Abstract #7333

PD-L1-directed ISACs target host immune cells to drive powerful antitumor immune responses in a manner distinct from conventional PD-1/PD-L1 blockade

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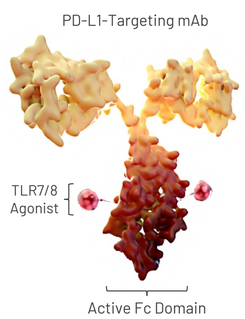
Background

Monoclonal antibodies targeting PD-1/PD-L1 have demonstrated clinical activity in numerous indications, but a large proportion of patients do not respond to anti-PD-(L)1 therapy, underscoring the need for alternative strategies. PD-L1 can be expressed by tumor cells as well as immune cells in the tumor microenvironment (TME) and tumor-draining lymph nodes. In particular, myeloid antigen-presenting cells (APCs) including macrophages and dendritic cells (DCs) can express high levels of PD-L1 and play a key role in regulating T cell responses through the PD-1/PD-L1 pathway. We are developing a novel class of molecules called Immune-Stimulating Antibody Conjugates (ISACs) that consist of tumor- or TME-targeting antibodies coupled to innate immune stimuli such as TLR7/8 agonists. Here, we present preclinical data demonstrating that PD-L1-targeted ISACs can engage PD-L1-expressing tumor cells as well as PD-L1-expressing myeloid cells to drive robust immune responses and antitumor activity superior to anti-PD-(L)1 mAbs. Our results indicate a critical role for immune cell-expressed PD-L1 in antitumor efficacy with a mechanism of action uncoupled from PD-1/PD-L1 blockade.

PD-L1 ISAC: Key Features



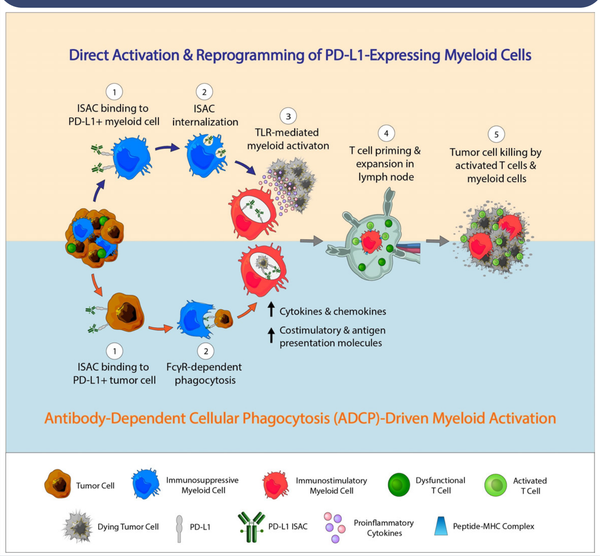
Next-Generation ISAC:
- Proprietary mAb and linker-payload
- Greater antitumor efficacy than PD-(L)1 blockers
- Favorable multi-dose safety profile in NHPs

Differentiated Mechanism of Action:
- Directly targets & reprograms PD-L1-expressing myeloid cells in the tumor microenvironment
- Enhances antigen presentation following phagocytosis of PD-L1-expressing tumor cells
- Elicits complete tumor regression and immunological memory

Broad Applicability:
- PD-L1 expression by either tumor or immune cells is sufficient to drive antitumor efficacy
- Opportunity in CPI-resistant indications

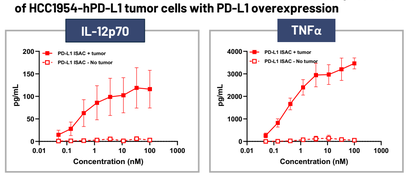
Combination Potential with CPIs:
- Distinct MoA that works by improving the function of antigen-presenting cells
- Complementary to T cell-directed CPI therapies

Mechanism of Action

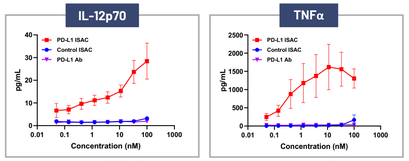


Myeloid Activation Through ADCP-Driven MoA

PD-L1 ISAC activates conventional dendritic cells (cDCs) in presence of HCC1954-hPD-L1 tumor cells with PD-L1 overexpression

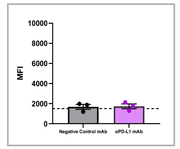


PD-L1 ISAC activates cDCs in presence of HCC1954 tumor cells with endogenous PD-L1 expression



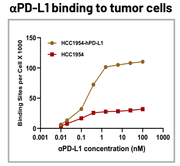
Conventional dendritic cell (cDC)-enriched primary human cells were cocultured with or without indicated tumor cells in the presence of PD-L1 ISAC. After 18 hours, supernatants were collected and analyzed for cytokine secretion. Data shown as mean with SEM for n=3 donors.

Peripheral blood cDCs lack PD-L1 expression



PD-L1 expression on healthy peripheral blood cDCs was evaluated by flow cytometry using commercial αPD-L1 antibody. Data shown as mean with SEM for n=3 donors.

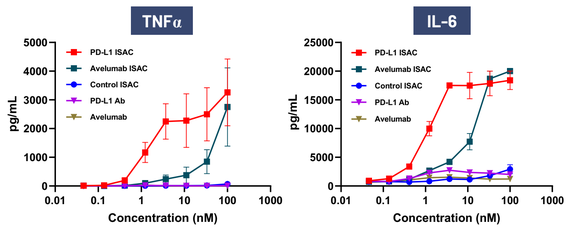
αPD-L1 binding to tumor cells



αPD-L1 mAb binding to tumor cell lines was quantified by flow cytometry using bead-based standards.

Direct Activation of PD-L1-Expressing Myeloid Cells

Monocyte-Derived Dendritic Cells Pretreated with IFNγ

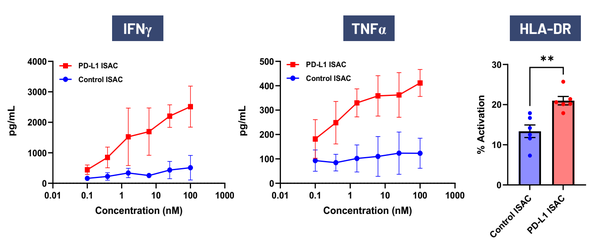


- PD-L1-targeted ISAC elicits strong cytokine responses from PD-L1-expressing dendritic cells
- ISACs made with proprietary αPD-L1 mAb outperform benchmark mAb (avelumab)
- Similar effects observed on macrophages polarized to express high levels of PD-L1

Human monocytes from healthy donors were differentiated into monocyte-derived dendritic cells with GM-CSF and IL-4. To induce PD-L1 expression, cells were primed with IFNγ for an additional 48 hours. Polarized myeloid cells were stimulated for 18 hours with indicated test article and supernatants were analyzed for cytokine secretion. Data shown as mean with SEM for n=3 donors.

PD-L1 ISAC-Activated Myeloid Cells Induce T Cell Responses

ISAC-Activated DCs + Allogeneic CD4⁺ T cells

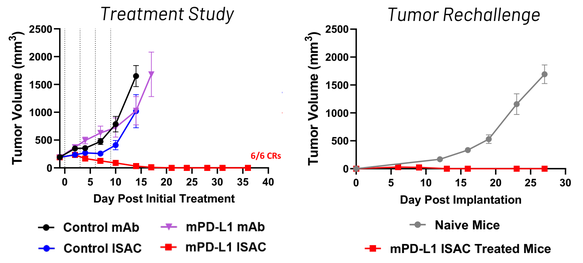


- PD-L1+ DCs activated w/ PD-L1 ISAC elicit strong T cell responses in mixed lymphocyte reaction
- ISAC-activated DCs induce T cell cytokine production & activation marker expression (HLA-DR)

Human Mo-DCs were primed with IFNγ for 48 hours to upregulate PD-L1 expression. IFNγ primed Mo-DCs were then cocultured with the indicated test articles and allogeneic CFSE-labeled CD4+ T cells at a 1:5 (DC:T cell) ratio for five days. T cell proliferation (measured by CFSE dilution) was assessed using flow cytometry, and multiplex cytokine analysis was performed using MSD. Data are presented as the mean ± SEM for n=6 donors.

PD-L1 ISAC Elicits Complete Responses & Immune Memory

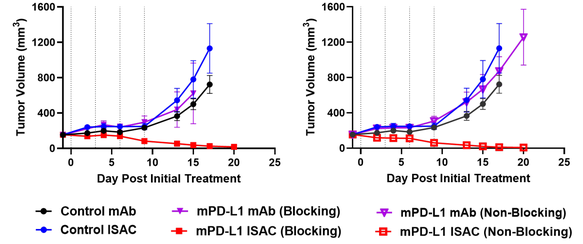
Syngeneic MB49 Tumor Model



- PD-L1 ISAC elicits complete responses (CRs) in model resistant to anti-PD-L1 mAb therapy
- Mice with CRs reject tumors upon rechallenge, indicating induction of immunological memory

Mice bearing 200 mm³ MB49 tumors on average were treated with 10 mg/kg of the indicated test article (Q3Dx4). Data are shown as mean and SEM with n=6 per group. MB49 tumor-bearing mice with CRs following treatment with mPD-L1 ISAC were rechallenged with MB49 cells ~1 month after tumor clearance (7 weeks post final dose). Naïve mice (n=5) were challenged with MB49 tumors as a control.

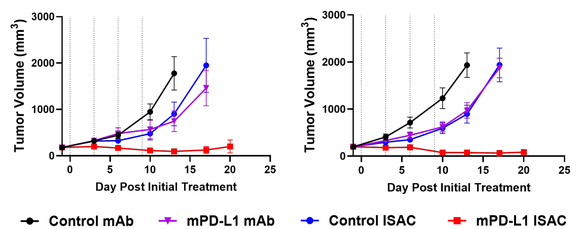
PD-1/PD-L1 Blockade Is Not Essential for Efficacy



- PD-L1 ISACs exhibit similar efficacy whether or not the mAb blocks PD-L1 binding to PD-1
- PD-L1 ISAC has distinct MoA from PD-1/PD-L1 mAbs that does not rely on checkpoint blockade

Mice bearing 200 mm³ MB49 tumors on average were treated with 5 mg/kg of the indicated test article (Q3Dx4). Data are shown as mean and SEM with n=6 per group. Commercially available mPD-L1 mAbs were used to prepare surrogate mPD-L1 ISACs. PD-L1-blocking mAb (clone 10F.9G2) blocks binding to PD-1, while non-blocking mAb (clone 10F.2H11) does not block binding to PD-1.

Tumor PD-L1 Expression Is Not Required for Efficacy

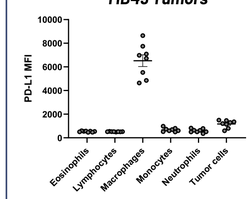


- PD-L1 ISAC shows similar efficacy whether or not tumor cells express PD-L1 in syngeneic model
- Targeting of PD-L1 on host immune cells is sufficient to drive antitumor efficacy

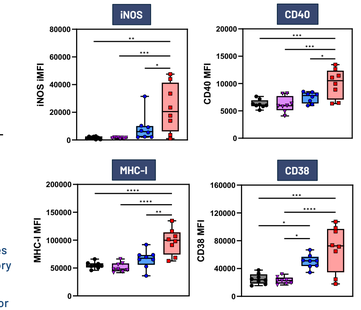
Mice bearing 200 mm³ MB49 tumors (parental or PD-L1 KO) on average were treated with 5 mg/kg of the indicated test article (Q3Dx4). Data are shown as mean and SEM with n=6 per group. PD-L1 KO MB49 cells were generated using CRISPR-Cas9 system. PD-L1 expression was confirmed on both tumor and immune cells from parental MB49 tumors via flow cytometry, while no PD-L1 expression was found on PD-L1 KO tumor cells (data not shown).

PD-L1 ISAC Activates PD-L1-Expressing TAMs *In Vivo*

PD-L1 Expression in MB49 Tumors



Tumor-Associated Macrophages (TAMs)

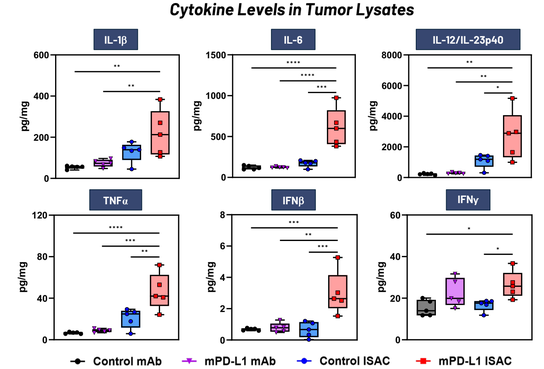


- PD-L1 expression is highest on TAMs, with much lower levels on tumor cells
- Treatment with PD-L1 ISAC upregulates markers associated w/ proinflammatory macrophage polarization
- Reprogrammed TAMs have potential for enhanced antigen presentation (MHC-I), T-cell costimulation (CD40), and cytotoxic activity (iNOS)

Mice bearing MB49 tumors were treated with a single dose of 5 mg/kg of the indicated test article with n=8 per group. Tumors were collected 14 hours post treatment and analyzed by flow cytometry. Median fluorescence intensity (MFI) or integrated MFI (iMFI) values are shown. Statistical analyses were performed using one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

PD-L1 ISAC Elicits Proinflammatory Cytokine Response in TME

Cytokine Levels in Tumor Lysates



- PD-L1 ISAC elicits profound increases in cytokines that promote antitumor immune responses
- Treatment with PD-L1 mAb had minimal effects on TAM polarization & cytokine milieu

Mice bearing MB49 tumors were treated with a single dose of 5 mg/kg of the indicated test article with n=5 per group. Tumors were collected 6 hours post treatment and lysate samples were analyzed using MSD multiplex assay. Statistical analyses were performed using one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Summary

- PD-L1 ISAC combines ADCP-driven MoA with direct activation of PD-L1-expressing myeloid cells to promote powerful antitumor immune responses
- PD-L1 ISACs elicit complete regressions and immunological memory in models that are resistant to PD-1/PD-L1 checkpoint inhibitor therapy
- Mechanistic studies indicate that PD-L1 expression by either tumor or immune cells is sufficient to drive antitumor efficacy
- PD-L1 ISACs directly activate and reprogram PD-L1-expressing myeloid cells in the TME to promote innate and adaptive antitumor immunity
- Blockade of the PD-1/PD-L1 axis is not required for PD-L1 ISAC efficacy but may be a supportive mechanism & complementary combination strategy
- Favorable safety profile demonstrated in non-GLP NHP toxicology studies supporting use in combination with SoC therapies & other agents