Tumor-targeted T-cell Activation via an Investigational PD-L1 x CD137 Bispecific Molecule

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Background

Background

Blockade of the PD-1/PD-L1 axis can improve outcome in a variety of cancers; yet, many patients, including subsets of patients with PD-L1+ tumors, do not benefit. The magnitude of immune activation promoted by PD-1/PD-L1 axis blockade can be further enhanced through concomitant T-cell co-stimulation such as that achieved through CD137 agonism; however, clinical applications of such an approach may be limited by toxicity associated with the systemic effects of CD137 agonists. Here we characterize PD-L1 and CD137 tumor expression supporting the development of a PD-L1 x CD137 bispecific molecule that provides PD-1 axis blockade coupled with tumor-targeted CD137 co-stimulation.

Methods

In situ hybridization (ISH) and multicolor flow cytometry was performed to characterize PD-L1 and CD137 expression in tumor biopsies. A PD-L1 x CD137 bispecific molecule (PD-L1 x CD137) was constructed based on PD-L1 blocking mAbs and CD137-engaging mAbs and was evaluated for binding to respective antigens. Its functional activity was evaluated in CD3 or SEB-driven T-cell activation systems, MLR assays and tumor microenvironment models. Anti-tumor activity in vivo was evaluated in combination with tumor targeted anti-CD3 based bispecific DART® molecules.

Results

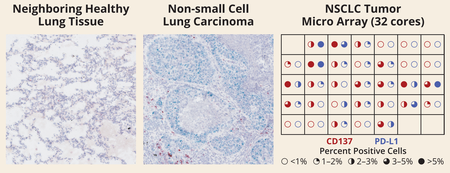
ISH revealed expression of PD-L1 in a significant proportion of surgically resected carcinomas; noteworthy, many such tumors contained CD137+ immune infiltrate adjacent to PD-L1+ cells. Moreover, ex vivo co-incubation of tumor and immune cells in the presence of CD3-based bispecifics or Fc-enhanced antibodies further induces PD-L1 and CD137 expression. PD-L1 x CD137 binds and blocks PD-L1, reversing PD-1-mediated T-cell inhibition equipotently to the effect of approved PD-L1 benchmark mAbs; it also binds CD137, but absent clustering supported by PD-L1+ cells, fails to induce CD137 signaling. In the presence of PD-L1-expressing cells, however, PD-L1 x CD137 drives CD137 activation and immune cell co-stimulation. Robust T-cell activation and cytokine secretion was induced by PD-L1 x CD137, with significantly greater activity than that observed with the combination of PD-L1 blocking and CD137 agonistic mAbs. Notably, when combined with tumor targeted immunotherapies, PD-L1 x CD137 supports enhanced activation of effector cells and anti-tumor activity.

Conclusions

These ex vivo data show that an investigational PD-L1 x CD137 bispecific can switch on CD137 co-stimulation in a PD-L1-dependent fashion. While tumor adaptive resistance via PD-L1 induction promotes tumor immune escape, PD-L1 x CD137 can exploit the checkpoint ligand up-regulation by contributing a co-stimulatory signal in addition to checkpoint blockade. PD-L1 x CD137 provides a potential therapeutic approach to overcome limitations of existing PD-1/PD-L1-targeting strategies either as monotherapy or in combination with complementary immune based therapeutic modalities, such as CD3 based bispecifics or Fc-enhanced mAbs.

Rationale

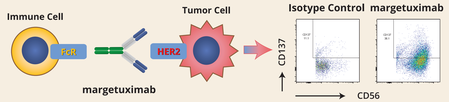
PD-L1 and CD137 Expression in Non-small Cell Lung Cancer and Adjacent Normal Tissue



Left: Expression of CD137 **(red)** and PD-L1 **(blue)** mRNA in tissue samples of healthy lung (left) or non-small cell lung carcinoma (right) visualized by RNAscope™.

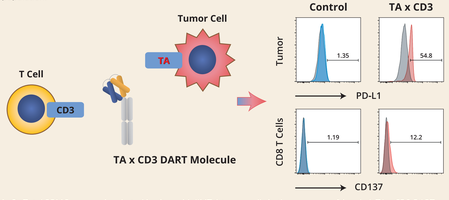
Right: PD-L1 and CD137 expression was analyzed by ISH in the array of 32 NSCLC tumor cores. Fraction of tumor stroma cells expressing CD137 and tumor cells expressing PD-L1 in each tumor core was calculated using HALO® Image analysis platform.

Immunotherapy Enhances CD137 Expression on Immune Cells and PD-L1 Expression on Tumor Cells



Left: Total PBMC were co-incubated in vitro with N87 tumor cells in the presence of 0.1 µg/mL Fc-enhanced anti-HER2 mAb (margetuximab).

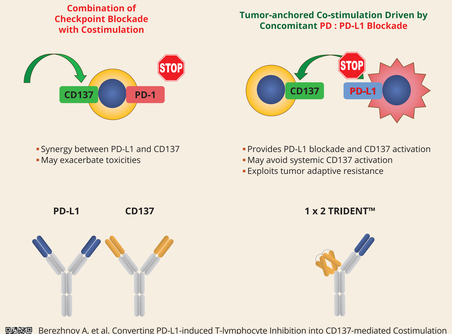
Right: CD137 expression on NK cells (gated CD3-CD56+ population) was measured after 48h of incubation population.



Left: Total PBMC were co-incubated in vitro with JIMT-1 tumor cells in the presence of 1 µg/mL TA x CD3 DART molecule as a T-cell activator.

Right: CD137 expression was measured on gated CD8 T cells (CD45+CD8+) after 48h of incubation, while PD-L1 expression was measured on CD45- tumor cells.

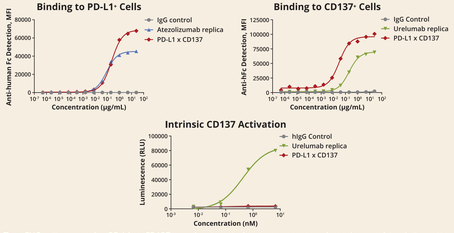
PD-L1 x CD137 Bispecific Molecule



Berezhnoy A, et al. Converting PD-L1-induced T-lymphocyte Inhibition into CD137-mediated Costimulation via PD-L1 x CD137 Bispecific DART® Molecules. Poster presented at 30th EORTC/AACR/NCI Symposium, November 13–16, 2018, Dublin, Ireland.

Results

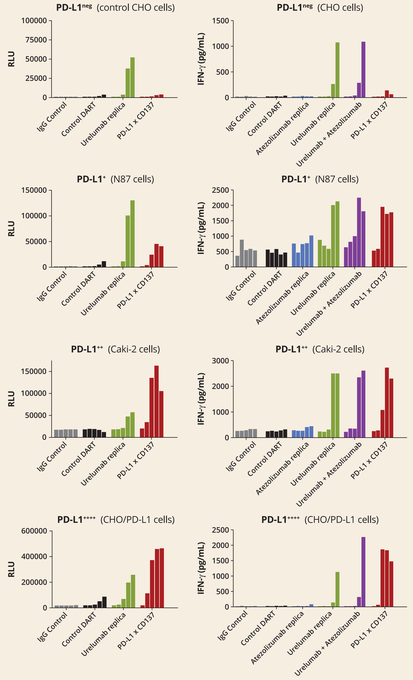
PD-L1 x CD137 Binds PD-L1 and CD137 but Lack Constitutive CD137 Agonistic Activity



Top: CHO cells expressing PD-L1 or CD137 were incubated with shown concentrations of test articles, followed by incubation with APC labeled goat anti-human polyclonal antibodies.

Bottom: Activation of CD137 was measured by NFkB-response element mediated luciferase expression in Jurkat-4-1BB$^{NFkB-luc}$ cells.

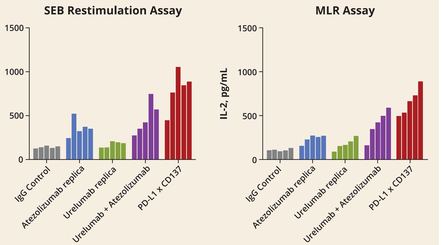
PD-L1 x CD137 Activates CD137 in a PD-L1-dependent Manner



CD137 reporter assay: 4-1BB Effector Cells that express CD137 and luciferase downstream of a response element, were co-cultured with tumor cells expressing different amounts of PD-L1 in the presence of indicated molecules at 0.0006, 0.006, 0.06, 0.6 and 6.7 nM. To facilitate PD-L1-mediated cross-linking of bispecific molecules, indicated target cells were added to effectors at 1:1 ratio.

Primary T cell activation: T cells were co-cultured with tumor cells for 72 hours. Indicated molecules were added to the co-culture at 0.01, 0.05, 0.26, 1.3 or 6.7 nM concentration.

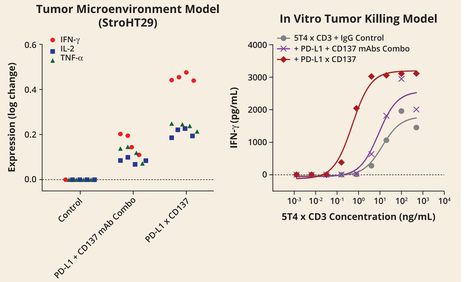
PD-L1 x CD137 Enhances Activation of Primary T Cells



SEB: PBMC were primed with 1 ng/mL of SEB for 48 hours, washed and re-stimulated with SEB for 48 hours in the presence of indicated molecules added at 0.01, 0.05, 0.26, 1.3 or 6.7 nM concentration.

MLR assay: Freshly isolated human CD4^{+} T cells were co-cultured with in vitro-differentiated APCs from a different donor in the presence of indicated molecules at 0.01, 0.05, 0.26, 1.3 or 6.7 nM concentration.

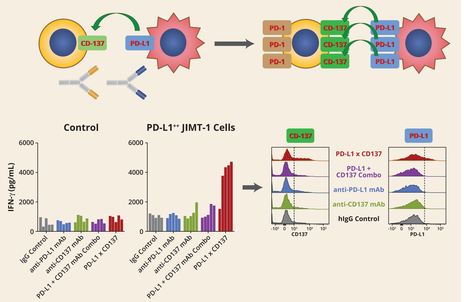
PD-L1 x CD137 Enhance Immune Responses in In Vitro Tumor Models



DiscoverX StroHT29 TME model: HT-29 colorectal cells were cultured with fibroblasts and PBMCs to recapitulate a stromal microenviroment (DiscoverX). Expression of depicted cytokines was measured upon treatment with 0.7, 2.2, 6.7, or 20 nM of mAbs or bispecific molecules.

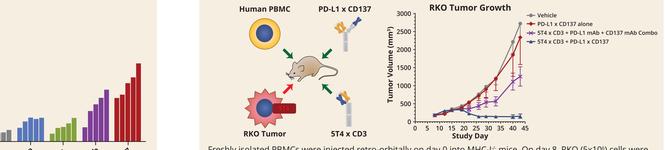
In vitro killing assay: T cells were co-incubated with 5T4-expressing RKO cells at 3:1 E:T ratio in the presence of indicated molecules at 1 µg/mL final concentration and 5T4 x CD3 DART at concentrations shown.

PD-L1 x CD137 Leverages PD-L1 Upregulation to Enhance CD137 Response



Freshly isolated primary human T cells were co-cultured with indicated cells for 72 hours. Indicated molecules were added to the co-culture at 0.01, 0.05, 0.26, 1.3 or 6.7 nM concentration. IFN-gamma was measured in supernatants while immunophenotypes of T cells and target cells was analyzed by flow cytometry. PD-L1 and CD137 expression in histogram insert is representative of cells treated with 1.3 nM of test articles.

PD-L1 x CD137 Inhibits Tumor Growth in Combination with Redirected T-Cell Killing



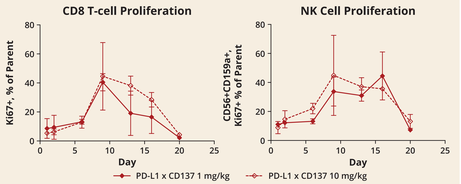
Freshly isolated PBMCs were injected retro-orbitally on day 0 into MHC-I^{-} mice. On day 8, RKO (5x10^{6}) cells were injected s.c. in a 1:1 mixture with Matrigel. On day 5 mice were treated with OKT4 to reduce the contribution of CD4 cells, and on day 14 treatment (IV) with ST4 x CD3 (twice weekly) and PD-L1 x CD137 (weekly) was initiated (N=8/group).

PD-L1 x CD137 in Non-human Primates

Finding	PD-L1 x CD137	
	1 mg/kg	10 mg/kg
AST changes	0/2	0/2
ALT changes	0/2	0/2
Bili changes	0/2	0/2
Creatinin changes	0/2	0/2
ALP changes	0/2	0/2

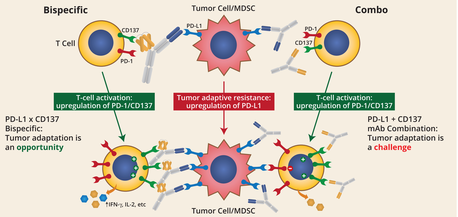
- No mortality or significant adverse findings

PD-L1 x CD137 Transiently Expands T Cells and NK Cells In Vivo



Cynomolgus monkeys were infused IV with 1 or 10 mg/kg of the indicated molecules. Ki67 expression by peripheral blood lymphocytes was quantified by flow cytometry.

PD-L1 x CD137 Exploits Tumor Immune Adaptive Resistance for Enhanced T-cell Activation



While tumor adaptive resistance via PD-L1 induction promotes immune escape, PD-L1 x CD137 bispecific molecules can exploit the checkpoint ligand up-regulation and further amplify T-cell activation by contributing a co-stimulatory signal.

Conclusions

- Tumors contain CD137+ immune infiltrate as well as PD-L1+ cells
- PD-L1 x CD137 bispecific blocks PD-L1 and activates CD137 in a PD-L1-dependent fashion ex vivo
- PD-L1 x CD137 provides a potential therapeutic approach to overcome limitations of existing PD-1/PD-L1-targeting strategies, either as monotherapy or in combination with complementary immune based therapeutic modalities, such as CD3 based bispecifics or Fc-enhanced mAbs

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