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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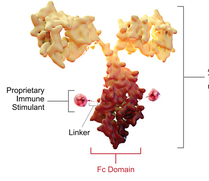
INTRODUCTION

Immune-stimulating antibody conjugates (ISACs) consist of tumor-targeting antibodies conjugated to immune stimulants and are designed to activate the innate and adaptive immune systems against tumor cells following systemic administration. PD-L1 is an immune checkpoint that regulates anti-tumor T cell responses and is expressed on tumor cells as well as tumor-infiltrating immune cells across many tumor types. We evaluated therapeutic properties of PD-L1-targeted TLR7/8 ISAC and preclinically demonstrated its potential as a multifunctional therapeutic that may improve efficacy of PD-L1/PD-1 inhibition by combining three 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).

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 antigen directs Boltbody™ ISACs 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 and adaptive anti-tumor immune response

PD-L1 ISAC:

- PD-L1 ISAC consist of PD-L1 targeting antibody conjugated to TLR7/8 agonist

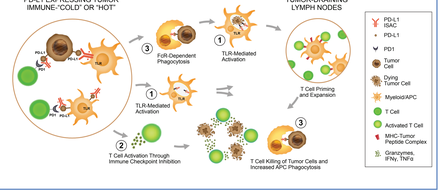
Clinical experience with ISACs:

- 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 tumor and immune cells

PD-L1 ISAC combines three mechanisms to activate innate and adaptive immune system and induce anti-tumor response:

1. TLR-mediated myeloid cell activation leading to innate and then adaptive immunity
2. T cell activation through immune checkpoint inhibition
3. Direct tumor cell killing by ADCP



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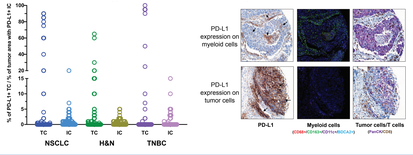
4. Huang R, et al. Modern Pathology. 2021;34:252–263. (https://doi.org/10.1038/s41379-020-00664-y).

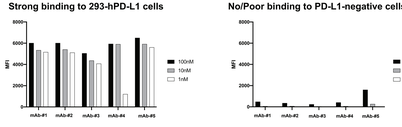
RESULTS

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



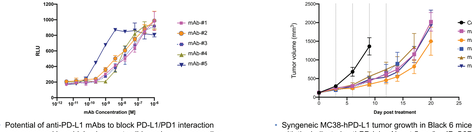
- Human non-small cell lung cancer (NSCLC), head and neck (H&N) and triple negative breast cancer (TNBC) microarray was stained with the PD-L1 IHC (22c3), a CD8/PanCK dual IHC, and a custom quadruplex IF assay for identification of myeloid APC sub-populations.
  TC – tumor cells; IC – immune cells
- Consistent with prior literature[3,4], PD-L1 expression was detected on tumor and immune cells across tumor types
- Representative images of PD-L1 expression on TC and IC in TNBC are shown

Proprietary anti-hPD-L1 mAbs are characterized by a specific binding to hPD-L1 on cell surface



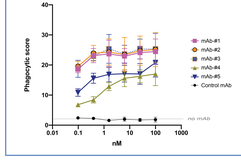
- Candidate anti-PD-L1 antibodies were incubated at 4°C with HEK-293 transduced with human PD-L1 or parental (no PD-L1 expression) HEK-293 cells. After unbound antibodies were removed by buffer wash, bound antibodies were detected using a fluorescent secondary antibody. Level of binding ("MFI") was determined by conventional flow cytometry.

Anti-hPD-L1 mAbs are potent PD-L1/PD1 blockers in vitro and in vivo



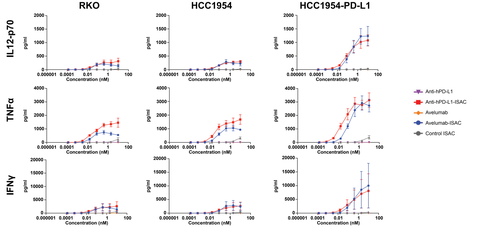
- Potential of anti-PD-L1 mAbs to block PD-L1/PD1 interaction was assessed by a bioluminescent cell-based assay, according to the vendor's protocol (Promega)
- Syngeneic MC38-hPD-L1 tumor growth in Black 6 mice following treatment with the indicated anti-PD-L1 mAbs at 5 mg/kg, IP, Q3Dx5, n=6 per group
- All tested anti-PD-L1 mAbs showed an anti-tumor growth inhibition

Anti-hPD-L1 mAbs induce potent ADCP in vitro



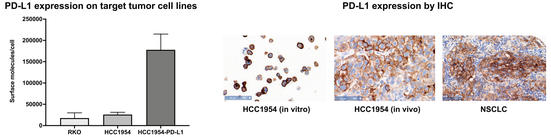
- Cell Tracker Green (CTG) labelled RKO cells were opsonized with indicated mAbs at the indicated concentrations
- Unbound mAbs were washed out, and the opsonized RKO cells were cocultured with M-CSF differentiated macrophages from healthy human donors at 1:1 E:T ratio
- Following the incubation, cells were stained with CD206 (macrophage specific marker) and analyzed by flow cytometry for CTG+CD206+ macrophages to assess level of phagocytosis
- Phagocytic score was calculated as the geometric mean fluorescence intensity (gMFI) of the CTG+CD206+ macrophages x% of CTG+CD206+ macrophages/10,000

Anti-hPD-L1 ISAC activates cDCs co-cultured with tumors expressing PD-L1 at physiological levels



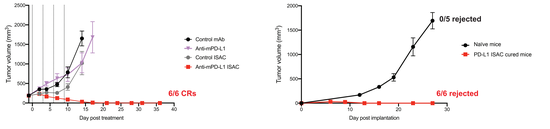
- cDC-enriched primary cells were co-cultured with HCC1954-PD-L1, HCC1954 or RKO tumor cells (5:1 E:T ratio) in the presence of indicated mAbs or ISACs. Supernatants were subsequently analyzed for cytokine expression. Data are shown as mean with SEM of n=3 donors.

Physiological PD-L1 expression level in RKO and HCC1954 tumor cell lines co-cultured with cDC



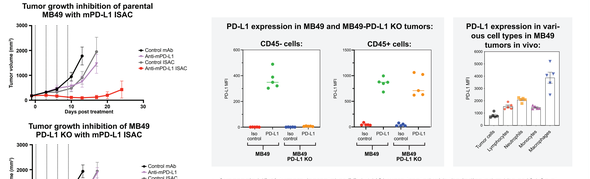
- Cell surface expression of PD-L1 in RKO, HCC1954 and PD-L1 over-expressing HCC1954-PD-L1 cells was quantified by flow cytometry using bead-based standards (Agilent QiFi kit).
- PD-L1 expression on HCC1954 cells cultured in vitro and in vivo was evaluated with PD-L1 IHC (22c3). For comparison, an example of PD-L1 expression in a human specimen of NSCLC is shown.

Anti-mPD-L1-ISAC shows profound efficacy vs anti-PD-L1 treatment and induces immunological memory



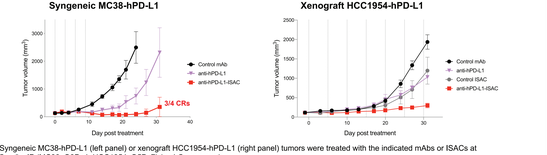
- Large (~200 mm³) syngeneic MB49 tumors were treated with the indicated mAbs or ISACs at 10 mg/kg IP, Q3Dx4 (n=6 per group)
- Treatment with PD-L1 ISAC resulted in improved anti-tumor efficacy compared to anti-PD-L1 treatment
- Treatment with anti-mPD-L1 ISAC resulted in complete responses (CRs) in all 6 animals
- All 6 mice that experienced complete regression of MB49 tumors following anti-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 the re-challenge. None of mice developed tumors
- Naïve mice were challenged with MB49 tumors as control. Tumors from all 5 naïve mice grew without regression

mPD-L1 ISAC can induce anti-tumor response through PD-L1 on immune cells



- Syngeneic 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
- **Right panels:** PD-L1 expression in indicated cells in parental and PD-L1 KO MB49 tumors in vivo was evaluated with a flow cytometry

PD-L1 ISAC improves anti-tumor response over anti-PD-L1 treatment in syngeneic and xenograft tumor models expressing hPD-L1



- Syngeneic MC38-hPD-L1 (left panel) or xenograft HCC1954-hPD-L1 (right panel) tumors were treated with the indicated mAbs or ISACs at 5mg/kg IP (MC38: Q3Dx4; HCC1954: Q5Dx7) (n=4-5 per group)
- Treatment with anti-hPD-L1 ISACs resulted in improved anti-tumor efficacy compared to anti-PD-L1 treatment

CONCLUSIONS

- **PD-L1-targeted ISAC is a novel multifunctional therapeutic that has improved efficacy of 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 induce 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 effective anti-tumor response that is substantially improved over the PD-L1 antibody blockade**
- **PD-L1 ISAC induces immunological memory**
- **Systemically delivered PD-L1 ISACs are well-tolerated in mice (data not shown)**
- **These data warrant continued research and development of PD-L1 ISAC with a potential to improve efficacy in patients including those with tumor types that do not responding to current immune checkpoint therapy**