Abstract #994

Discovery of a PD-L1-directed ISAC optimized for the activation of PD-L1-expressing myeloid cells to drive potent antitumor immune responses

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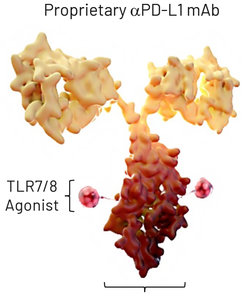
Background

PD-L1-directed immune-stimulating antibody conjugates (ISACs) have shown compelling efficacy in preclinical models, demonstrating the potential to treat tumors resistant to conventional immune checkpoint inhibitors (ICIs) and/or improve responses to ICIs when used in combination. PD-L1 is a unique target for ISACs and other antibody-drug conjugates as it can be expressed on both tumor and immune cells. Myeloid cells such as 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 have shown that PD-L1 expression by tumor cells is dispensable for antitumor efficacy with PD-L1 ISACs in syngeneic models, indicating a critical role for PD-L1-expressing immune cells in the mechanism of action. However, the key immune cell targets of PD-L1 ISACs and the properties affecting their immunostimulatory activity remained unclear. Here, we show that PD-L1 ISACs activate PD-L1-expressing myeloid cells in vitro and in vivo, with powerful immunostimulatory effects observed in both tumors and tumor-draining lymph nodes. Further, we demonstrate that our lead PD-L1 ISAC utilizing a dual TLR7/8 agonist payload and Fc-competent antibody has the potential for stronger myeloid cell activation and antitumor efficacy than alternative embodiments.

PD-L1 Boltbody™ ISAC: Key Features

Proprietary αPD-L1 mAb



Next-Generation ISAC:

- Anti-PD-L1 human IgG1 conjugated to potent TLR7/8 agonist through non-cleavable linker
- Greater antitumor activity than PD-(L)1 blockers with potential to combine for enhanced efficacy
- Favorable multi-dose safety profile in NHPs

Differentiated Mechanism of Action:

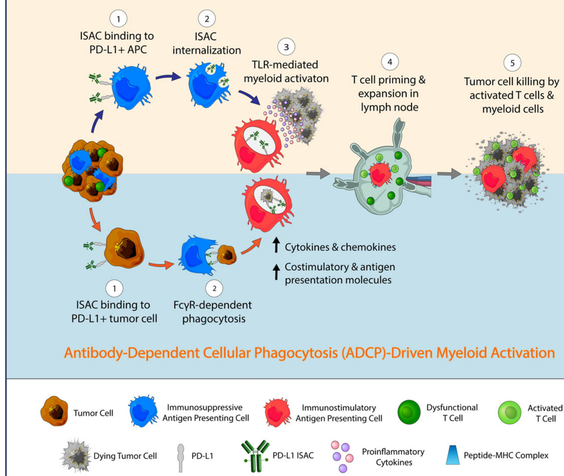
- Distinct MoA from PD-(L)1 inhibitors—checkpoint blockade not required
- Targets & reprograms PD-L1-expressing myeloid cells in tumors & lymphoid tissues
- Drives robust proinflammatory cytokine responses & increases in antigen presentation machinery
- Elicits complete tumor regression and immunological memory

Broad Applicability:

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

Mechanism of Action

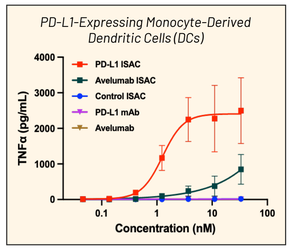
Direct Activation & Reprogramming of PD-L1-Expressing Myeloid Cells



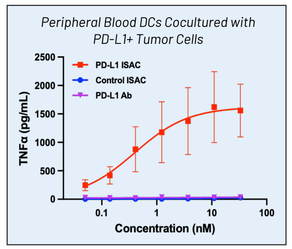
Antibody-Dependent Cellular Phagocytosis (ADCP)-Driven Myeloid Activation

PD-L1 ISAC Activates Myeloid Cells Through Two Key MoAs

Direct Activation of PD-L1-Expressing Myeloid Cells



ADCP-Driven Activation of Myeloid Cells

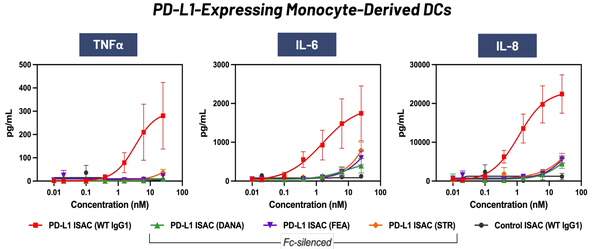


- PD-L1 ISACs can activate myeloid cells directly if they express PD-L1, or indirectly through an ADCP-driven mechanism when in the presence of PD-L1-expressing tumor cells
- Bolt's PD-L1 ISAC utilizing proprietary anti-PD-L1 mAb shows activity superior to avelumab ISAC using same linker-payload

(Left) Human monocytes from healthy donors were differentiated into DCs with GM-CSF and IL-4 and subsequently treated IFNγ to induce PD-L1 expression. Cells were incubated overnight with indicated test article and supernatants were analyzed for cytokine secretion. Data shown as mean with SEM (n=3). (Right) Conventional DC-enriched primary cells from healthy donor blood were cocultured with HCC1954 cells overnight in the presence of indicated test article. Supernatants were analyzed for cytokine secretion. Data shown as mean with SEM (n=3).

Fc-Competent PD-L1 ISAC Activates PD-L1+ Myeloid Cells

PD-L1-Expressing Monocyte-Derived DCs

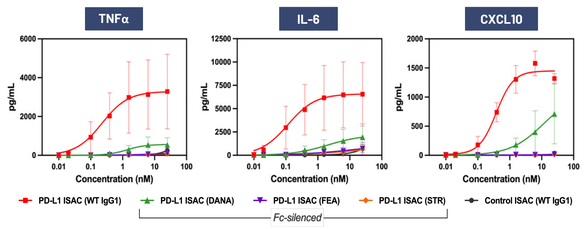


- PD-L1 ISAC with wild-type IgG1 Fc domain robustly activates PD-L1-expressing myeloid cells
- Much weaker responses observed with a series of PD-L1 ISACs made using Fc-silenced mAbs

Human monocytes from healthy donors were differentiated into DCs 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 the indicated test article and supernatants were analyzed for cytokine secretion. PD-L1 ISACs were prepared using IgG1 Fc variants (indicated in parentheses) of proprietary mAb and non-cleavable TLR7/8 agonist linker-payload. Data are shown as mean with SEM (n=3).

Robust ADCP-Driven Myeloid Activation Requires Active Fc

Peripheral Blood DCs Cocultured with PD-L1+ Tumor Cells

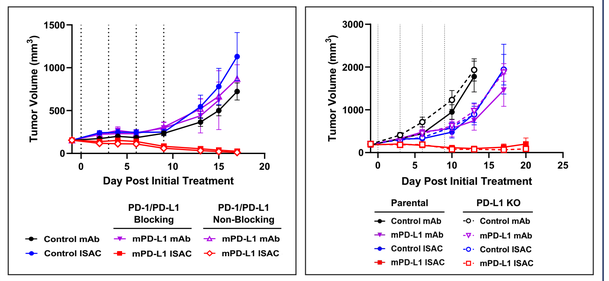


- Fc-competent ISAC elicits robust activation of myeloid cells in presence of PD-L1+ tumor cells
- Fc-silenced PD-L1 ISACs elicit weaker responses, with activity closer to control ISAC

Conventional DC-enriched primary human cells were isolated from healthy donor blood and cocultured with HCC1954 tumor cells that overexpress PD-L1. Cocultures were stimulated overnight with indicated test article and supernatants were analyzed for cytokine secretion. PD-L1 ISACs were prepared using IgG1 Fc variants (indicated in parentheses) of proprietary mAb and non-cleavable TLR7/8 agonist linker-payload. Data are shown as mean with SEM (n=3).

PD-L1 ISAC Elicits Antitumor Activity Through Unique MoA

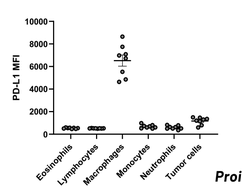
Syngeneic MB49 Tumor Model



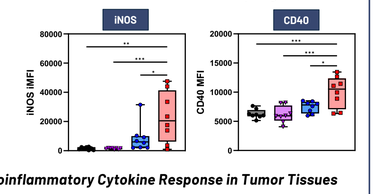
(Left) Mice bearing MB49 tumors (~200 mm³) were treated with 5 mg/kg of indicated test article (Q3Dx4). mPD-L1 ISACs were prepared using Fc-competent anti-mPD-L1 mAbs (rat IgG2b) that block (clone 10F.9G2) or do not block (clone 10F.2H11) binding to PD-1. (Right) Mice bearing parental or PD-L1 KO MB49 tumors were treated with 5 mg/kg of indicated test article (Q3Dx4). Data shown as mean with SEM (n=6/group).

PD-L1 ISAC Activates PD-L1-Expressing TAMs in TME

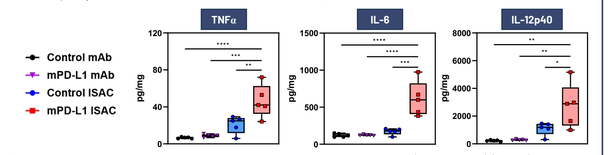
High PD-L1 Expression by Macrophages in MB49 Tumors



Proinflammatory Repolarization of Tumor-Associated Macrophages (TAMs)



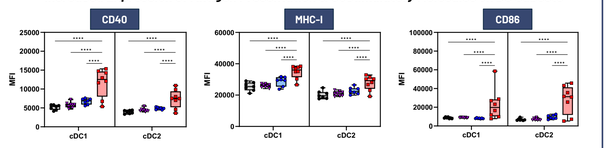
Proinflammatory Cytokine Response in Tumor Tissues



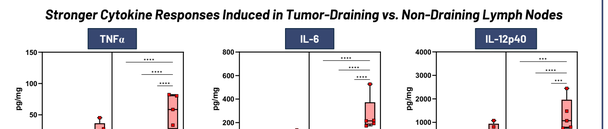
Mice bearing MB49 tumors were treated with a single dose of 5 mg/kg of the indicated test article (n= 5-8 per group). (Top row) Tumors were collected 14 hr post treatment and analyzed by flow cytometry. Median fluorescence intensity (MFI) or integrated MFI (iMFI) values are shown. (Bottom row) Tumors were collected 6 hr post treatment and cytokine levels were measured in tissue lysates. Statistical analyses were performed using one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

PD-L1 ISAC Activates Dendritic Cells in Tumor-Draining LNs

Increased Expression of Antigen Presentation & Costimulatory Molecules on DC Subsets



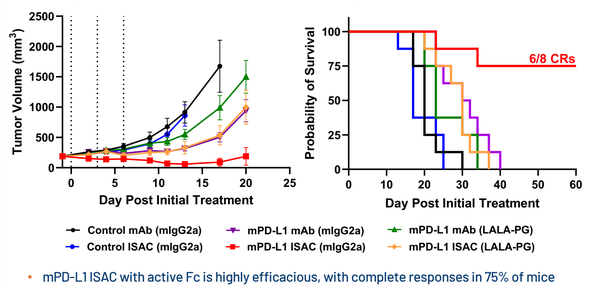
Stronger Cytokine Responses Induced in Tumor-Draining vs. Non-Draining Lymph Nodes



Mice bearing MB49 tumors were treated with a single dose of 5 mg/kg of the indicated test article (n=5-8/group). (Top row) Conventional DC subsets (cDC1 and cDC2) in tumor-draining lymph nodes (LNs) were analyzed by flow cytometry at 14 hr post treatment. Median fluorescence intensity (MFI) values are shown. (Bottom row) Cytokine levels in lysates from tumor-draining and contralateral non-draining LNs were measured at 6 hr post treatment. Statistical analyses were performed using one-way ANOVA. ***p < 0.001, ****p < 0.0001.

Fc-Competent PD-L1 ISAC Demonstrates Superior Efficacy

Syngeneic MB49 Tumor Model

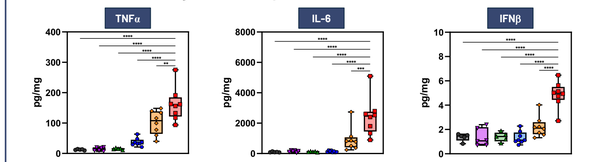


- mPD-L1 ISAC with active Fc is highly efficacious, with complete responses in 75% of mice
- Fc-silenced mPD-L1 ISAC (LALA-PG variant) shows weaker efficacy, similar to mPD-L1 mAb

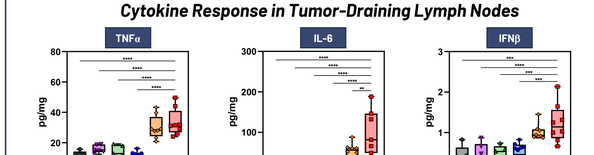
Mice bearing MB49 tumors (~200 mm³) were treated with 5 mg/kg of the indicated test article (Q3Dx3). mPD-L1 ISACs were prepared using commercially sourced variants of anti-mPD-L1 mAb clone 10F.9G2 as wild-type mIgG2a or Fc-silenced version (LALA-PG). 6 out of 8 mice treated with Fc-competent ISAC achieved long-term complete responses. Data are shown as mean with SEM (n=8/group).

Fc-Competent PD-L1 ISAC Elicits Enhanced Immune Responses

Cytokine Response in Tumor Tissues



Cytokine Response in Tumor-Draining Lymph Nodes



- mPD-L1 ISACs elicit proinflammatory cytokine responses in tumors & tumor-draining LNs
- Fc-competent mPD-L1 ISAC exhibits enhanced immunostimulatory effects, especially in tumors

Mice bearing MB49 tumors were treated with a single dose of 5 mg/kg of indicated test article (n=8/group). Tumors and tumor-draining lymph nodes were collected 6 hr post treatment and cytokine levels were measured in tissue lysates. Statistical analyses were performed using one-way ANOVA with multiple comparisons testing between mPD-L1 ISAC (mIgG2a) and other groups. **p < 0.01, ***p < 0.001, ****p < 0.0001.

Summary

- Bolt's PD-L1 ISAC, comprising a proprietary anti–PD-L1 mAb & TLR7/8 agonist linker-payload, was designed to achieve robust activation of key human myeloid cell populations to drive powerful antitumor immune responses
- Treatment with PD-L1 Boltbody™ ISAC activates & reprograms myeloid cells in tumors & tumor-draining lymph nodes, leading to a potent adaptive immune response that clears tumors & prevents recurrence in preclinical models
- Mechanistic studies demonstrate a highly differentiated MoA—distinct from & potentially complementary to checkpoint inhibitors & cytotoxic ADCs
- Bolt's PD-L1 ISAC with an intact Fc domain & TLR7/8 dual agonist payload has the potential for enhanced immunostimulatory effects & antitumor activity compared to other formats
- Favorable safety profile demonstrated with PD-L1 Boltbody™ ISACs in non-GLP NHP studies, supporting use in combination with SoC therapies & other agents
- Ready for IND-enabling studies & available for partnering