INCAGN02390, a Novel Antagonist Antibody That Targets the Co-Inhibitory Receptor TIM-3

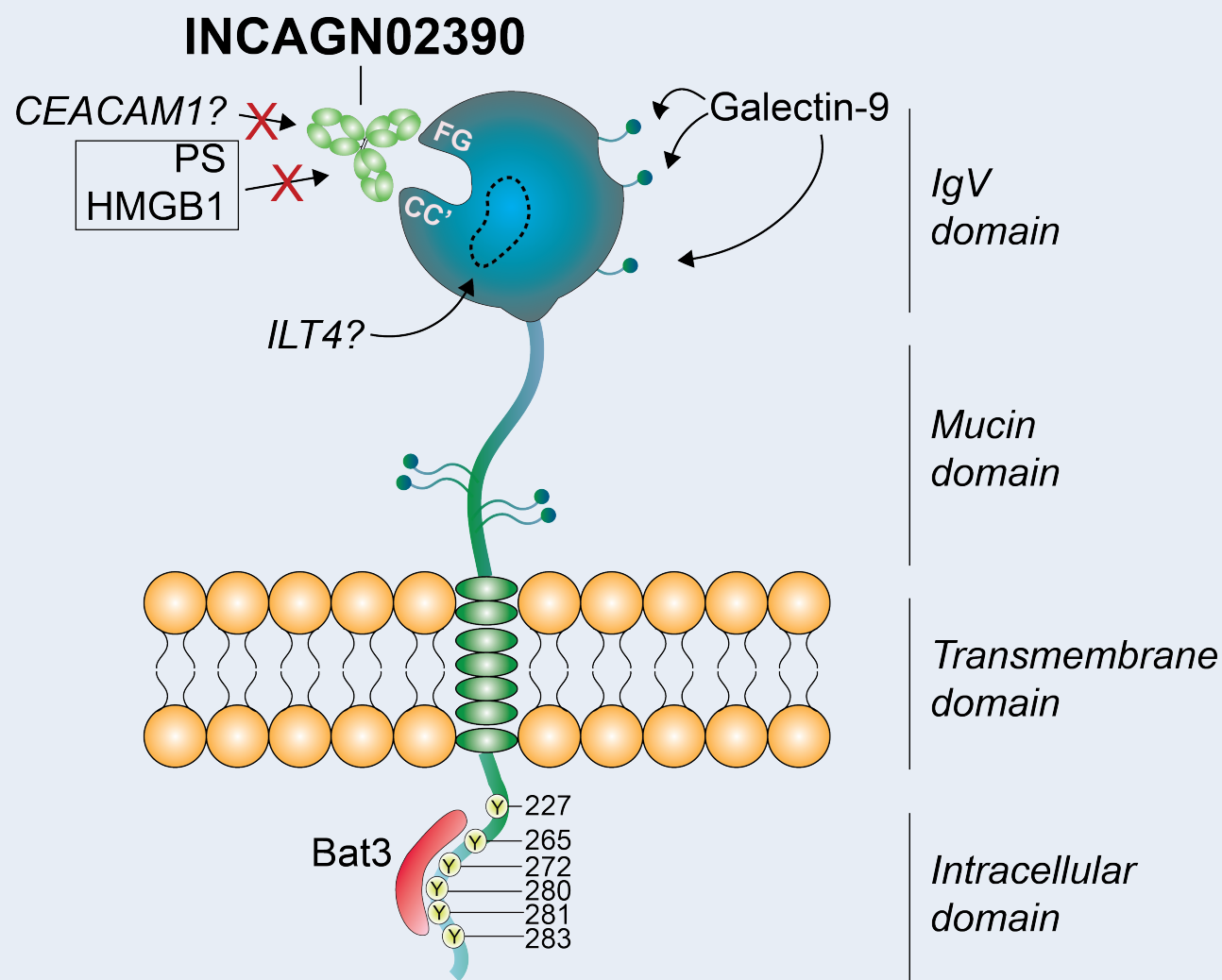Jeremy Waight,[1] Priyadarshini Iyer,[1] Ekaterina Breous-Nystrom,[1] Christina Riordan,[1] David A. Savitsky,[1] Mark Findeis,[1] Dennis Underwood,[1] Joseph Connolly,[1] Benjamin Morin,[1] Michele Sanicola-Nadel,[1] Horacio Nastri,[2] Peggy Scherle,[2] Gregory Hollis,[2] Reid Huber,[2] Robert Stein,[1] Marc van Dijk,[1] Jennifer Buell,[1] Nicholas S. Wilson[1]

[1]Agenus Inc. or subsidiary thereof (current or former employee), Lexington, MA; [2]Incyte Research Institute, Wilmington, DE
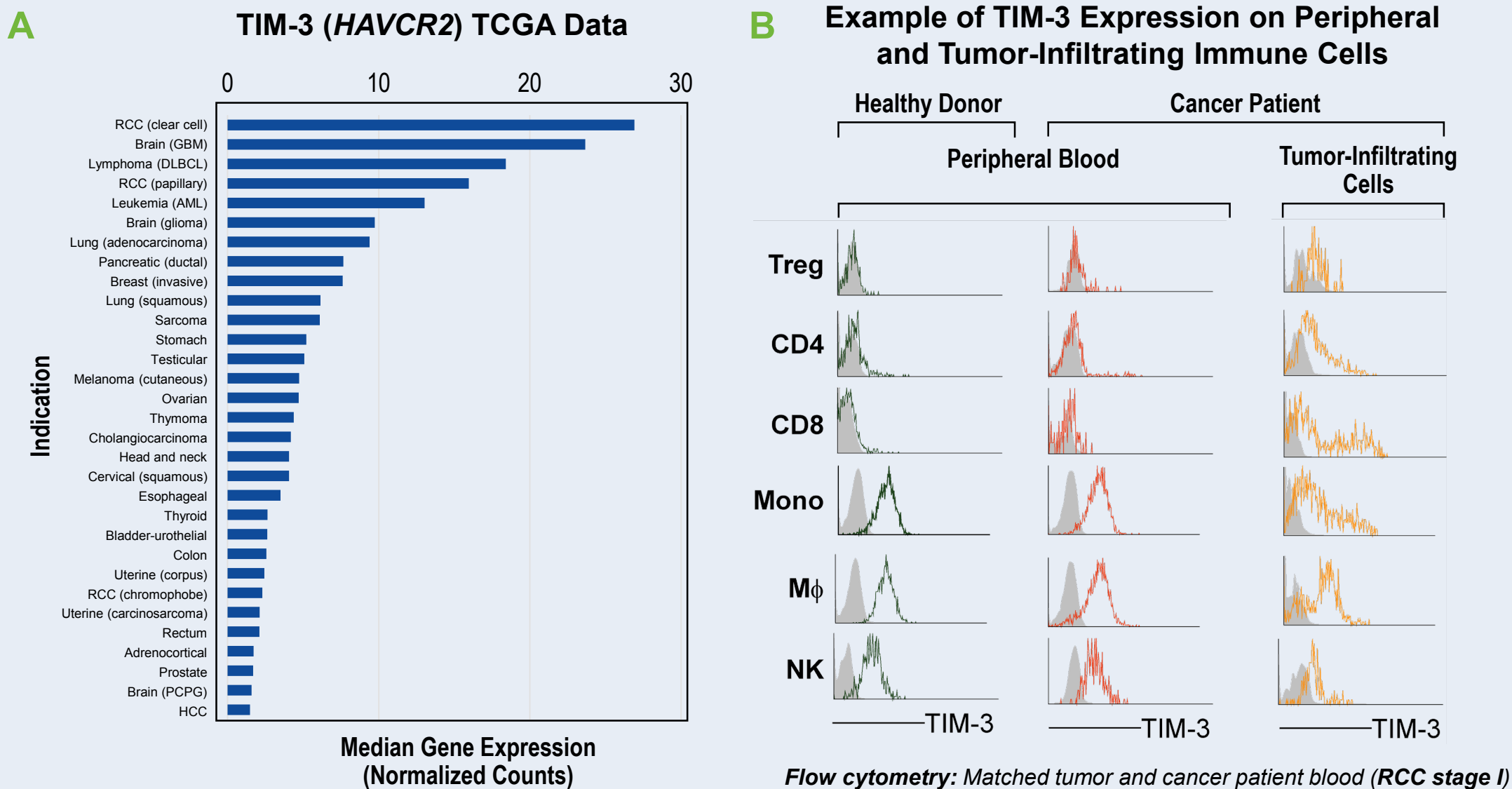
Abstract

Unprecedented rates of durable clinical responses have been observed for antibody-based therapeutics targeting immune checkpoint proteins such as cytotoxic T lymphocyte antigen-4 (CTLA-4) or programmed death receptor-1 (PD-1). Nonetheless, a significant number of patients experience de novo resistance or relapse due to adaptive resistance mechanisms. T-cell immunoglobulin and mucin domain containing-3 (TIM-3) is an inhibitory receptor involved in immune tolerance often co-opted by tumors to prevent successful antitumor responses. Accordingly, TIM-3 is frequently expressed on myeloid and 'exhausted' T and NK cells within the tumor microenvironment. Targeting the TIM-3 pathway in preclinical models has provided additional rationale for pharmacologic modulation of this axis in cancer patients. INCAGN02390 is a novel and fully human Fc-engineered IgG1κ antibody developed to antagonize the TIM-3 pathway for the treatment of human malignancies. INCAGN02390 forms a high-affinity interaction with TIM-3, occluding access to the CC'/FG binding cleft and blocking phosphatidylserine binding. In addition, INCAGN02390 elicits rapid receptor internalization, potentially obviating interactions with other described or undescribed ligands. INCAGN02390 also enhances IFNγ production from T cells undergoing tonic TCR stimulation when combined with PD-1 blockade. Finally, to demonstrate combinatorial potential, we show potent antitumor activity of an anti-mouse TIM-3 antibody in concert with other checkpoint antibodies in vivo. In summary, these data support the assessment of INCAGN02390 in patients with advanced or metastatic solid tumors.

TIM-3 Is a Single-Variable Immunoglobulin Domain (IgV)–Containing Inhibitory Receptor With Multiple Reported Ligands

Many proteins have been reported to interact with the IgV and mucin domain of TIM-3 (HAVCR2), including CEACAM1,[1] ILT4,[2] HMGB1,[3] Galectin-9,[4] and phosphatidylserine (PS).[5] However, the relative importance of the individual ligands and distinct downstream signaling events remains to be elucidated.
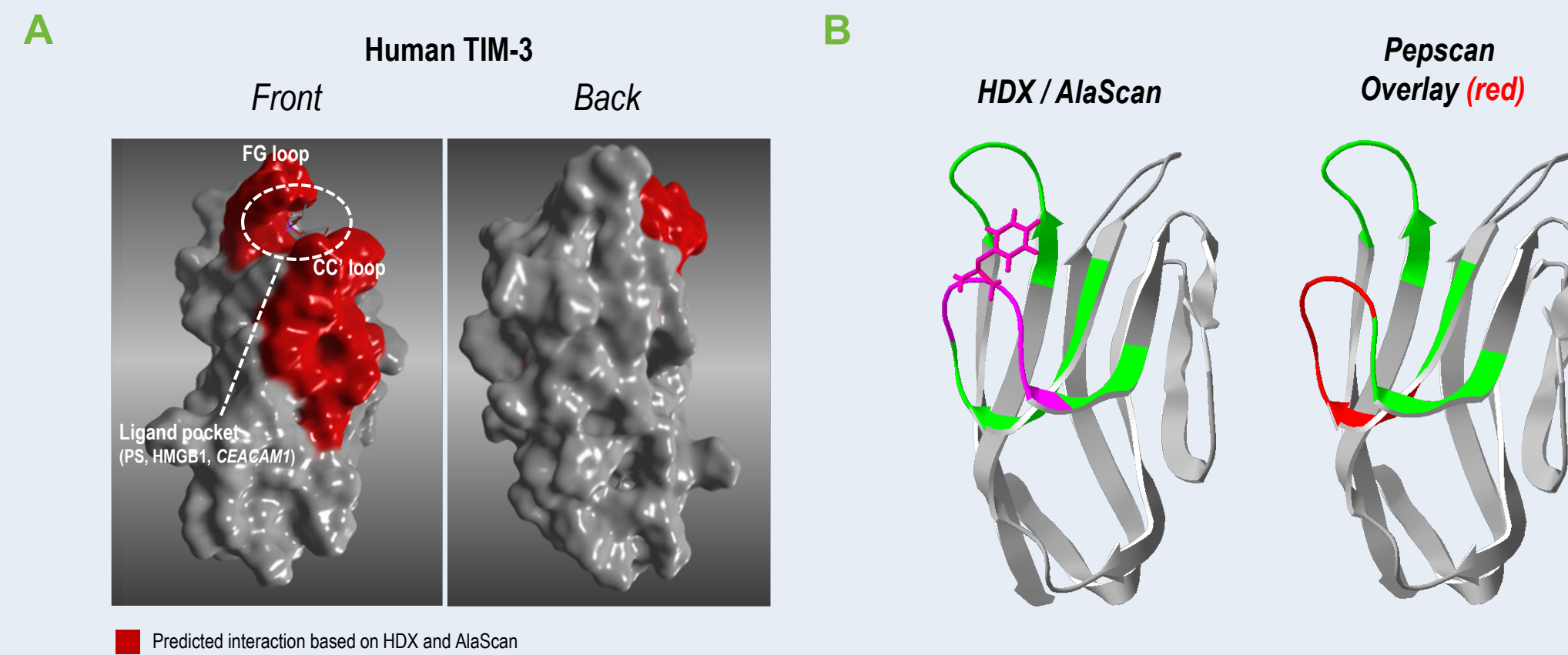
TIM-3 Is Expressed in a Range of Human Malignancies and Infiltrating Immune Cells

AML, acute myeloid leukemia; DLBCL, diffuse large B-cell lymphoma; GBM, glioblastoma; HCC, hepatocellular carcinoma; Mono, monocytes; MΦ, macrophages; NK, natural killer cells; PCPG, pheochromocytoma and paraganglioma; RCC, renal cell carcinoma; Treg, regulatory T cells.
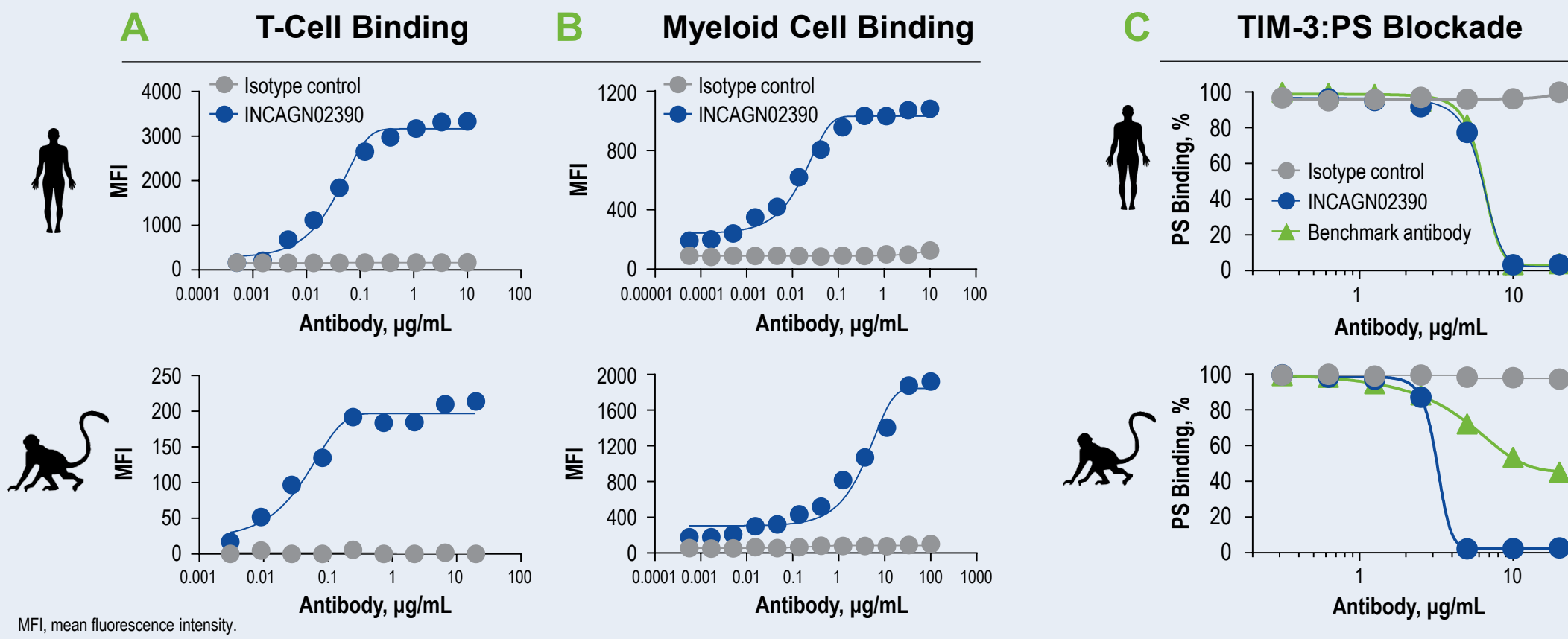
A. Median gene expression of TIM-3 in various tumor types profiled by The Cancer Genome Atlas (TCGA). Gene expression was determined using whole-genome RNA-seq of 11,000 patient tumor biopsies across 31 tumor types.
B. Representative flow cytometry histograms demonstrating TIM-3 expression on various immune populations within the tumor microenvironment and from peripheral blood of healthy or cancer patients.

Example shown: RCC (stage I), n = 8 separate indications characterized (and 4 individual RCC samples).

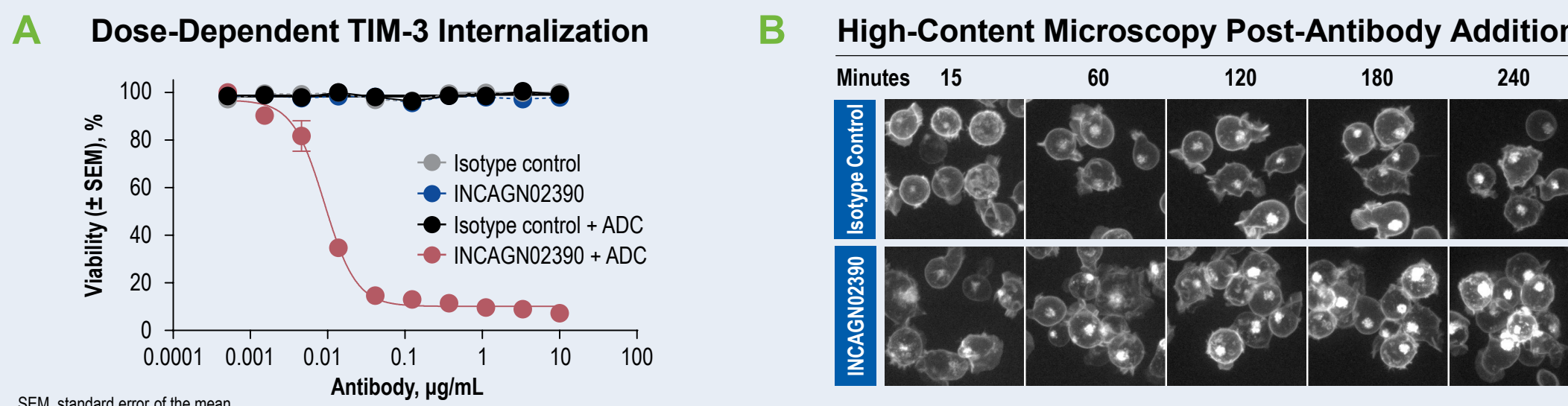## INCAGN02390 Obstructs the CC'/FG Binding Cleft of TIM-3

A. Modeling of the binding site of INCAGN02390 on human TIM-3 as determined by hydrogen deuterium exchange (HDX) and alanine scanning (AlaScan). Based on HDX characterization, INCAGN02390 occludes the CC'/FG loops of human TIM-3, potentially interfering with multiple predicted TIM-3 ligand interactions.
B. HDX epitope of INCAGN02390 on human TIM-3 extracellular domain (green and magenta), highest difference in HDX (magenta), and sidechain of Phe40 identified by AlaScan are shown. Structure is based on murine crystal structure PDB 2OYP modeled to human sequence (SwissModel entry i.d. abe2cb4efdbde42fab6c00d84cb1a578_UP000063_1). Epitope identified by Pepscan is overlaid in red.

INCAGN02390 Demonstrates Dose-Dependent Binding to TIM-3 and Blockade of PS:TIM-3 Interactions

MFI, mean fluorescence intensity.

Dose-dependent binding of phycoerythrin-conjugated INCAGN02390 or isotype control to (A) CD3/CD28-stimulated CD3+ T cells or (B) unstimulated CD14+ myeloid cells from human and cynomolgus monkey peripheral blood mononuclear cells (PBMCs). (C) Inhibition of recombinant human and cynomolgus monkey TIM-3 (Fc) binding to irradiated mouse WR19L cells (PS, induced by 30 Gy of irradiation). Binding of TIM-3 Fc to irradiated murine cells was detected by flow cytometry. The species cross-reactivity of PS allowed the use of irradiated mouse WR19L to reduce potential off-target binding of human or cynomolgus TIM-3 Fc to target cells.
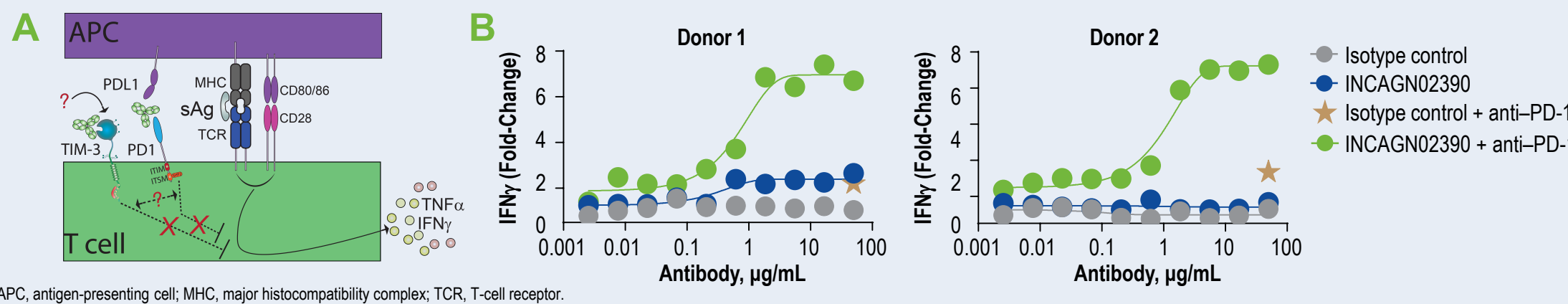
INCAGN02390 Elicits Rapid Internalization of TIM-3

SEM, standard error of the mean.

INCAGN02390-mediated TIM-3 internalization characterized by (A) antibody drug conjugate (ADC) and (B) microscopy-based internalization analysis.

A. Dose-dependent TIM-3 internalization by INCAGN02390. Jurkat-TIM-3+ cells were exposed to monomethyl auristatin E–conjugated or unconjugated INCAGN02390 or isotype control for 3 days. Internalization was indirectly assessed by viability of Jurkat-TIM-3+ cells using CellTiter-Glo® (Promega Corporation, Madison, WI).
B. Kinetic analysis of INCAGN02390-induced TIM-3 internalization by high-content microscopy. HaloTag® technology (engineered Jurkat-TIM-3+ cells [Promega]) was used to assess internalization. For microscopy, each antibody was used at 10 µg/mL.
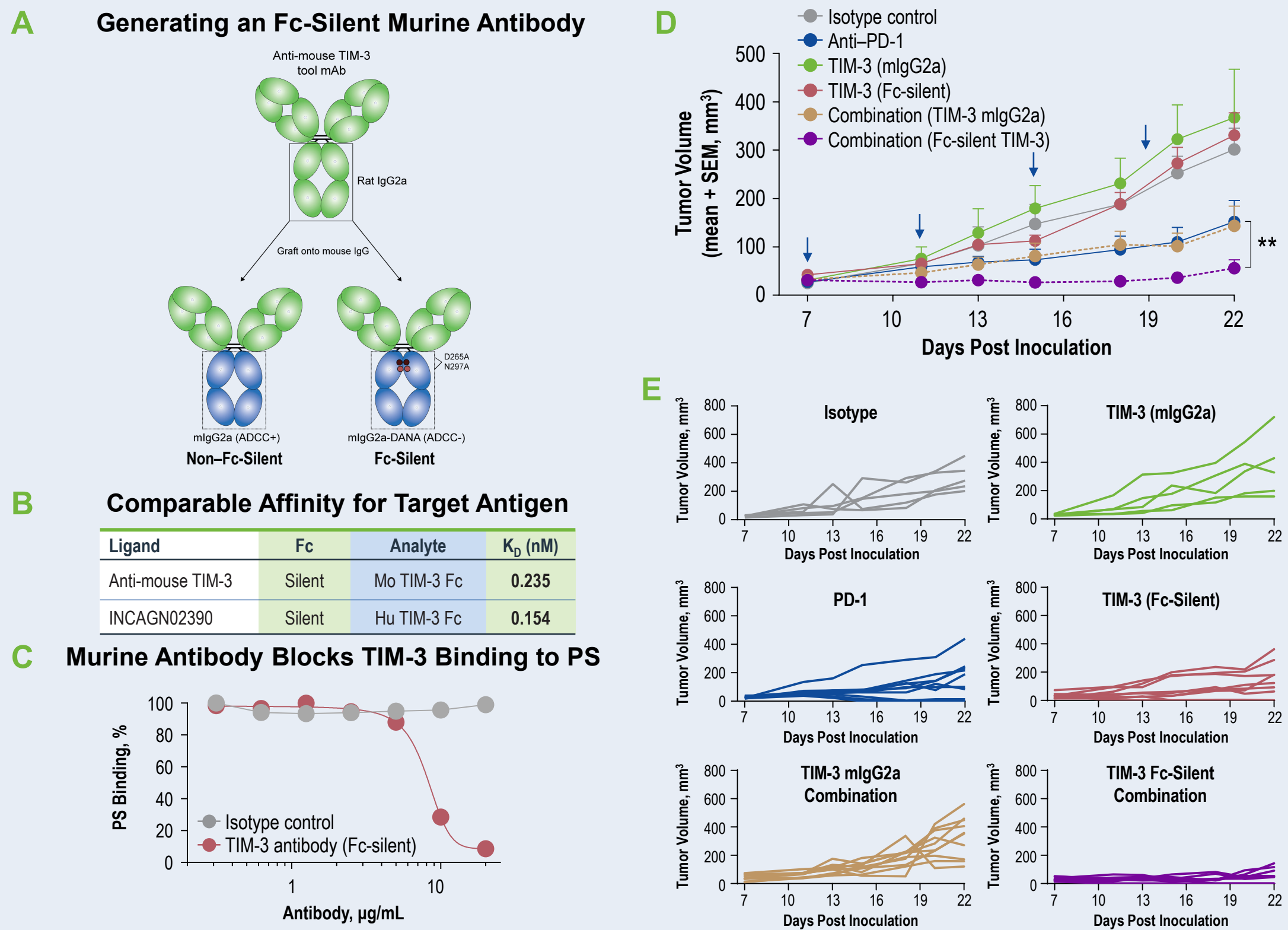
INCAGN02390 Cooperates With Anti–PD-1 to Enhance T-Cell Function

APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor.

A. An illustration of the functional effects elicited by TIM-3 and PD-1 blockade following staphylococcal enterotoxin A (SEA peptide, sAg) stimulation of human PBMCs.
B. IFNγ production from SEA-stimulated human PBMCs following blockade with INCAGN02390 and/or anti–PD-1 (pembrolizumab, 5 µg/mL). Healthy donor PBMCs were stimulated with 100 ng/mL SEA + antibody for 8 days. IFNγ production was assessed by AlphaLISA® (PerkinElmer Ltd., Llantrisant, UK).

Increased Efficacy With Fc-Silent Anti-Mouse TIM-3 Antibody in Combination With Anti–PD-1

Ligand	Fc	Analyte	K_D (nM)
Anti-mouse TIM-3	Silent	Mo TIM-3 Fc	0.235
INCAGN02390	Silent	Hu TIM-3 Fc	0.154

ADCC, antibody-dependent cell-mediated cytotoxicity; SPR, surface plasmon resonance.

Characterization of Fc-modified (Fc-silent, D265A-N297A) anti-mouse TIM-3 antibody. (A) Illustration of the generated anti-TIM-3 Fc variants (mIgG2a and mIgG2a-D265A-N297A). (B) SPR-based affinity assessment of anti-TIM-3 antibodies and (C) inhibition of recombinant mouse TIM-3 (human IgG Fc) binding to irradiated mouse WR19L cells (PS, induced by 30 Gy of irradiation). Binding of TIM-3 Fc to irradiated murine cells was detected by flow cytometry. (D) Tumor growth in C57Bl/6 mice (n = 5) inoculated subcutaneously with MC-38 colon cells (1×10^{5} cells/mouse) as assessed with and without bi-weekly anti–PD-1 (RMP1-14, 10 mg/kg) and/or anti–TIM-3 (RMT3-23 variants; 10 mg/kg, days 7, 11, 15, 19 [blue arrows]) treatment. Tumor growth curves for individual mice are shown in (E). ** P < 0.01 for comparison of anti–PD-1 + TIM-3 combinations with anti–PD-1 alone using 2-way analysis of variance or Student t test.

Conclusions

- INCAGN02390 is a fully human Fc-engineered IgG1κ antibody (aglycosylated, N297A) identified by Retrocyte Display® technology
- INCAGN02390 binds near the CC'/FG cleft of the TIM-3 IgV domain, disrupting TIM-3:phosphatidylserine binding
- INCAGN02390 induces rapid receptor internalization, potentially disrupting multiple TIM-3:ligand interactions
- INCAGN02390 combines with anti–PD-1 antibody to enhance the functional activity of suboptimally stimulated human PBMCs, in vitro
- In the MC-38 mouse model of colon adenocarcinoma, Fc-engineered TIM-3 antibody (aglycosylated, D265A-N297A) demonstrated significant tumor control when combined with an anti–PD-1 antibody

References

1. Huang YH, et al. Nature. 2015;517:386–90. 2. Feldman I, et al. Patent application (W02016111947A3). 2015. 3. Chiba S, et al. Nat Immunol. 2012;13:832–42. 4. Kikushige Y, et al. Cell Stem Cell. 2015;17:341–52. 5. Sabatos-Peyton CA, et al. Oncoimmunology. 2017;7:e1385690.

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