DISCOVERY AND CHARACTERIZATION OF INCB106385, A NOVEL A_{2A}/A_{2B} ADENOSINE RECEPTOR ANTAGONIST, AS A CANCER IMMUNOTHERAPY

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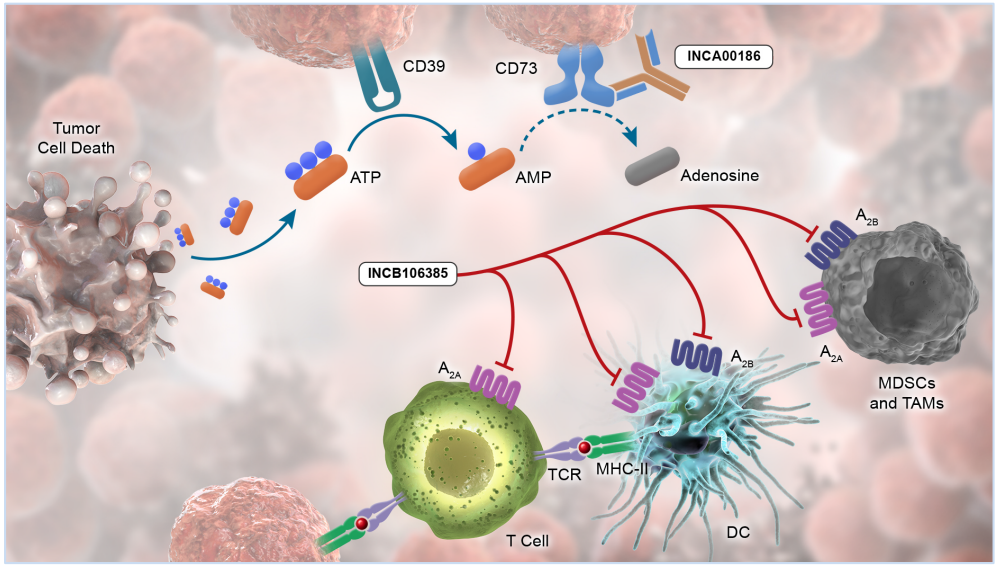
BACKGROUND



AMP, adenosine monophosphate; ATP, adenosine triphosphate; DC, dendritic cell; MDSC, myeloid-derived suppressor cells; MHC-II, major histocompatibility class 2; TAM, tumor-associated macrophage; TCR, T-cell receptor; TME, tumor microenvironment.

- In the TME, concentration of the immunosuppressive metabolite, adenosine, is tightly regulated by the enzyme CD73
- High levels of adenosine in the TME suppress antitumor immunity by binding to the A_{2A} and A_{2B} receptors expressed on T cells, DCs, and MDSCs
- Increased flux through the adenosine pathway has been linked to resistance to immune checkpoint therapies
- Pharmacologic inhibition at multiple nodes of the adenosine pathway is expected to reverse the immunosuppressive activity of adenosine and improve antitumor immunity
- INCB106385 is a novel small-molecule antagonist of A_{2A} and A_{2B} receptors, and INCA00186 is a monoclonal antibody targeting CD73

INCB106385: A CLINICAL BEST-IN-CLASS A_{2A}/A_{2B} ANTAGONIST

- Highly potent and selective A_{2A} and A_{2B} antagonism to overcome elevated adenosine levels in the TME
- Dual targeting of A_{2A} and A_{2B} provide broad spectrum coverage of T cells, DCs, myeloid cells, and potentially tumor cells directly
- Minimal brain penetration (<1% total plasma concentration)
- Highly accessible to tumor cells (tumor/plasma ratio >1)
- Excellent ADME profiles in humans

Compound	A_{2A} Binding (Ki, nM)	A_{2B} Binding (Ki, nM)	WB pCREB (IC_{50}, nM)	Brain Penetration
INCB106385	**0.24**	**9.5**	**4.3**	**<1%**
CPI-444[1,2]	3.54	NA	>3000	NA
AZD4635[2,3]	1.5	64	2600	NA
AB928[4]	1.4	2.4	80	~1%

[1] Willingham et al, Cancer Immunology Research, 2018.
[2] AB928, a dual antagonist of A_{2A}R and A_{2B}R adenosine receptors for the treatment of cancer (SITC, 2017).
[3] Preclinical pharmacodynamics and antitumor activity of AZD4635, a novel adenosine 2A receptor inhibitor that reverses adenosine mediated T cell suppression (AACR, 2017).
[4] Arcus Corporation press release (2020).

ADME, absorption, distribution, metabolism, and excretion; IC_{50}, half maximal inhibitory concentration; Ki, inhibition constant; NA, not available; pCREB, phospho–cyclic-AMP response element binding protein; WB, whole blood. Data on file (Incyte Corporation).

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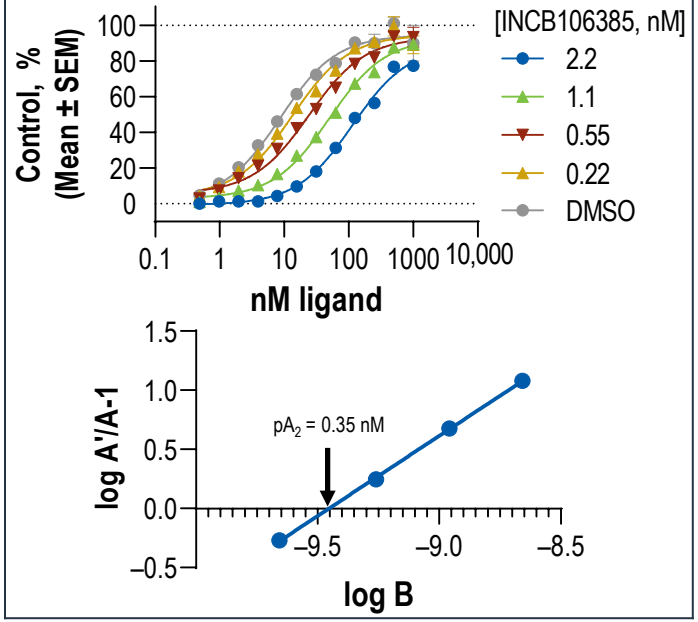
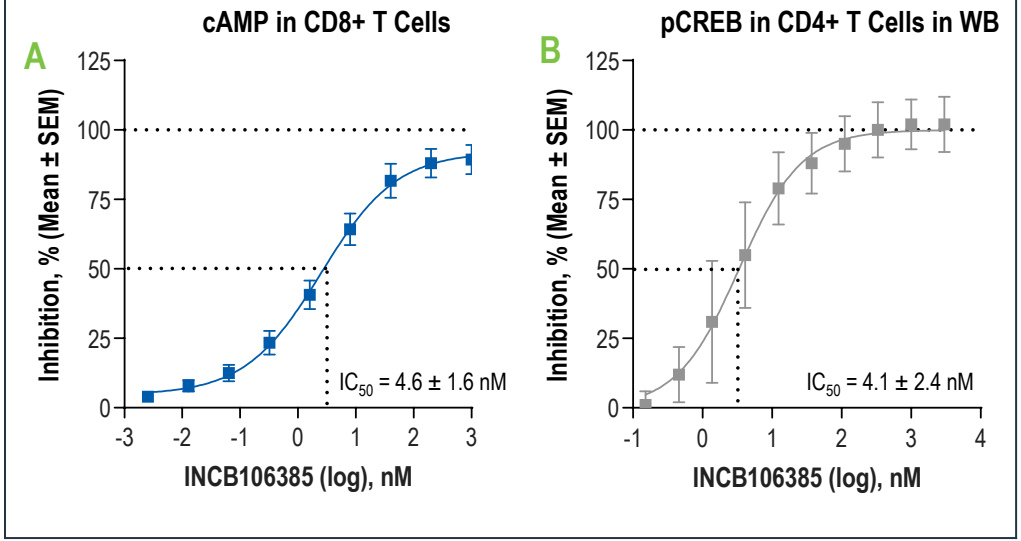
INCB106385 IS A POTENT AND COMPETITIVE ANTAGONIST OF A_{2A} AND A_{2B}

Schild Analysis Demonstrates INCB106385 Is a Competitive Antagonist



In the Presence of 5 µM NECA, INCB106385 Potently Inhibits (A) T-Cell cAMP Production and (B) pCREB in a WB Assay



cAMP, cyclic adenosine monophosphate; NECA, 5'-N-Ethylcarboxamidoadenosine; SEM, standard error of the mean. Data on file (Incyte Corporation).

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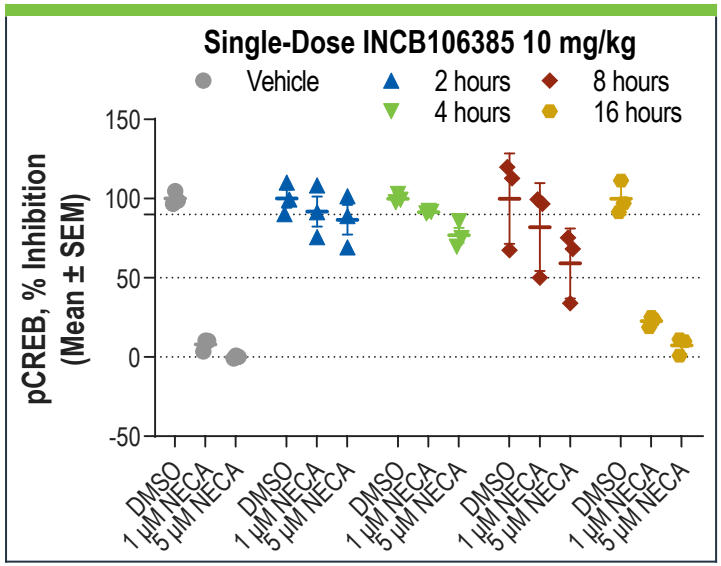
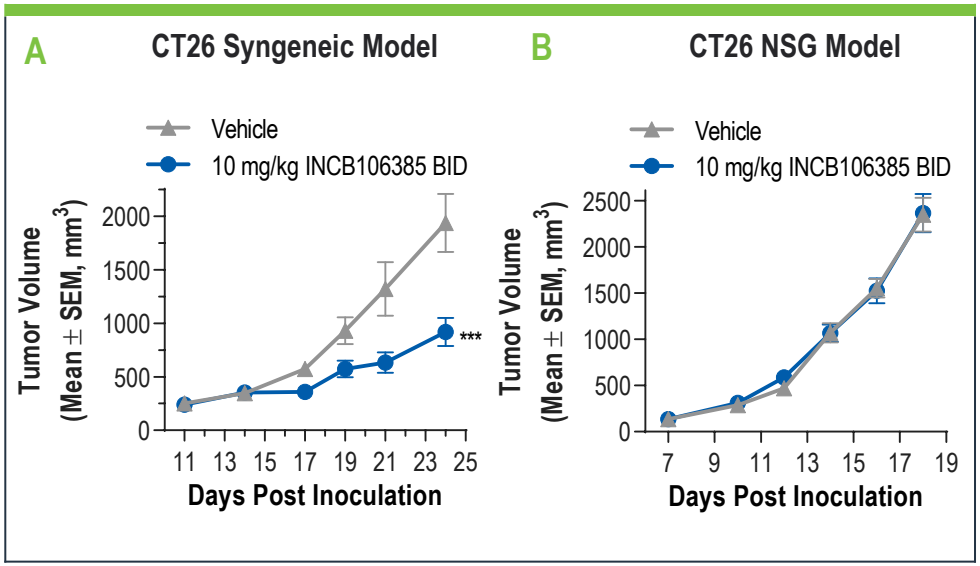
IN VIVO INCB106385 INHIBITS ADENOSINE PATHWAY SIGNALING AND INCREASES ANTITUMOR IMMUNE ACTIVITY

Pharmacodynamic Effect of INCB106385



- After a single oral dose of 10 mg/kg INCB106385 *in vivo*, pCREB levels in CD8+ T cells were measured in an *ex vivo* flow cytometry assay in the presence of 5 µM NECA treatment at indicated time points

In Vivo Efficacy of INCB106385



- In the CT26 syngeneic model (A), single-agent INCB106385 administered orally BID at 10 mg/kg achieved 52% tumor growth inhibition (***$P < 0.001$ by ANOVA with Fisher's least significant difference test)
- In the CT26 NSG model (B), which lacks T cells, B cells, and natural killer cells, INCB106385 yielded no efficacy with orally BID dosing at 10 mg/kg

ANOVA, analysis of variance; BID, twice daily. Data on file (Incyte Corporation).

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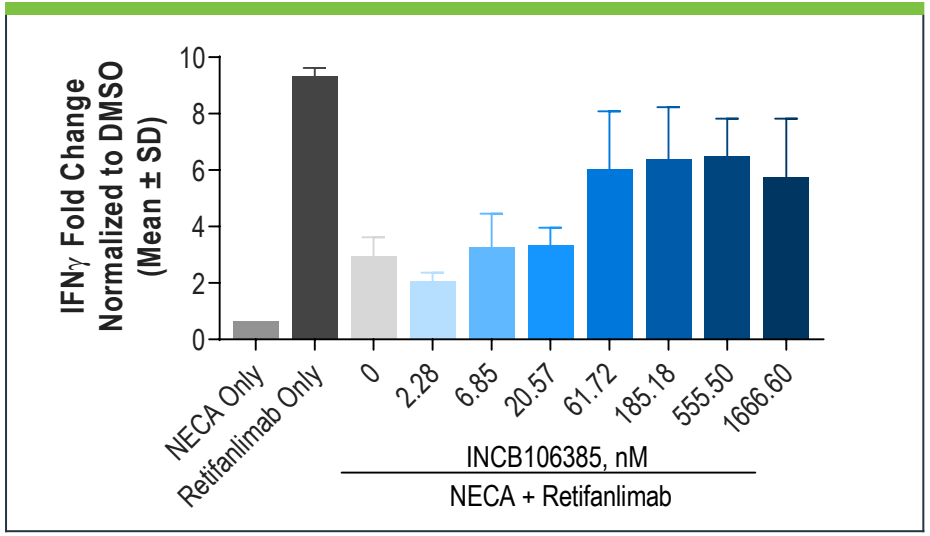
COMBINED A_{2A}/A_{2B} AND PD-1 INHIBITION RESTORES T-CELL ACTIVATION

INCB106385 and Retifanlimab Combination Reversed Suppression of T-Cell Activation in the Presence of High Levels of NECA



- In a co-culture system composed of primary T cells and PD-L1–expressing aAPC CHO-K1 cells, the effect of retifanlimab (PD-1 antibody) on T-cell activation was suppressed by 2 µM NECA
- INCB106385 in combination with retifanlimab reversed the suppressive effect of NECA and restored IFNɣ production in a dose-dependent manner

aAPC, artificial antigen presenting cells; IFN, interferon; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; SD, standard deviation.
Data on file (Incyte Corporation).

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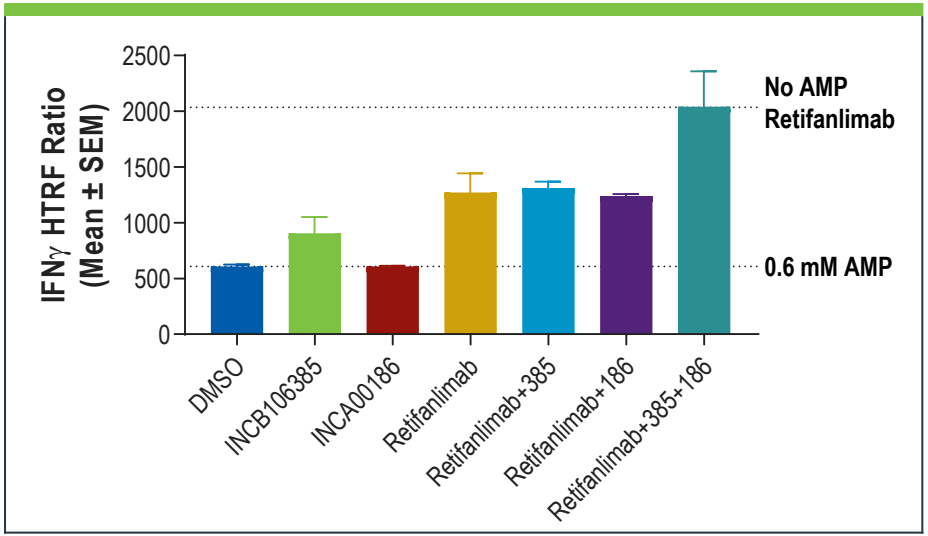
TRIPLE COMBINATION OF A_{2A}/A_{2B}, CD73, AND PD-1 BLOCKADE REVERSES ADENOSINE IMMUNOSUPPRESSION IN HUMAN T CELLS

Triple Combination of INCB106385, INCA00186, and Retifanlimab Yielded Maximal T-Cell Activation Under High Concentration of AMP



- In an MLR assay, T-cell activation measured by IFNγ production was suppressed by 0.6 mM AMP
- Triple combination of INCB106385 (100 nM), INCA00186 (CD73 antibody, 5 µg/mL), and retifanlimab (PD-1 antibody, 5 µg/mL) reversed the suppressive effect of AMP and restored IFNγ production

HTRF, homogeneous time-resolved fluorescence; MLR, mixed lymphocyte reaction. Data on file (Incyte Corporation).

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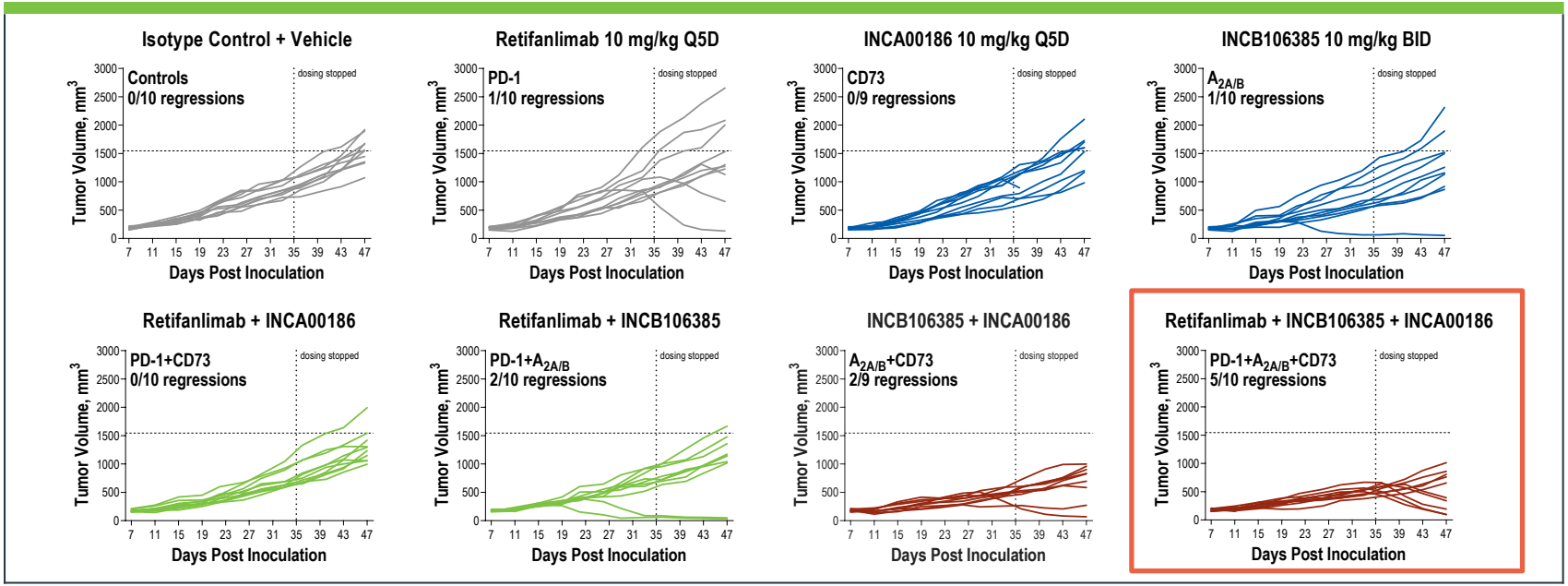
INCB106385 in Combination With PD-1 And CD73 Antibody Achieved Highest Antitumor activity in a Humanized Cancer Model



- In human CD34+ engrafted NSG model engrafted with MDA-MB-231 tumors, triple combination of INCB106385, INCA00186, and retifanlimab yielded the highest response (5/10 regressions)

Tumors were considered regressions if the tumor volume on day 47 was less than 500 mm^3 and there were at least 2 measurements with decreasing tumor volumes. Q5D, every 5 days. Data on file (Incyte Corporation).

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CONCLUSIONS

- INCB106385 is a potent and selective dual antagonist of adenosine receptor A_{2A} and A_{2B} and antagonizes downstream signaling of adenosine receptors
- INCB106385 restored effector T-cell activity in the presence of high concentrations of adenosine, which mimics the TME
- INCB106385 inhibited pharmacodynamic effect *in vivo* and displayed antitumor activity in the CT26 syngeneic tumor model
- INCB106385 overcame adenosine-mediated immunosuppression, in combination with retifanlimab and INCA00186 *in vitro*
- Triple combination of INCB106385, retifanlimab, and INCA00186 displayed enhanced antitumor activity in MDA-MB-231 humanized model *in vivo*
- Preclinical characterization of INCB106385 supports the ongoing clinical trial of this compound in patients with cancer (NCT04580485)

DISCLOSURES

- **Wang, Fanuka, Hansbury, Mason, Harris, Stevens, Maddage, Ren, Gao, Kurzeja-Lipinski, Yang, Conlen, Stump, Feldman, Thekkat, Lin, Covington, Yeleswaram, Qi, Wang, Yao, Kim, Wee, Chen, Koblish:** Employment or former employment, and stock ownership – *Incyte Corporation*

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