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INCA33890, a Novel TGFβR2×PD-1 Bispecific Antibody Conditionally Antagonizes TGFβ Signaling in Primary Immune Cells Co-expressing PD-1

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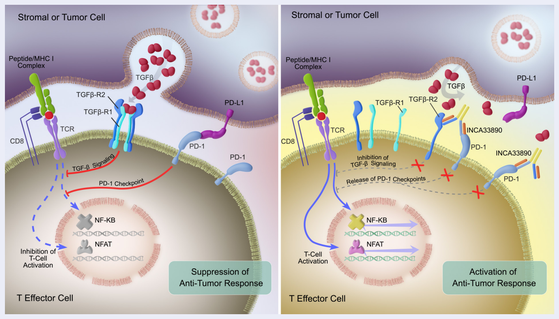
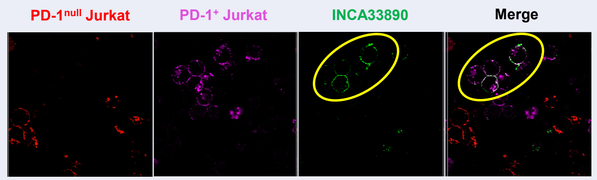
Abstract

Transforming growth factor-β (TGFβ) signaling is common in many solid tumors and is initiated by binding of the high-affinity canonical ligands TGFβ1, 2, or 3 to TGFβR2, which forms a heteromeric receptor complex with TGFβR1.[1] Activation of the pathway results in potent suppression of immune cell–mediated antitumor immunity and has been reported to predict poor response to programmed cell death protein-1 (PD-1) and its ligand (PD-L1) targeted therapy in patients.[2,3] However, TGFβ drug development has been hampered by the occurrence of adverse events.

INCA33890 is a dual PD-1– and TGFβR2-binding bispecific Biclonics® antibody, developed to antagonize the TGFβ signaling pathway specifically in cells co-expressing PD-1 and TGFβR2. Additionally, it potently antagonizes the PD-1 axis independently of TGFβR2 co-expression. The cell-selective action of INCA33890 was designed to mitigate risks of the known adverse effects associated with TGFβ-pathway inhibition in tissues requiring active TGFβ signaling. INCA33890 mediates its specificity through a PD-1 binding arm with a >10-fold higher affinity relative to the TGFβR2-binding arm. Consistent with this profile, in isogenic Jurkat cells expressing TGFβR2 ± PD-1, INCA33890 potently inhibits TGFβ1-induced pSMAD activation in a PD-1–dependent manner. Additionally, in 2 independent PD-1 reporter assays, INCA33890 inhibited SHP recruitment and enhanced nuclear factor of activated T-cell activation with a potency within an order of magnitude to that of pembrolizumab. In mixed leukocyte reaction assays with exhausted primary human T cells, INCA33890 was found to induce a similar level of antitumor cytokine production as the combination of pembrolizumab and an anti-TGFβR2 antagonist monoclonal antibody (mAb). Treatment of primary ovarian ascites with INCA33890 ex vivo induced interferon-gamma (IFN-γ) production in all donors tested, whereas pembrolizumab had no activity. Similarly, in human CD34⁺ cell–engrafted NSG mice, INCA33890 significantly inhibited the growth of human MDA-MB-231 and A375 subcutaneous xenograft tumors, whereas pembrolizumab or an anti-TGFβR2 mAb had little or no monotherapy activity. INCA33890 had a balanced pharmacokinetic and potency profile and was well tolerated in nonhuman primates (NHPs) at exposures required for projected pharmacodynamic activity; there was no evidence of adverse effects in NHPs due to TGFβ-pathway blockade. Collectively, these results provide compelling data for an effective and specific approach to simultaneously antagonizing TGFβ and PD-1 signaling in tumors. Clinical development of INCA33890 in checkpoint inhibitor–resistant and other cancers has been initiated.

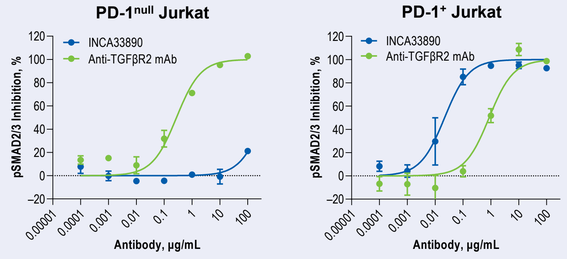


INCA33890 Selectively Binds to Cells Co-expressing TGFβR2 and PD-1



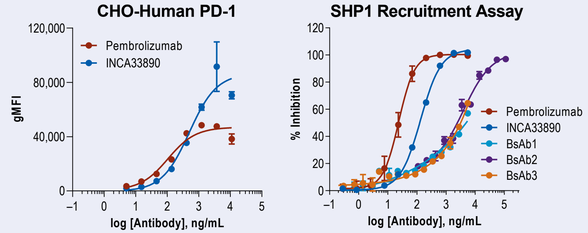
A mixture of isogenic Jurkat cells with (magenta) or without (red) PD-1 were fluorescently labeled with either WGA-AF647 or WGA-AF594, mixed, and stained with AF488-conjugated INCA33890. INCA33890 selectively bound to PD-1–expressing Jurkat cells.

INCA33890 Conditionally Inhibits pSMAD Activation in PD-1⁺ Jurkat Cells



An isogenic pair of Jurkat cells with or without PD-1 was stimulated with TGFβ1 in the presence or absence of INCA33890 or an anti-TGFβR2 mAb. Whereas the anti-TGFβR2 mAb inhibited pSMAD2/3 activation with similar potency in both cell lines, INCA33890 demonstrated high potency and selectivity only in PD-1–expressing Jurkat cells.

High-Affinity PD-1 Arm of INCA33890 Demonstrates Potent PD-1 Antagonism

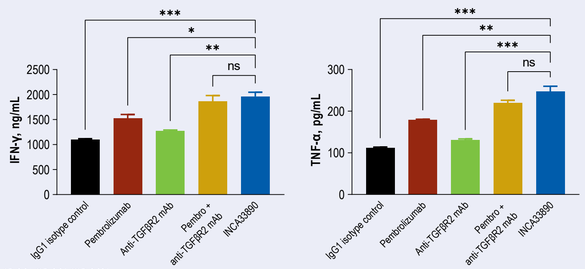


BsAb, bispecific antibody; gMFI, geometric mean fluorescence intensity.

CHO cells were engineered to over-express human PD-1, and INCA33890 demonstrated equipotent binding to these cells compared with pembrolizumab as measured by flow cytometry (left panel).

INCA33890 inhibited PD-1–mediated signaling to a similar extent as pembrolizumab in a PD-1–expressing reporter cell line, whereas other bispecific antibodies with lower-affinity PD-1 arms showed much weaker activity.

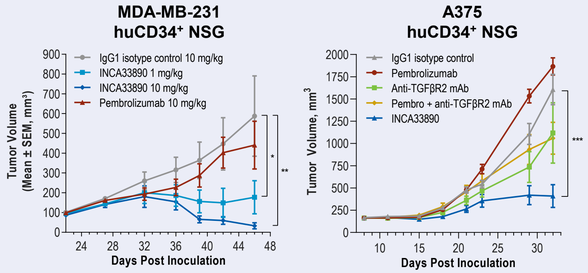
INCA33890 Induces IFN-γ and TNF-α in an Exhausted MLR Assay



*P<0.05; **P<0.01; ***P<0.001.
IFN-γ, interferon-gamma; MLR, mixed leukocyte reaction; ns, not significant; Pembro, pembrolizumab; TNF-α, tumor necrosis factor-alpha.

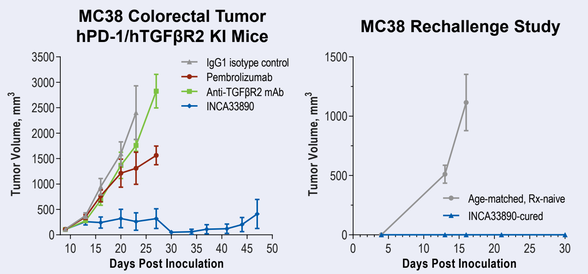
Exhausted mixed leukocyte reaction assay was set up by co-culturing pre-activated T cells with dendritic cells from other donors, in the presence or in the absence of various antibodies (10 μg/mL). INCA33890 induced a similar level of IFN-γ and TNF-α as the combination of anti–PD-1 and anti-TGFβR2 mAbs.

INCA33890 Inhibits Tumor Growth in PD-1–Resistant Preclinical Mouse Models



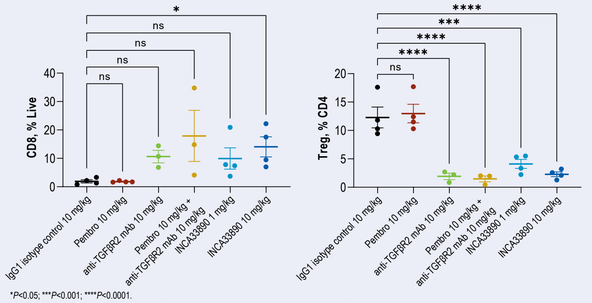
*P<0.05; **P<0.005; ***P<0.0005.

CD34⁺ cell–engrafted NSG mice bearing MDA-MB-231 (n=7/group) or A375 (n=10/group) xenografts were treated with various antibodies via intraperitoneal injection (10 mg/kg every 5 days), and tumor volumes were monitored. These 2 preclinical models were not responsive to pembrolizumab, and A375 xenografts were not responsive to anti-TGFβR2 mAb. INCA33890 significantly inhibited the growth of both MDA-MB-231 and A375 xenografts.



Transgenic mice with humanized PD-1 and TGFβR2 (hPD-1/hTGFβR2) bearing MC38 tumors (n=9/group) were treated via intraperitoneal injections with various antibodies (10 mg/kg twice weekly), and the tumor volumes were monitored over time. Both pembrolizumab and anti-TGFβR2 mAb had minimal effect to reduce tumor volume, whereas INCA33890 significantly inhibited tumor growth and induced complete responses in some mice ($P<0.001$). Those cured animals (N=4) developed immunity against MC38 from reoccurrence.

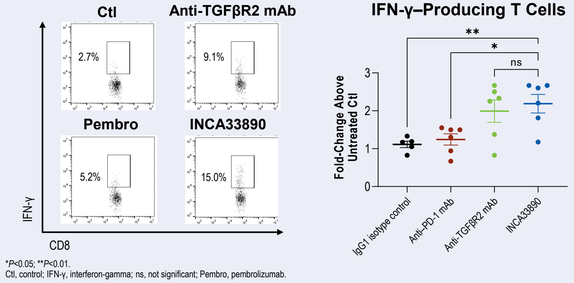
INCA33890 Increases Number of CD8 TILs While Reducing CD4 Treg



*P<0.05; ***P<0.001; ****P<0.0001.
ns, not significant; Pembro, pembrolizumab; TIL, tumor-infiltrating T lymphocyte; Treg, regulatory T cell.

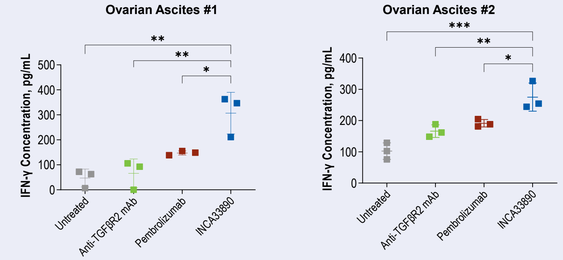
Transgenic hPD-1/hTGFβR2 mice bearing MC38 tumors were treated via intraperitoneal injections with various antibodies. Tumors were harvested 1 week post-treatment for flow cytometric analysis. INCA33890 showed a dose-dependent increase in number of CD8 T cells and a decrease in CD4 regulatory T cells.

INCA33890 Induces IFN-γ Production in Ex Vivo Patient Samples



*P<0.05; **P<0.01.
Ctl, control; IFN-γ, interferon-gamma; ns, not significant; Pembro, pembrolizumab.

NY-eso-specific T cells (Ly95) were cocultured with A549-eso cells in the presence of tumor digests from non-small cell lung cancer (NSCLC) patients. After antibody treatment (20 μg/mL), IFN-γ production of Ly95 T cells was analyzed. INCA33890 significantly increased the percentage of IFN-γ–producing Ly95 T cells over the isotype and pembrolizumab-treated samples.



***P<0.001; **P<0.01; *P<0.05.
IFN-γ, interferon-gamma.

Ovarian ascites were treated with various antibodies (10 μg/mL) for 4 days, and the culture supernatants were collected for assessment of IFN-γ production. INCA33890 significantly induced IFN-γ in all patients. Results for 2 representative ovarian ascites samples are shown.

Conclusions

- **INCA33890 is a Biclonic® antibody engineered to antagonize TGFβ-mediated signaling in T cells co-expressing TGFβR2 and PD-1, while demonstrating weak binding to normal cells expressing only TGFβR2 to avoid systemic toxicity**
- **High-affinity PD-1–binding arm of INCA33890 grants potent binding to PD-1⁺ T cells independent of TGFβR2 expression**
- **INCA33890 was shown to reactivate T cells to produce cytokines in multiple primary T-cell assays, including ex vivo samples from NSCLC and ovarian cancer patients**
- **Dual PD-1 and TGFβR2 blockade achieved greater antitumor efficacy than the anti–PD-1 and anti-TGFβR2 benchmark monoclonal antibodies in 3 preclinical mouse models, and increased CD8⁺ T-cell infiltration**
- **Clinical development of INCA33890 in checkpoint inhibitor–resistant and other cancers has been initiated**

Disclosures

Wang, Kulkarni, Soloviev, Huo, Weber, Harvey, He, Chang, Horsey, Volgina, Zhang, Pandey, Yang, Rios-Doria, Nastri, Mayes: Employment and stock ownership – *Incyte Corporation*. **Mondal:** Former employment and stock ownership – *Incyte Corporation*. **Klooster, Garcia de Vinuesa, Hendriks, de Kruif, Geuijen:** Employment and stock ownership – *Merus N.V.* **Albelda, Powell, Erulasnov:** Research funding – *Incyte Corporation*.

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