PD-L1-targeted ISAC combines myeloid cell activation, immune-checkpoint inhibition and ADCP to improve anti-tumor efficacy over anti-PD-L1 antibodies in preclinical models

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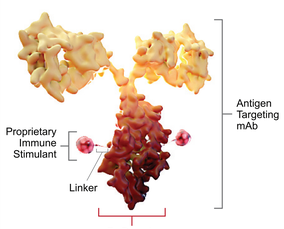
INTRODUCTION

Immune-stimulating antibody conjugates (ISACs) consist of tumor-targeting antibodies conjugated to immune stimulants (such as TLR7/8 agonists) and are designed to activate the innate and adaptive immune systems against tumor cells following systemic administration. PD-L1 is an immune checkpoint molecule that regulates anti-tumor T cell responses and is expressed on tumor cells as well as tumor-infiltrating immune cells across many tumor types. Antibody-mediated blockade of the PD-L1/PD-1 axis is a clinically validated therapeutic strategy in oncology; however, there remains a large proportion of patients that do not benefit from anti-PD-L1/PD-1 therapy. We evaluated PD-L1-targeted TLR7/8 ISACs in preclinical studies and demonstrate their potential to improve upon the therapeutic activity of PD-L1/PD-1 inhibitors by combining three complementary mechanisms of action into a single molecule: TLR-mediated myeloid cell activation, T cell activation through immune checkpoint inhibition, as well as antibody-dependent cellular phagocytosis (ADCP).

Immune-stimulating antibody conjugates (ISACs) consist of tumor-targeting antibodies conjugated to immune stimulants



Immune-stimulating antibody conjugates (ISACs):[1]

- Systemically delivered, tumor-targeting therapeutics
- Antibody against a tumor-associated antigen directs Boltbody™ ISAC to the tumor
- Proprietary immune stimulant activates myeloid antigen-presenting cells
- Myeloid cells kill tumor cells, create a "hot" tumor microenvironment, and initiate an innate & adaptive anti-tumor immune response

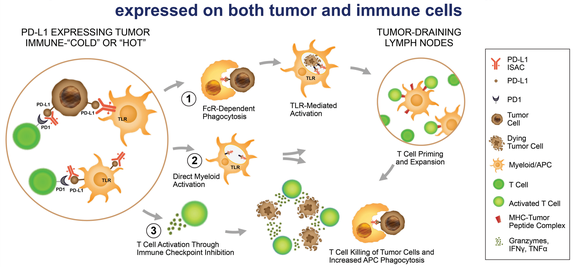
PD-L1 ISAC:

- PD-L1 targeting antibody conjugated to TLR7/8 agonist

Clinical experience with ISACs:

- BDC-1001[2] (HER2 ISAC) is currently being evaluated in a Phase 1/2 clinical study

Proposed mechanism of action: PD-L1 ISAC can act through PD-L1 expressed on both tumor and immune cells



PD-L1 ISAC combines three mechanisms to activate the innate and adaptive immune systems to drive anti-tumor immunity:

1. Phagocytosis of PD-L1+ tumor cells followed by TLR-mediated myeloid activation and subsequent T cell activation
2. Direct targeting of PD-L1+ myeloid cells and activation through TLR stimulation
3. T cell activation through immune-checkpoint inhibition

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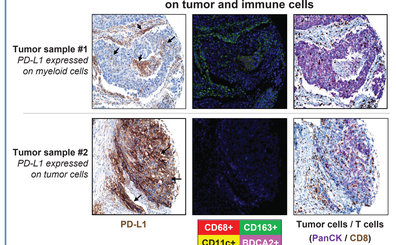
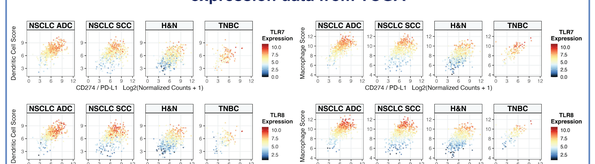
RESULTS

PD-L1 is an immune checkpoint molecule expressed by both tumor and immune cells



- Serial sections from two different human triple-negative breast cancer (TNBC) tumors were stained with PD-L1 IHC (22c3), a CD8/PanCK dual IHC, and a custom quadruplex IF assay for identification of myeloid APC sub-populations.
- Consistent with prior literature,[3,4] PD-L1 expression was detected on tumor and immune cells.

PD-L1 correlates with myeloid cell scores and TLR7/8 in bulk gene expression data from TCGA



- Tumors with high myeloid cell scores and TLR7/TLR8 expression also show high PD-L1 gene expression
- Targeting PD-L1 with an ISAC may provide for TLR-mediated myeloid cell activation in addition to immune-checkpoint blockade

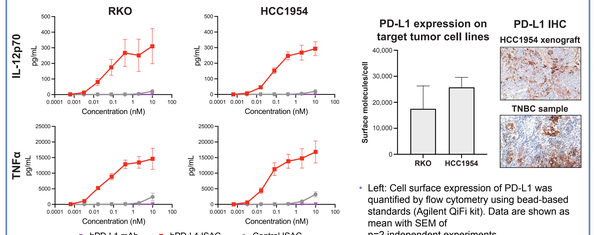
Gene expression data from The Cancer Genome Atlas (TCGA) was downloaded via Xena browser (xenabrowser.net). Gene expression was related to myeloid cell scores[5] based on their corresponding TCGA sample ID numbers. Data include NSCLC adenocarcinoma (NSCLC ADC, N=522), NSCLC squamous cell carcinoma (NSCLC SCC, N=527), head and neck cancer (H&N, N=560), and triple-negative breast cancer (TNBC, N=127)

PD-L1 and TLR7/8 are co-expressed by tumor-associated myeloid cells

- PD-L1 expression is enriched in myeloid lineage cells relative to other cell types, and overlaps with expression of TLR7 and/or TLR8
- TLR7 is expressed by myeloid cells as well as B cells and plasma cells, whereas TLR8 expression is restricted to myeloid cells

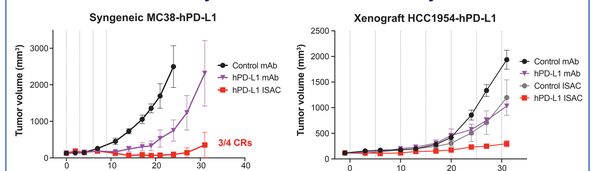
Cells from colorectal tumors and adjacent normal tissues (n=62 patients; 371,223 cells) were analyzed by single-cell RNA sequencing (scRNA-Seq), as originally described by Pelka[6] (dataset GSE178341). Data are visualized using t-distributed stochastic neighbor embedding (t-SNE). Plots depicting major cell clusters and expression levels of the indicated genes were obtained from the Broad Single Cell Portal (https://singlecell.broadinstitute.org/single_cell).

PD-L1 ISAC activates cDCs co-cultured with tumor cells expressing physiological levels of PD-L1



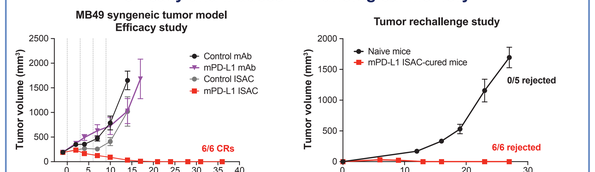
- Conventional dendritic cell (cDC)-enriched primary human cells were co-cultured with RKO (human colon cancer) or HCC1954 (human breast cancer) tumor cells (10:1 E:T ratio) in the presence of indicated mAbs or ISACs. After 18 hours, supernatants were collected and analyzed for cytokine secretion. Data shown as mean with SEM for n=3 donors.
- Left: Cell surface expression of PD-L1 was quantified by flow cytometry using bead-based standards (Agilent QiFi kit). Data are shown as mean with SEM of n=2 independent experiments.
- Right: PD-L1 expression on HCC1954 tumors in vivo was evaluated with PD-L1 IHC (22c3, Dako). For comparison, an example of PD-L1 expression in a human TNBC specimen is shown.

Human PD-L1-specific ISAC targeting only tumor cells exhibits improved activity over naked hPD-L1 antibody



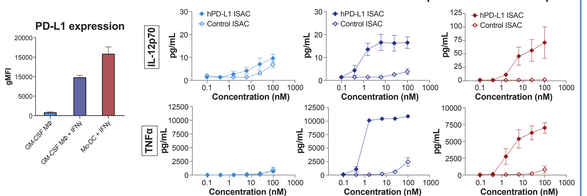
- Syngeneic MC38-hPD-L1 (left panel) or xenograft HCC1954-hPD-L1 (right panel) tumors were treated with the indicated mAbs or ISACs at 5 mg/kg IP (MC38, Q3Dx4; HCC1954, Q5Dx7) (n=4-5 per group).
- The PD-L1 mAbs and ISACs used react specifically with human but not mouse PD-L1, and thus target tumor but not immune cell expressed PD-L1.
- Treatment with hPD-L1 ISACs resulted in improved anti-tumor efficacy compared to naked hPD-L1 antibodies, with complete response (CRs) in 3 of 4 mice in the syngeneic model.

mPD-L1 ISAC targeting both tumor and immune cells shows profound efficacy and induces immunological memory



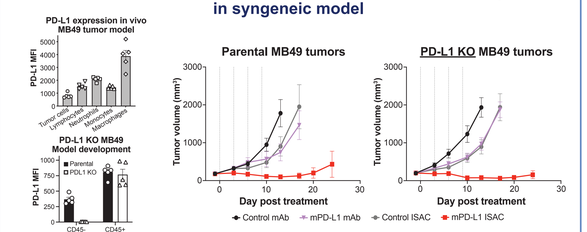
- Large (~200mm³) syngeneic MB49 tumors were treated with the indicated mAbs or ISACs at 10 mg/kg IP, Q3Dx4 (n=6 per group).
- Treatment with mPD-L1 ISAC resulted in improved anti-tumor efficacy compared to mPD-L1 mAb, resulting in complete responses (CRs) in all 6 animals.
- All 6 mice that experienced complete regression of MB49 tumors following mPD-L1 ISAC treatment were re-challenged with MB49 cells ~1 month after tumor clearance (~7 weeks post final dose). No new treatment was given after re-challenge. None of the mice developed tumors.
- Naïve mice were challenged with MB49 tumors as a control. Tumors from all 5 naïve mice grew normally.

PD-L1 ISACs can directly activate PD-L1-expressing myeloid cells



- Human monocytes were differentiated into macrophages with GM-CSF (GM-CSF MΦ) or monocyte-derived dendritic cells (Mo-DC) with GM-CSF and IL-4.
- To induce PD-L1 expression, cells were primed with IFNγ for an additional 48 hr. PD-L1 expression was assessed by flow cytometry. Data shown as average geometric mean fluorescence intensity (gMFI) values with SEM for n=3 donors.
- Polarized myeloid cells were stimulated overnight with hPD-L1 or control ISACs, followed by cytokine analysis by multiplex immunoassay. Data shown as mean with SEM for n=3 donors.

mPD-L1 ISAC retains activity in the absence of tumor PD-L1 expression in syngeneic model



- PD-L1 expression by tumor immune cells in MB49 tumors was assessed by flow cytometry (n=5).
- PD-L1 knockout (KO) MB49 cells were generated by CRISPR/Cas9, and lack of tumor PD-L1 in vivo was confirmed by flow cytometry (n=5 per tumor type).
- Mice bearing MB49 tumors (parental or PD-L1 KO) were treated with the indicated mAbs or ISACs at 5 mg/kg IP, Q3Dx4.
- Treatment with PD-L1 ISAC resulted in improved anti-tumor efficacy even in the absence of PD-L1 expression on tumor cells, indicating critical role for PD-L1 on immune cells.

CONCLUSIONS

- **PD-L1-targeted ISAC is a novel multifunctional therapeutic that has improved efficacy over PD-L1/PD-1 inhibition preclinically in syngeneic and xenograft tumor models**
- **PD-L1 ISAC combines three mechanisms of action (MoA): myeloid cell activation, immune-checkpoint inhibition, and ADCP, and can mediate its MoA through PD-L1 expressed on either tumor or immune cells**
- **PD-L1 ISACs induce robust, target-dependent activation of the immune system that leads to an effective anti-tumor response that is substantially improved over PD-L1 antibody blockade**
- **PD-L1 ISAC treatment induces immunological memory following complete tumor regression in syngeneic models**
- **Systemically delivered PD-L1 ISACs are well tolerated in mice (data not shown)**
- **These data support continued research and development of PD-L1 ISAC with the potential to improve efficacy in patients, including those with tumor types that do not respond to current immune checkpoint therapy**