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

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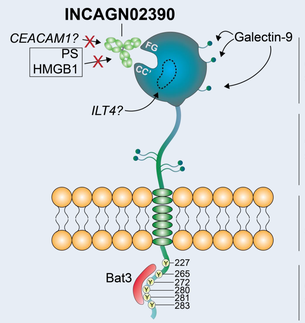
## Abstract

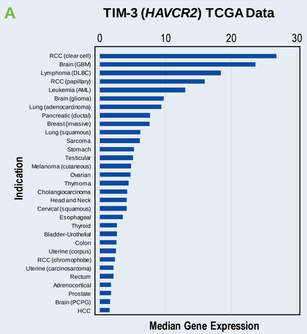
Unprecedented rates of durable clinical responses have been observed for antibody-based therapeutics targeting immune checkpoint proteins such as cytotoxic T lymphocyte antigen-4 (CTLA-4) or programmed death receptor-1 (PD-1). Nonetheless, a significant number of patients experience *de novo* resistance 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.

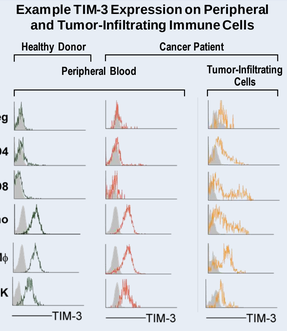
## TIM-3 Is a Single Variable Immunoglobulin Domain (IgV)-Containing Inhibitory Receptor With Multiple Reported Ligands




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 (Gal-9),[4] and phosphatidylserine (PS).[5] However, the relative importance of the individual ligands and distinct downstream signaling events remain to be elucidated.

## TIM-3 Is Expressed in a Range of Human Malignancies and Infiltrating Immune Cells




***Flow cytometry:***
*Matched tumor and cancer patient blood (**RCC stage I**)*

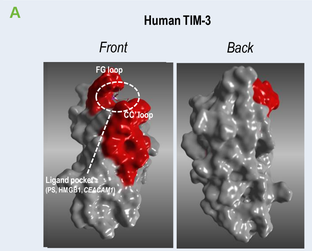
Mono, monocytes; MΦ, macrophages; NK, natural killer cells; Treg, regulatory T cells.

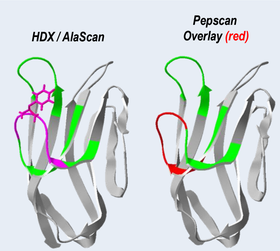
A. Median gene expression of TIM-3 in various tumor types profiled by The Cancer Genome Atlas (TCGA). Gene expression was determined using whole genome RNA-seq of 11,000 patient tumor biopsies across 31 tumor types.

B. Representative flow cytometry histograms demonstrating TIM-3 expression on various immune populations within the tumor microenvironment and from peripheral blood of healthy patients or cancer patients.

**Example shown:** Renal cell carcinoma (RCC; stage I), N=8 separate indications characterized (and 4 individual RCC samples).

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

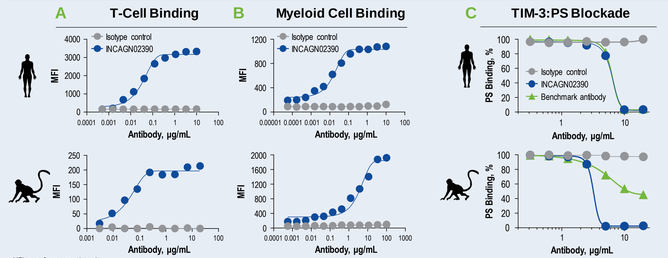



A. Modeling of the binding site of INCAGN02390 on human TIM-3 as determined by hydrogen deuterium exchange (HDX) and Alanine scanning (AlaScan). Based on HDX characterization, INCAGN02390 occludes the CC'/FG loops of human TIM-3, potentially interfering with multiple predicted TIM-3 ligand interactions.

B. HDX epitope of INCAGN02390 on hTIM-3 extracellular domain (green and magenta); highest difference in HDX (magenta); sidechain of Phe40 identified by AlaScan is 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.

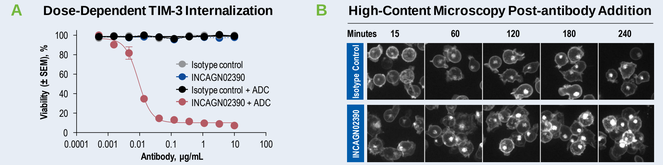
## INCAGN02390 Demonstrates Dose-Dependent Binding to TIM-3 and Blockade of PS-TIM-3 Interactions




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

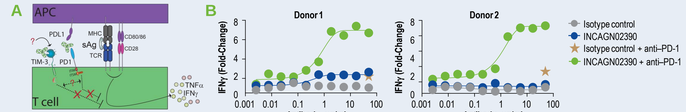



INCAGN02390-mediated TIM-3 internalization characterized by (A) antibody drug conjugate (ADC)- and (B) microscopy-based internalization analysis.

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

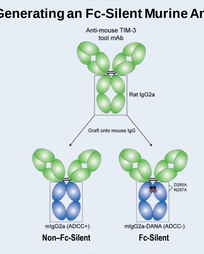



APC, antigen presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor.

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.

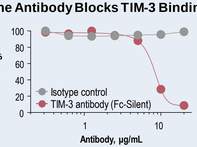
B. IFNγ production from SEA-stimulated human PBMCs following blockade with INCAGN02390 and/or anti–PD-1 (pembrolizumab, 5 μg/mL). Healthy donor PBMCs were stimulated with 100 ng/mL SEA + antibody for 8 days. IFNγ production was assessed by AlphaLISA (Perkin-Elmer, Llantrisant, UK).

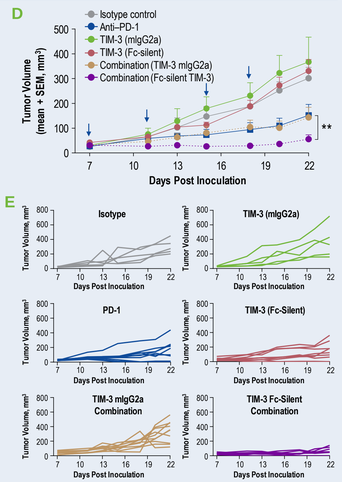
## Increased Efficacy With Fc-Silent Anti-Mouse TIM-3 Antibody in Combination With Anti–PD-1




B Comparable Affinity for Target Antigen

| 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-mouse 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 (1×10⁵ 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 ) 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 two-way ANOVA or Student *t* test.

## Conclusions

- **INCAGN02390 is a fully human Fc-engineered IgG1κ antibody (aglycosylated, N297A)**
- **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 anti–PD-1 antibody**

**Disclosures**
**Waight, Iyer, Breous-Nystrom, Riordan, Savitsky, Findeis, Underwood, Connolly, Morin, Sanicola-Nadel, Stein, van Dijk, Buell, Wilson:** Present and former employment and stock ownership –*Agenus Inc.* **Nastri, Scherle, Hollis, Huber:** Employment and stock ownership – *Incyte Corporation.*

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**References**
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