INCAGN02385 Is an Antagonist Antibody Targeting the Co-inhibitory Receptor LAG-3 for the Treatment of Human Malignancies

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Abstract

Lymphocyte activation gene 3 (LAG-3) is a cell surface receptor that negatively regulates antigen-specific T-cell responses. LAG-3 expression is generally restricted to populations of recently activated and chronically stimulated exhausted T cells, and is often correlated with T-cell dysfunction across several human malignancies. Accordingly, the LAG-3 pathway has been identified as a potential barrier to productive tumor-specific T-cell immunity generated by programmed cell death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) blockade. The antitumor activity from targeting the LAG-3 pathway in preclinical models has provided further rationale for pharmacologic modulation of the LAG-3 axis in cancer patients. INCAGN02385 is an Fc-engineered IgG1κ antibody chosen for development based on its high-affinity binding to human LAG-3, cross-reactivity with cynomolgus monkey LAG-3, and ability to potently block LAG-3 binding with its major histocompatibility complex (MHC) class II ligand. INCAGN02385 also enhances T-cell responsiveness to T-cell receptor (TCR) stimulation alone or in combination with PD-1/PD-L1 axis blockade. INCAGN02385 was well tolerated in cynomolgus monkeys and demonstrated the expected pharmacokinetic profile. Altogether, these data support assessment of INCAGN02385 in patients with solid tumors.

LAG-3 Antagonist Antibody INCAGN02385 Attenuates Inhibitory Signaling to Enhance T-Cell Tumor Immunity

LAG-3 consists of 4 Ig-like extracellular domains (D1–D4) that interact with a conserved region on MHC class II via the D1 domain.[1,2] Engagement with ligand induces cell-intrinsic co-inhibitory signaling to limit antigen-specific T-cell responses.[3,4] The inhibitory function of the LAG-3 requires an intact 'KIEELE' motif within the intracellular domain of the protein.[5] Tumor immunity is enhanced when LAG-3 antagonism is combined with concomitant blockade of the PD-1 pathway.[6]
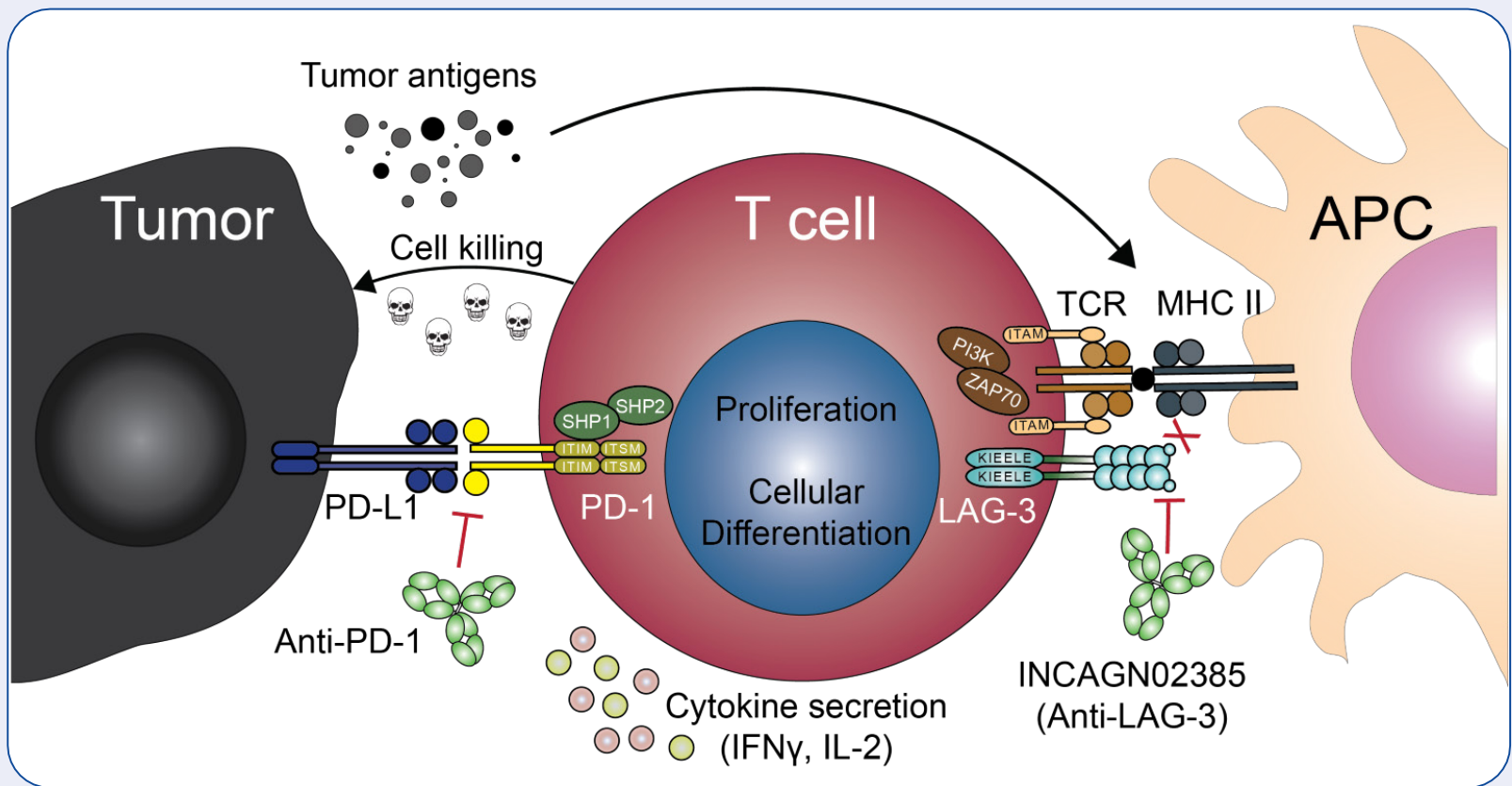
APC, antigen-presenting cell; IFN, interferon; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; ITSM, immunoreceptor tyrosine-based switch motif; PI3K, phosphatidylinositol-3-kinases; SHP, Src homology region 2 domain-containing phosphatase; TCR, T-cell receptor; ZAP, Zeta-chain-associated protein kinase.

INCAGN02385 Binds to the D1 Domain of LAG-3 to Disrupt MHC Class II Binding

Model of human LAG-3 based on the crystal structure of a complete ternary complex of T-cell receptor, peptide-HLA and CD4 (PDB entry 3T0E) was generated using SWISS-MODEL (Biozentrum, the Center for Molecular Life Sciences, University of Basel, Switzerland). Residues 23-428 (UniProt numbering) of human LAG-3 (UniProtKB - P18627) were used as the target sequence, and the structure of human CD4 from PDB entry 3T0E was used as a template. The peptide backbones of LAG-3 and HLA-DR are represented by the yellow and gray ribbon diagrams, respectively. The interaction surface of the LAG-3 model with HLA-DR was calculated using Molecular Operating Environment software (Chemical Computing Group, Montreal, QC, Canada), and is shown as an electrostatic surface. Residues in LAG-3 D1 domain identified as having reduced HDX due to INCAGN02385 binding (determined using mass spectrometry) are indicated by the space-filling model of specific residues 45-55 (SPTIPLQDLSL, green).
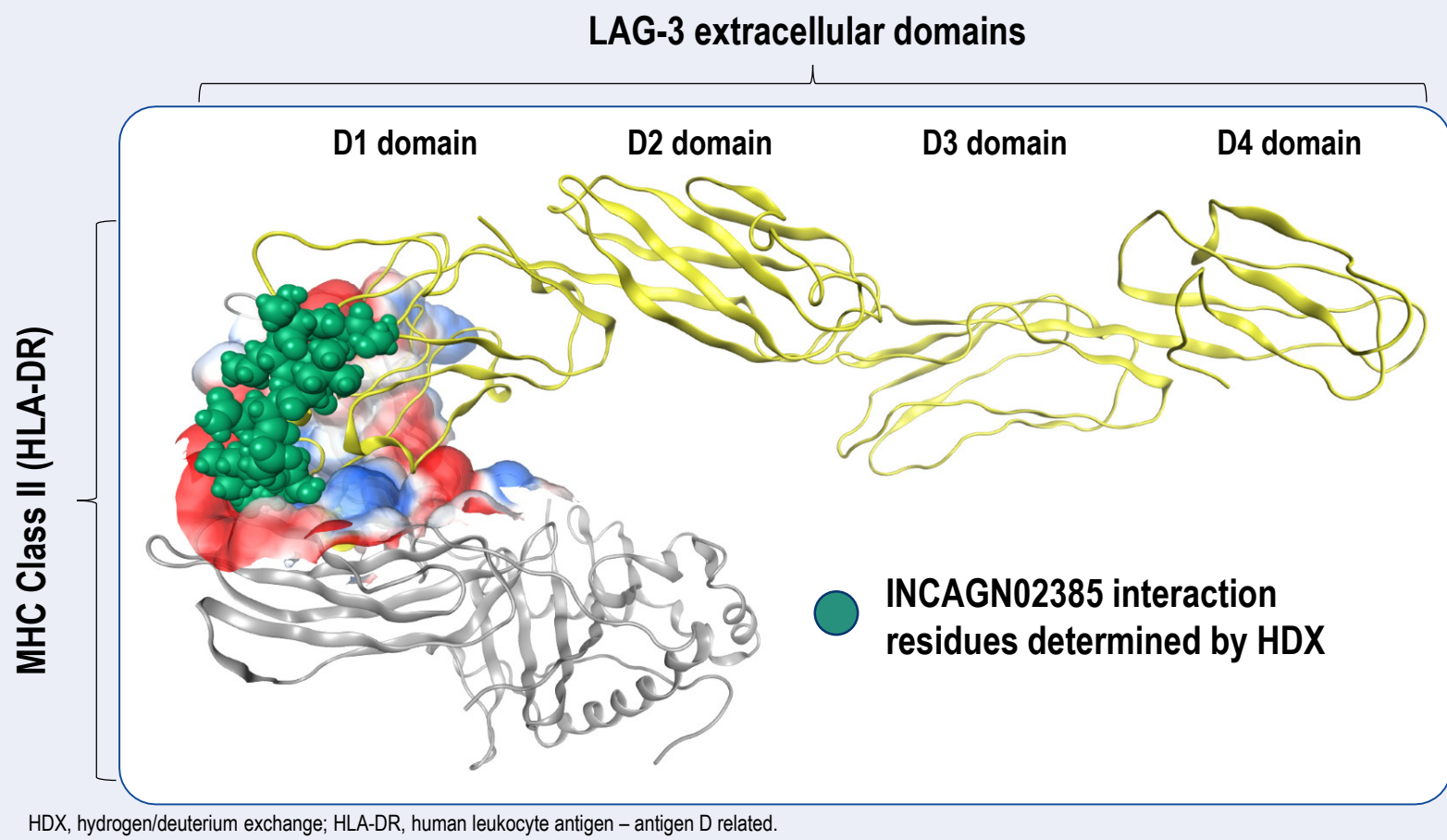
HDX, hydrogen/deuterium exchange; HLA-DR, human leukocyte antigen – antigen D related.

INCAGN02385 Demonstrates Dose-Dependent Binding to Cell-Expressed LAG-3 and Disrupts Binding to MHC Class II

A Primary CD4+ and CD8+ T-Cell Binding

B LAG-3: MHC Class II Blockade
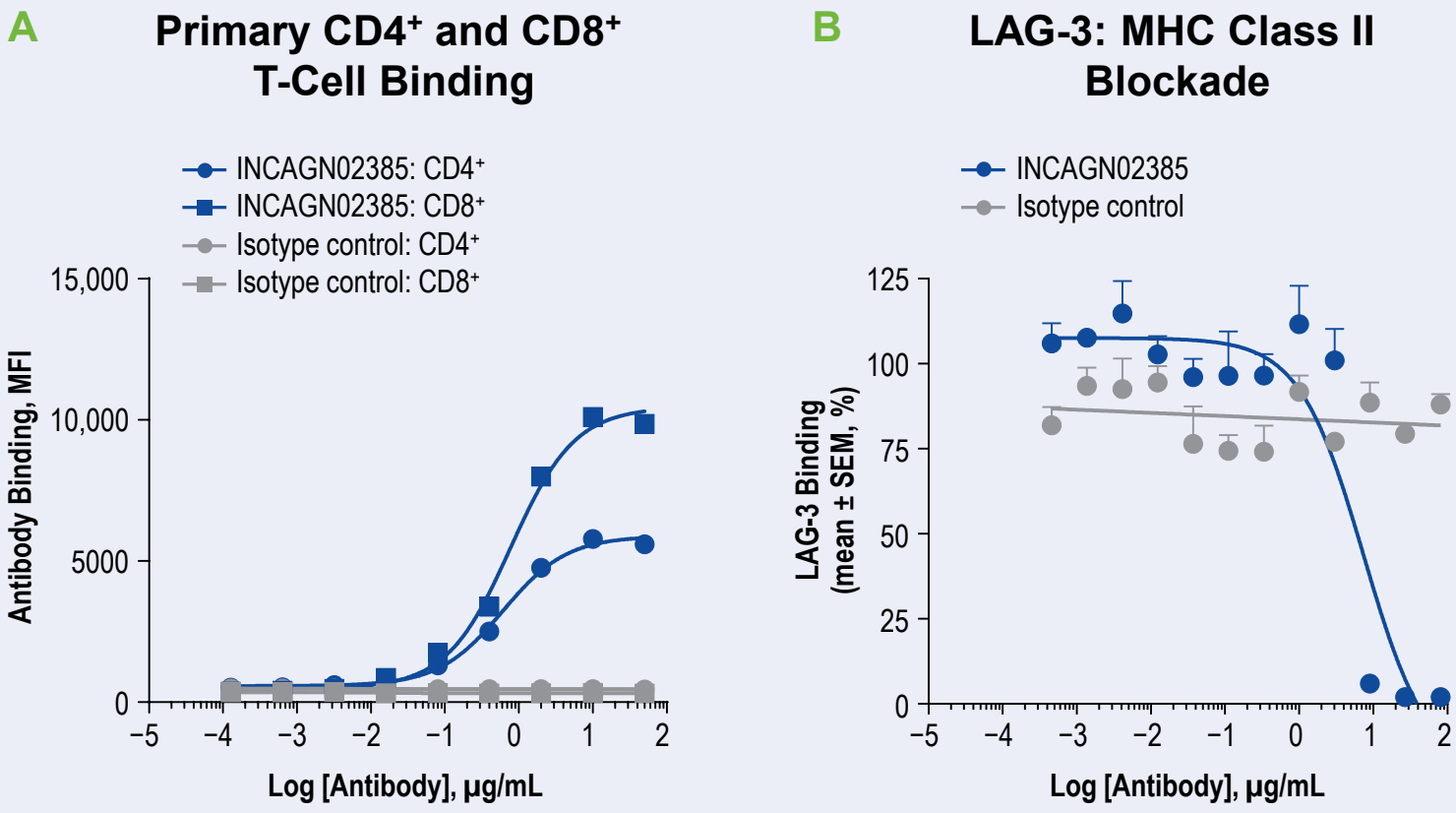
MFI, mean fluorescence intensity; SEM, standard error of the mean.

A. Dose-dependent binding of INCAGN02385 to Staphylococcus enterotoxin A (SEA) stimulated primary human CD4+ and CD8+ T cells. INCAGN02385 binding was detected using a fluorochrome-conjugated anti-human Fc secondary antibody and analyzed by flow cytometry.

B. Binding of a fixed-concentration multimerized LAG-3-His to MHC class II+ Raji (human Burkitt's lymphoma) cells following pre-incubation with a dose range of INCAGN02385. Data were collected by flow cytometry.

INCAGN02385 Antagonizes Tumor Cell–Mediated T-Cell Inhibition to Restore TCR-NFAT Signaling

A Schematic of Assay

B Luciferase Reporter Activation of TCR-NFAT Signaling
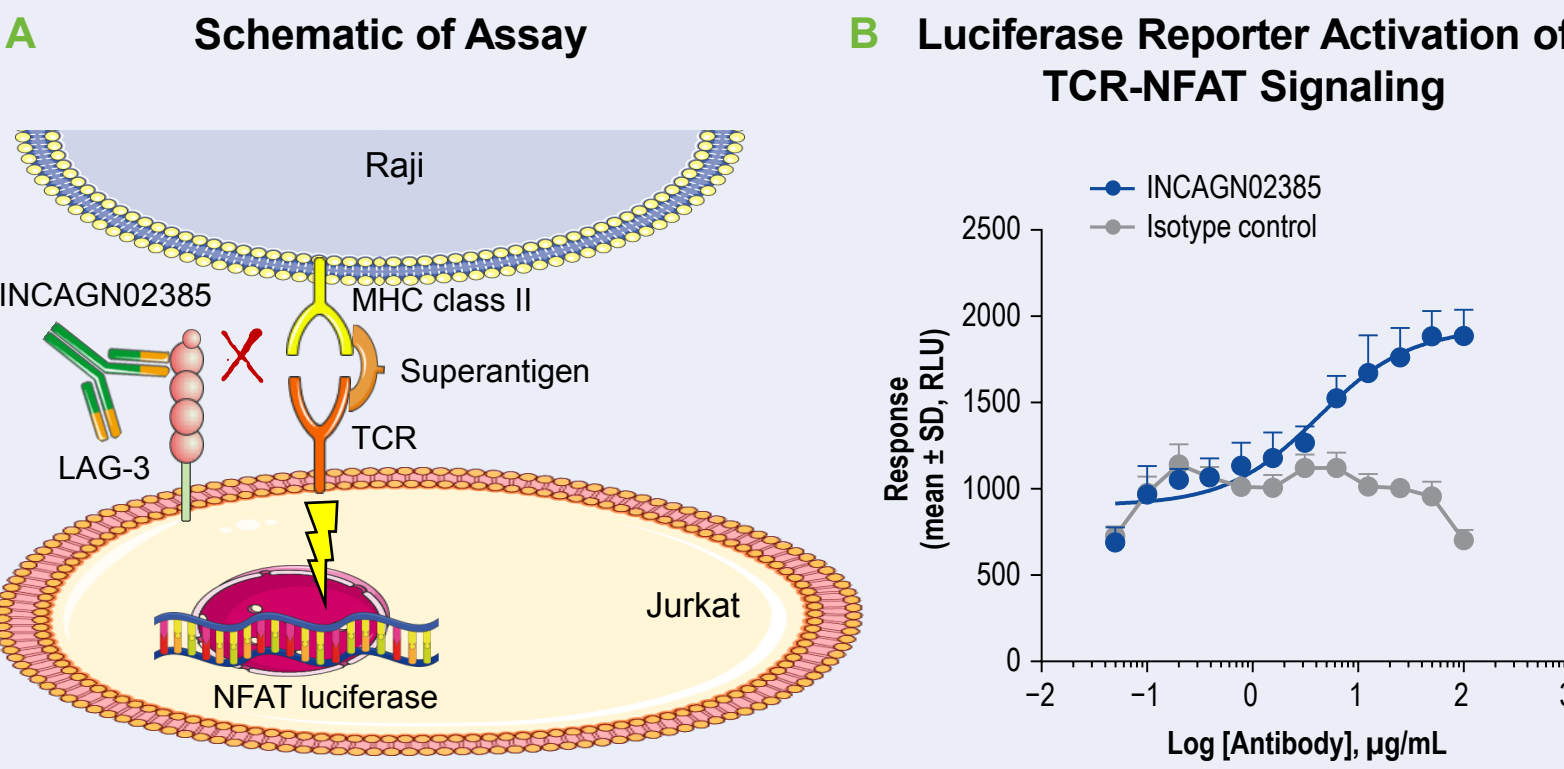
NFAT, nuclear factor of activated T cells; RLU, relative luciferase unit; SD, standard deviation.

A. INCAGN02385 inhibits LAG-3 inhibitory signaling induced at the interface of T-cell and hematologic tumors expressing MHC class II.

B. INCAGN02385 restores TCR-NFAT signaling in a Jurkat-LAG-3-NFAT-luciferase reporter T-cell line (BPS Bioscience, San Diego, CA) co-cultured with Raji cells and a superantigen peptide. Average and SD of 6 technical replicates from 1 of 4 independent experiments are shown.

INCAGN02385 Enhances T-Cell Cytokine Secretion in Combination With Other Checkpoint Modulators
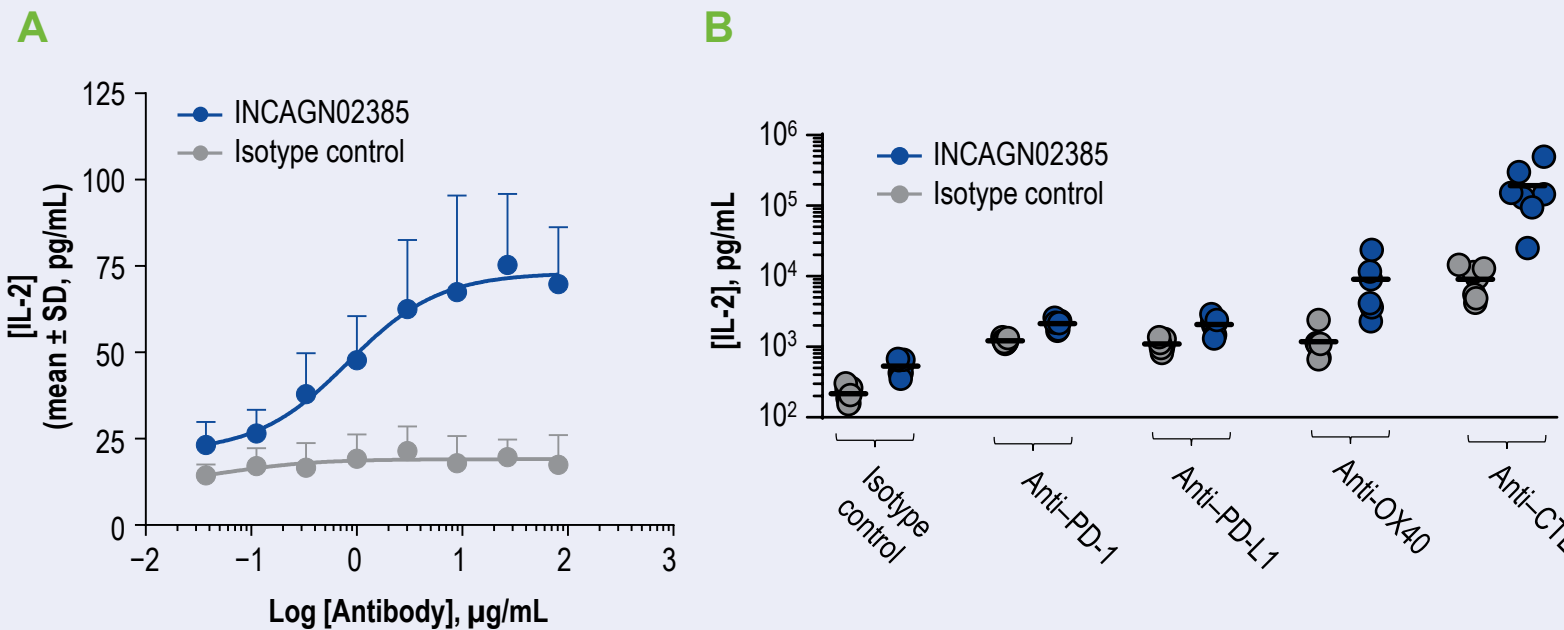
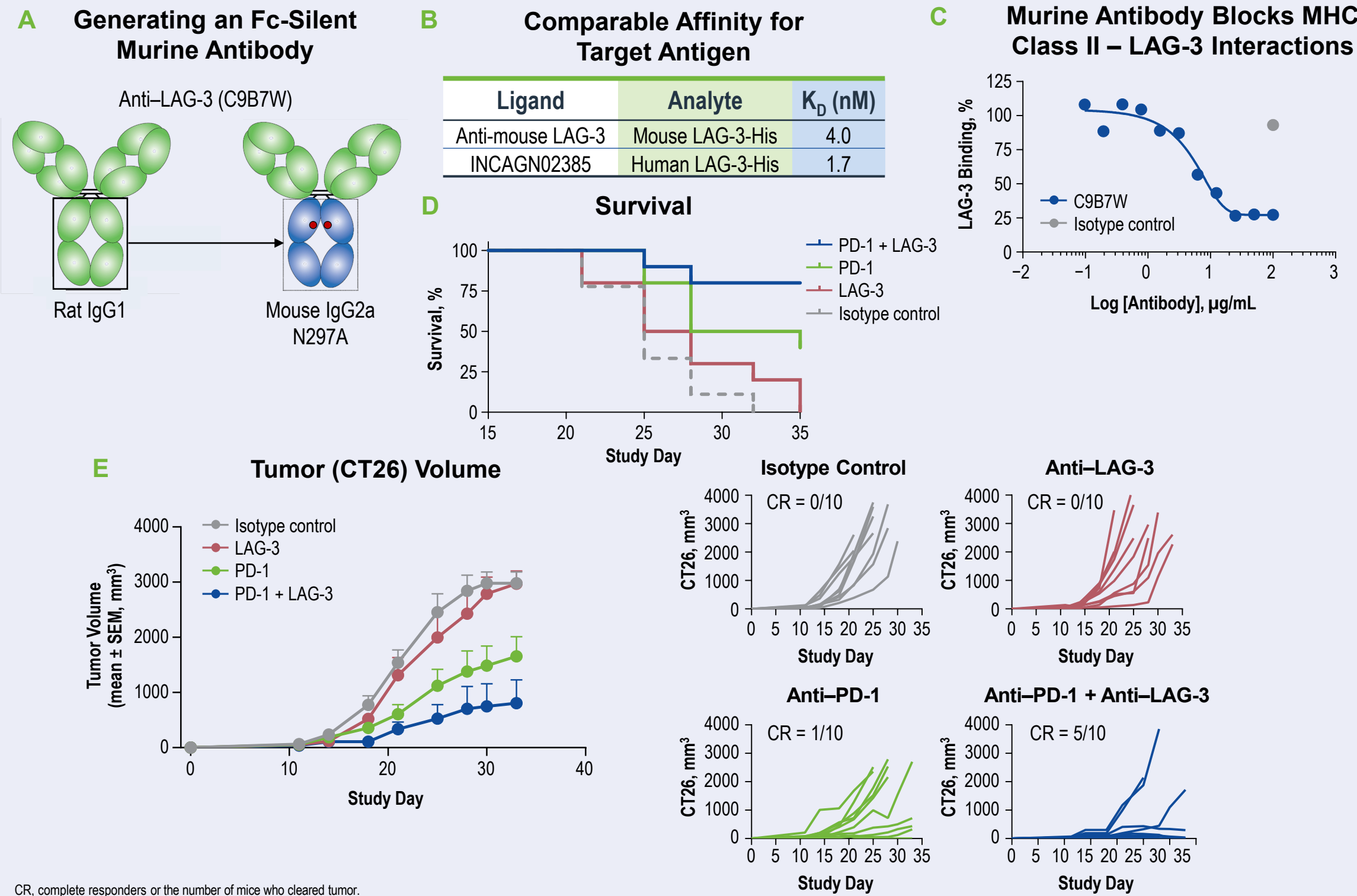CTLA-4, cytotoxic T lymphocyte associated antigen 4.

INCAGN02385 enhanced T cell–induced IL-2 production in primary peripheral blood mononuclear cell (PBMC) cultures. PBMCs were stimulated with superantigen peptide in combination with a (A) dose range or (B) fixed concentration (10 µg/mL) of INCAGN02385 alone and in combination with anti–PD-1 (pembrolizumab), anti–PD-L1 (MSB0010718C analog), anti-OX40 (INCAGN1949), or anti–CTLA-4 (ipilimumab). Cultured supernatants were evaluated for IL-2 levels by AlphaLISA® (PerkinElmer, Waltham, MA) after 5 days. An average of 6 technical replicates are shown. Representative of 1 of 2+ experiments.

INCAGN02385 Promotes T-Cell Cytotoxicity of Exhausted T Cells

A Antigen-Specific Target Cells

B Antigen-Nonspecific Target Cells
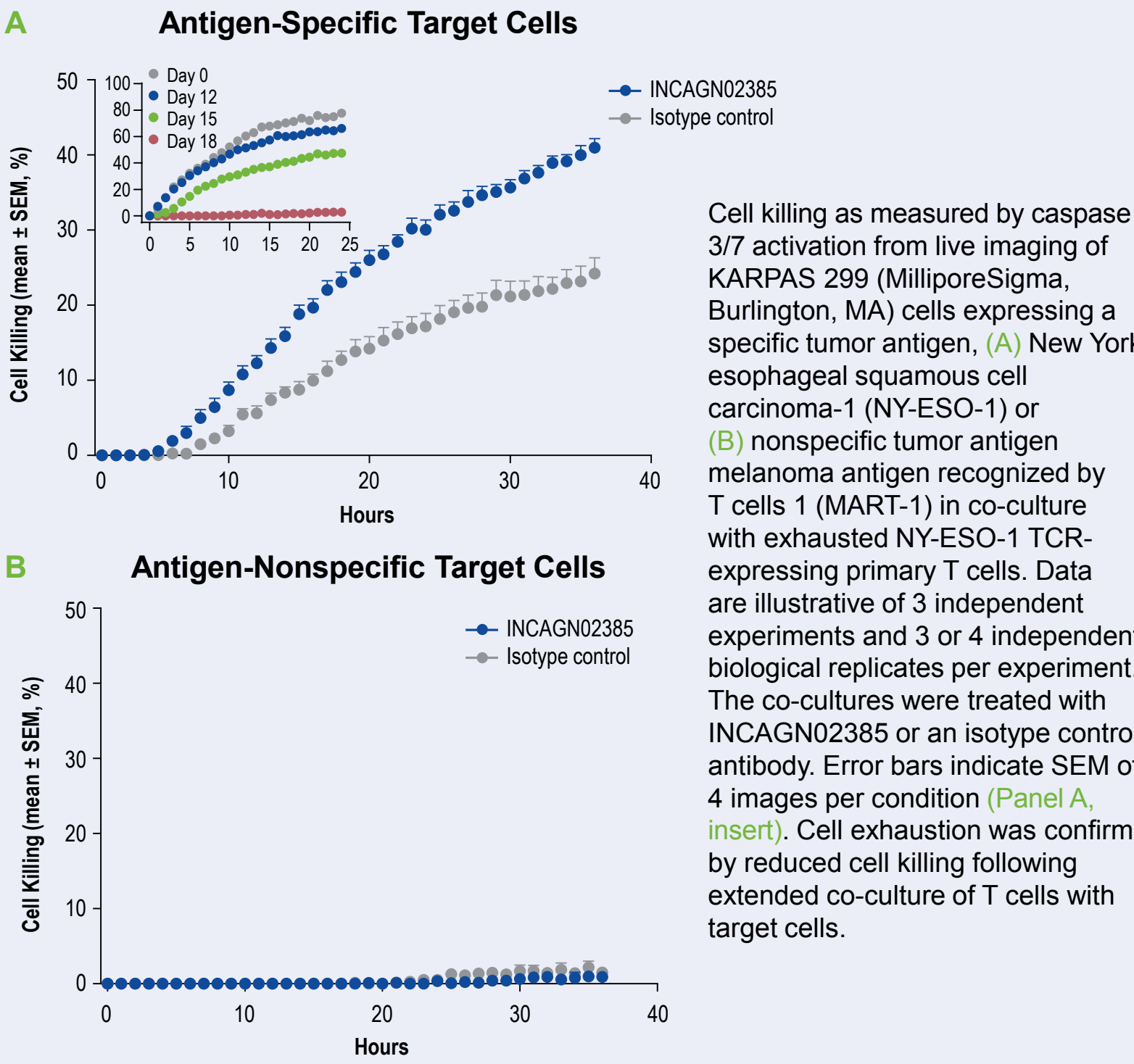
Cell killing as measured by caspase 3/7 activation from live imaging of KARPAS 299 (MilliporeSigma, Burlington, MA) cells expressing a specific tumor antigen, (A) New York esophageal squamous cell carcinoma-1 (NY-ESO-1) or (B) nonspecific tumor antigen melanoma antigen recognized by T cells 1 (MART-1) in co-culture with exhausted NY-ESO-1 TCR-expressing primary T cells. Data are illustrative of 3 independent experiments and 3 or 4 independent biological replicates per experiment. The co-cultures were treated with INCAGN02385 or an isotype control antibody. Error bars indicate SEM of 4 images per condition. (Panel A, insert). Cell exhaustion was confirmed by reduced cell killing following extended co-culture of T cells with target cells.

Increased Tumor Control With Fc-Silent Anti-Mouse LAG-3 Antibody and PD-1 Inhibition

A Generating an Fc-Silent Murine Antibody

Anti–LAG-3 (C9B7W)
Rat IgG1 → Mouse IgG2a N297A

B Comparable Affinity for Target Antigen

Ligand	Analyte	K_D (nM)
Anti-mouse LAG-3	Mouse LAG-3-His	4.0
INCAGN02385	Human LAG-3-His	1.7

C Murine Antibody Blocks MHC Class II – LAG-3 Interactions

D Survival

E Tumor (CT26) Volume

CR, complete responders or the number of mice who cleared tumor.

(A) Illustration of the anti-mouse LAG-3 Fc engineered (mouse IgG2a N297A) antibody. (B) Surface plasmon resonance–based affinity assessment of anti–LAG-3 antibodies and (C) inhibition of multimerized mouse LAG-3-His binding to MHC class II+ A20 (mouse B-cell lymphoma). (D, E) Syngeneic CT26 tumors subcutaneously transplanted in groups of 9–10 Balb/c mice were treated with isotype (mouse aglycosylated IgG2a), anti–PD-1 (rat IgG2a), anti–LAG-3 (mouse aglycosylated IgG2a), or combination anti–PD1 plus anti–LAG3 antibodies for 6 cycles every 3–4 days at 0.2 mg/mouse. Survival and tumor growth in aggregate and for individual animals are shown.

Conclusions

- INCAGN02385 is a humanized Fc-engineered IgG1κ antibody (aglycosylated, N297A)
- INCAGN02385 binds the D1 domain of LAG-3, disrupting LAG-3-MHC class II binding
- INCAGN02385 restores TCR-NFAT signaling, enhances cytokine secretion of activated T cells, and augments target cell killing by exhausted T cells
- INCAGN02385 combines with modulators of PD-1, PD-L1, OX40, and CTLA-4 to enhance the functional activity of suboptimally stimulated human PBMCs in vitro
- In a mouse model of colon adenocarcinoma (CT26), an Fc-engineered anti-mouse LAG-3 antibody (aglycosylated, N297A) demonstrated significant tumor control when combined with an anti–PD-1 antibody

References
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