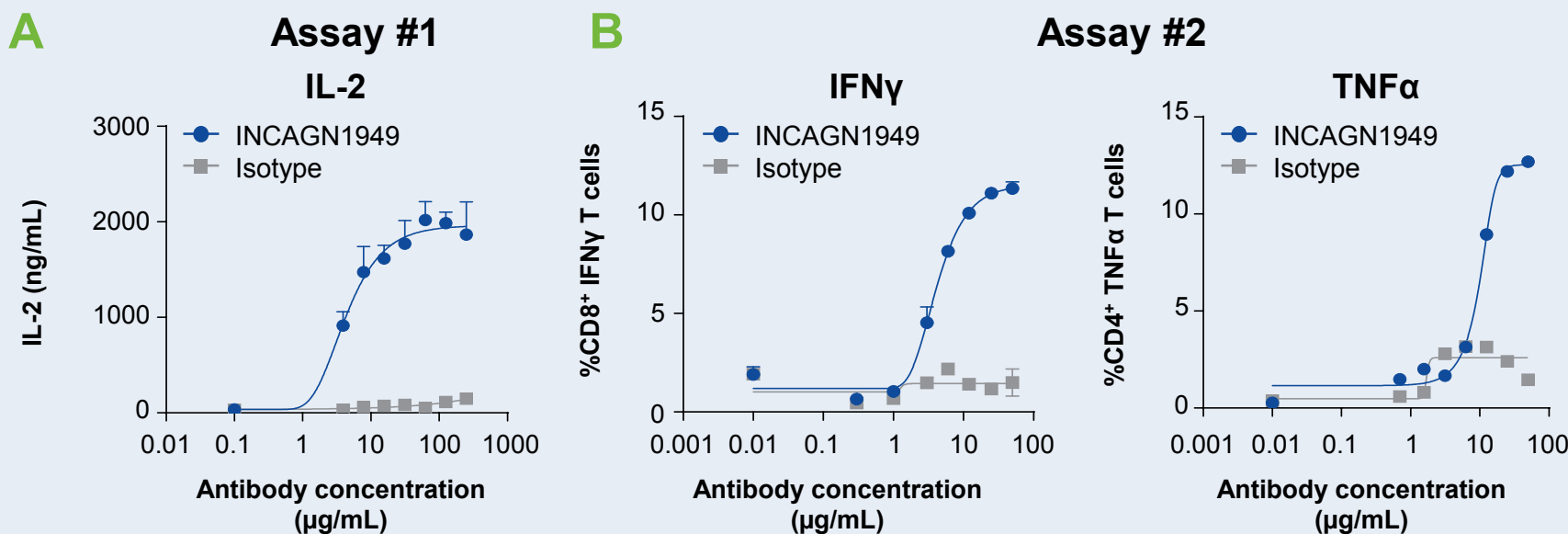
IL-2 secretion by human primary T cells in the presence of superantigen and INCAGN1949 as compared to other anti-OX40 antibody variants (Ab1-Ab10) or isotype control (* significant $P<0.05$, Mann-Whitney test).

INCAGN1949 Mediates Effective OX40 Clustering



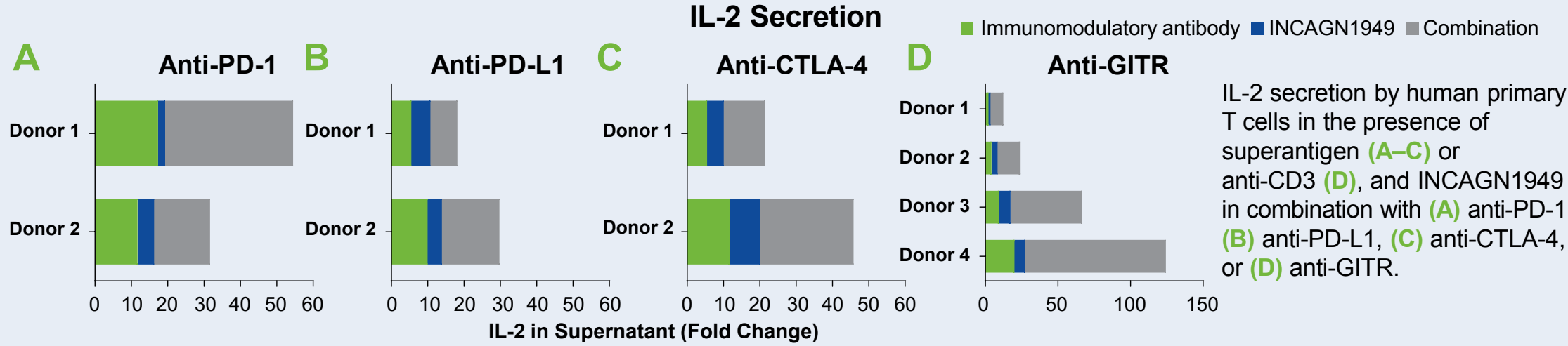
OX40-mCherry stably transfected Jurkat cells were co-cultured with plate-bound isotype control (A) or INCAGN1949 antibody (B). Arrows point to OX40 clustering. Confocal stack images are shown.

INCAGN1949 Enhances Primary T Cell Function Across a Broad Range of Concentrations

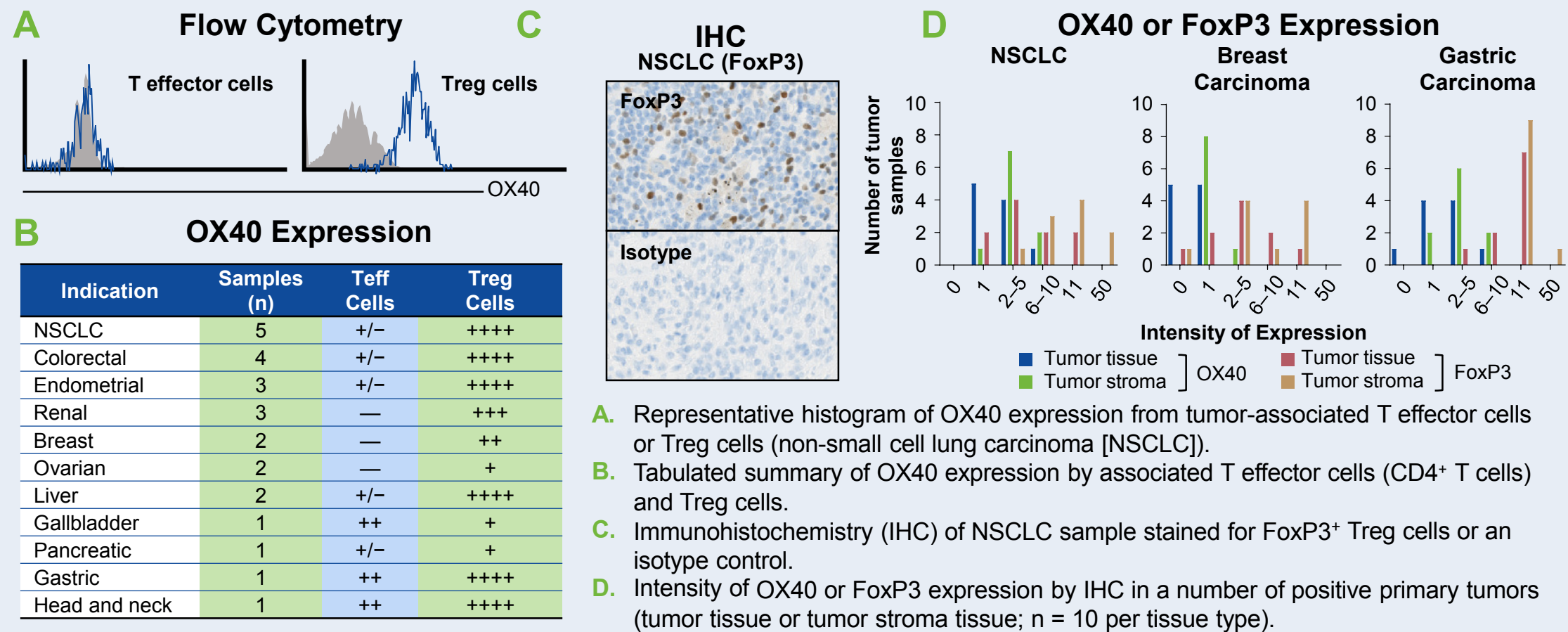


- A. IL-2 secretion by human primary T cells in the presence of superantigen (Assay #1) and INCAGN1949 or isotype control (representative of n = 8 healthy donors).
- B. Intracellular cytokine (IFNγ and TNFα) readout using flow cytometry post-anti-CD3 antibody stimulation (Assay #2) and plate bound INCAGN1949 or isotype control (representative of n = 10 healthy donors).

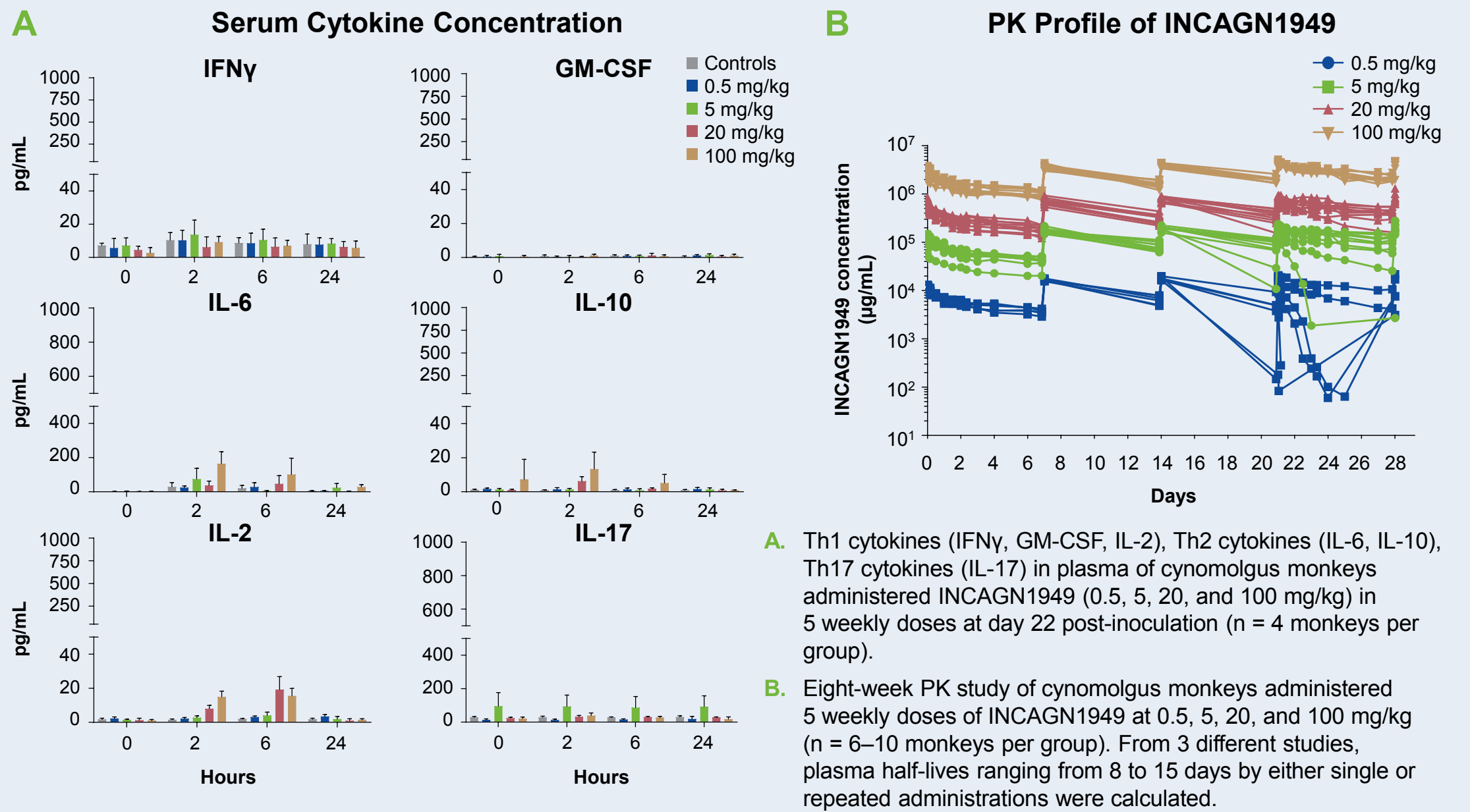
INCAGN1949 Cooperates With Other Immunomodulatory Antibodies to Enhance T Cell Function



OX40 Is Selectively Expressed by Intratumoral Treg Cells in a Range of Primary Tumors

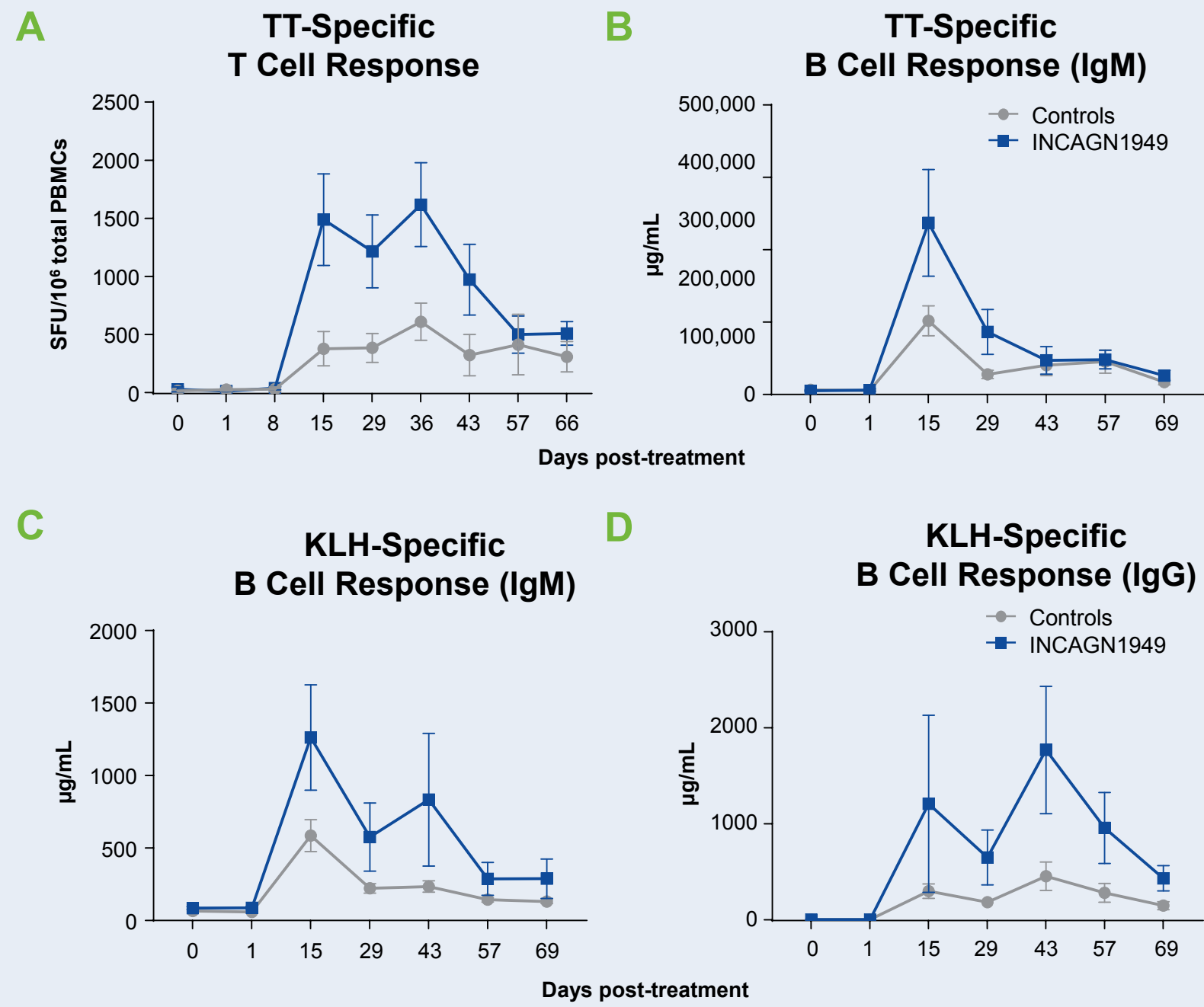


INCAGN1949 Is Well Tolerated *In Vivo* and Demonstrates a Linear PK Profile in Cynomolgus Monkeys



- A. Th1 cytokines (IFNγ, GM-CSF, IL-2), Th2 cytokines (IL-6, IL-10), Th17 cytokines (IL-17) in plasma of cynomolgus monkeys administered INCAGN1949 (0.5, 5, 20, and 100 mg/kg) in 5 weekly doses at day 22 post-inoculation (n = 4 monkeys per group).
- B. Eight-week PK study of cynomolgus monkeys administered 5 weekly doses of INCAGN1949 at 0.5, 5, 20, and 100 mg/kg (n = 6–10 monkeys per group). From 3 different studies, plasma half-lives ranging from 8 to 15 days by either single or repeated administrations were calculated.

T Cell-Dependent Antibody Response (TDAR) in Cynomolgus Monkeys After Co-administration of Vaccines and INCAGN1949



Cynomolgus monkeys were vaccinated against tetanus toxoid (TT) or keyhole limpet hemocyanin (KLH) and administered 5 mg/kg INCAGN1949 or vehicle at day 1 and day 29; a vaccination booster was also given at day 29 (n = 6 monkeys per group). (A) TT-specific IFNγ T cell responses; (B) TT-specific IgM antibody responses; (C) KLH-specific IgM antibody responses; and (D) KLH-specific IgG antibody responses.

Summary

- INCAGN1949 shows an optimal agonist profile across a broad dose range, which is mediated by its ability to engage and cluster OX40 on T cells
- INCAGN1949 cooperates with other immunomodulatory antibodies (both agonist and antagonist) to enhance T cell responsiveness
- Primary human tumors contain populations of FOXP3-expressing OX40^{high} regulatory T cells, with the potential to be selectively depleted by the IgG1 Fc region of INCAGN1949
- INCAGN1949 has a linear PK profile in non-human primates and was well tolerated
- INCAGN1949 co-administered with reporter vaccines in non-human primates promotes enhanced T cell-mediated B cell immune responses
- INCAGN1949 is an effective agonist of the OX40 pathway and has confirmed immunomodulatory activity *in vivo*
- INCAGN1949 is currently in Phase 1 clinical development (NCT02923349)

References

- Croft M. *Nat Rev Immunol*. 2003;3:609–620.
- Bulliard Y, et al. *Immunol Cell Biol*. 2013;92:475–480.
- Croft M. *Annu Rev Immunol*. 2010;28:57–78.
- Voo KS, et al. *J Immunol*. 2013;191:3641–3650.
- Piconese S, et al. *J Exp Med*. 2008;205:825–839.
- Weinberg A. *Cancer Res*. 2013;73:7189–7198.

Author Disclosures

Ana Gonzalez, Mariana Manrique, Ekaterina Breous, David Savitsky, Jeremy Waight, Lukasz Swiech, Thomas Horn, Christopher Clarke, Yuqi Liu, Shiwen Lin, Jennifer Buell, Robert Stein, Marc van Dijk, Nicholas S. Wilson: Agencus Inc.; Employment and Stock Ownership. Jennifer Pulini, Kevin Heller, Reid Huber, Peggy Scherle, Gregory Hollis: Incyte Corporation; Employment and Stock Ownership. Taha Merghoub, Daniel Hirschhorn-Cymerman, Gerd Ritter, David Schaer: None.

Acknowledgments

The authors would like to thank Joseph Connolly and Zhenyu Li for their help characterizing and producing INCAGN1949. Layout and printing support was provided by Evidence Scientific Solutions, Philadelphia, PA, funded by Incyte Corporation.

