Converting PD-L1-induced T-lymphocyte Inhibition into CD137-mediated Costimulation via PD-L1 x CD137 Bispecific DART® Molecules

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Abstract 216

Abstract

Background

PD-1/PD-L1 axis blockade is a clinically proven cancer therapeutic strategy, but can be insufficient to fully activate tumor-specific T cells. CD137 co-stimulation synergistically increases the activity of PD-1 blockade in mouse tumor models. Clinical application of such an approach, however, may be limited by toxicity associated with the systemic administration of CD137 agonists. Here we demonstrate that bispecific DART molecules comprising anti-PD-L1 and CD137 mAb specificities provide PD-1 axis blockade concomitantly with PD-L1-dependent CD137 co-stimulation.

Materials and Methods

PD-L1 x CD137 bispecific DART and TRIDENT™ molecules were constructed based on PD-L1 blocking mAbs and CD137-engaging mAbs and evaluated for binding to their respective antigens and in reporter assays, as well as in CD3 or SEB-driven T-cell activation and MLR assays. Anti-tumor redirected T-cell activity was evaluated in combination with anti-CD3 based DART molecules. RNAseq was performed to characterize T-cell gene expression.

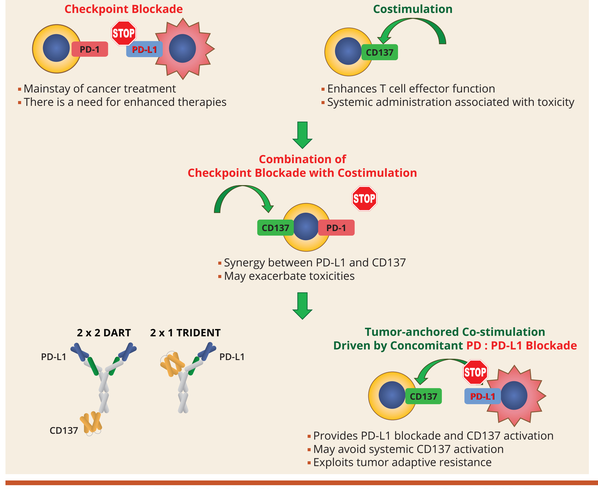
Results

PD-L1 x CD137 DART and TRIDENT molecules bind and block PD-L1, reversing PD-1-mediated T-cell inhibition equipotently to the effect of approved PD-L1 benchmark mAbs. They also bind CD137, but, without secondary cross-linking or clustering induced by PD-L1^{+} cells, fail to induce CD137 signaling. In the presence of PD-L1-expressing cells, however, PD-L1 x CD137 DART molecules drive CD137 activation and immune cell co-stimulation. Robust T-cell activation and cytokine secretion was induced by PD-L1 x CD137 DART proteins, with significantly greater activity than that observed with the combination of PD-L1 blocking and CD137 agonistic mAbs. Notably, when combined with tumor targeted anti-CD3 based DART molecules, PD-L1 x CD137 bispecific molecules enhance activation of effector cells in the presence of tumor cells and increase tumor growth inhibition. Transcriptome studies revealed a gene expression profile uniquely induced by the PD-L1 x CD137 bispecific protein but not by the mAb combination.

Conclusions

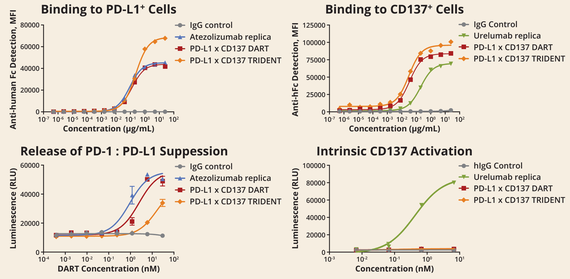
These data show that PD-L1 x CD137 bispecific DART and TRIDENT molecules can switch on CD137 co-stimulation in a PD-L1-dependent fashion. While tumor adaptive resistance via PD-L1 induction promotes immune escape, PD-L1 x CD137 DART molecules can exploit the checkpoint ligand up-regulation and further amplify checkpoint blockade by contributing a co-stimulatory signal. Further investigations as a potential therapeutic approach to overcome limitations of existing PD-1/PD-L1-targeting strategies is warranted.

Rationale



Results

PD-L1 x CD137 DART and TRIDENT Molecules Bind CD137 and Block PD-L1

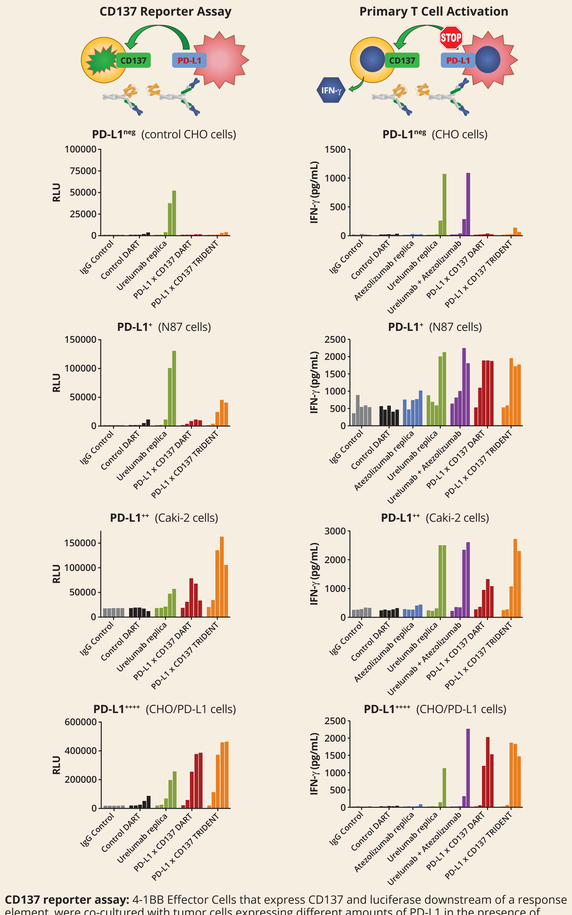


Top: CHO cells expressing respective targets were incubated with shown concentrations of test articles, followed up by incubation with APC labeled goat-antihuman polyclonal antibodies.

Bottom left: Release of PD-L1 : PD-1 suppression upon addition of mAbs was measured by NFAT response element-mediated luminescence in Jurkat-PD-1$^{NFAT\text{-}luc}$ cells.

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

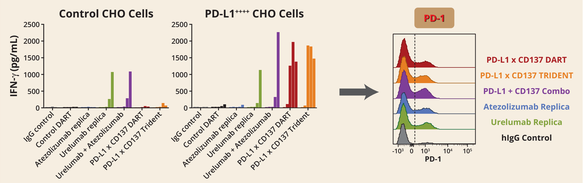
PD-L1 x CD137 DART and TRIDENT Molecules Activate CD137 in a PD-L1-dependent Manner

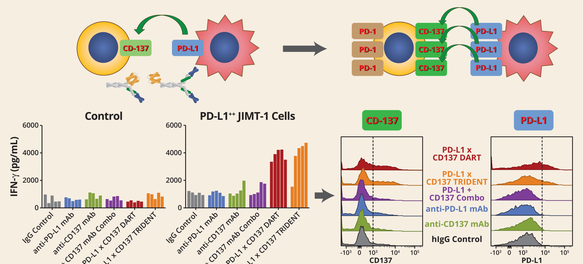


CD137 reporter assay: 4-1BB Effector Cells that express CD137 and luciferase downstream of a response element, were co-cultured with tumor cells expressing different amounts of PD-L1 in the presence of indicated molecules at 0.0006, 0.006, 0.06, 0.6 and 6.7 nM. To facilitate PD-L1-mediated cross-linking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

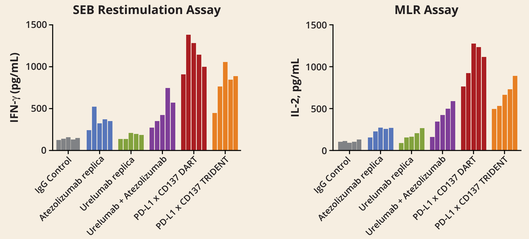
PD-L1 x CD137 DART and TRIDENT Molecules Can Overcome Adaptive Tumor Resistance





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. Interferon-gamma was measured in supernatants while immunophenotypes of T cells and target cells had been analyzed by flow cytometry. PD-L1 and CD137 expression in histogram insert is representative example of cells treated with 1.3 nM of test articles.

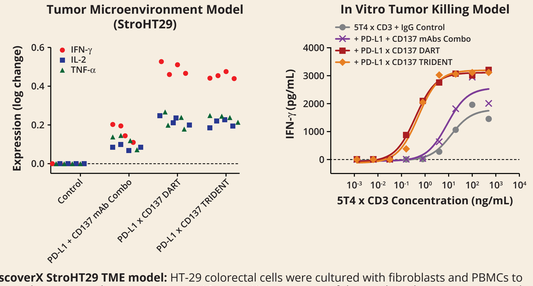
PD-L1 x CD137 DART and TRIDENT Molecules Enhance Activation of Primary T Cells



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.

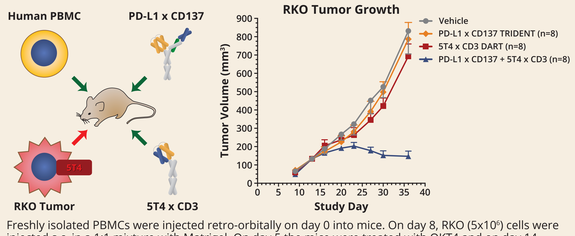
PD-L1 x CD137 DART and TRIDENT Molecules Enhance Immune Responses In Vitro Tumor Models



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.

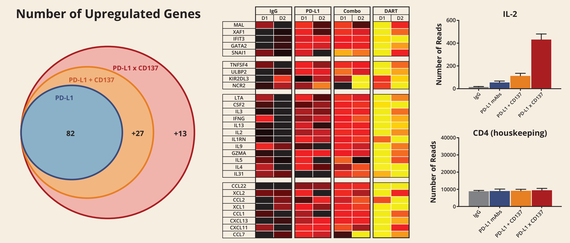
In vitro killing assay: T cells were co-incubated with 5T4-expressing RKO cells at 3:1 E:T ratio in the presence of indicated molecules at 1 µg/mL final concentration and 5T4 x CD3 DART at concentrations shown.

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



Freshly isolated PBMCs were injected retro-orbitally on day 0 into mice. On day 8, RKO (5x10^6) cells were injected s.c. in a 1:1 mixture with Matrigel. On day 5 the mice were treated with OKT4 and on day 14 treatment (IV) with 5T4 x CD3 (twice weekly) and PD-L1 x CD137 (weekly) was initiated. (N=8/group).

PD-L1 x CD137 DART Molecule Induces Enhanced Gene Expression Compared to mAbs Combination

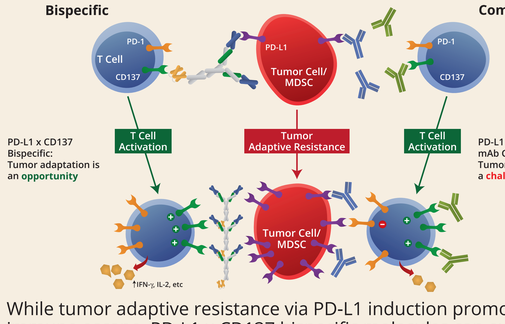


T cells were re-isolated from 72h MLR assay, and RNAseq was performed using Illumina HiSeq platform. Gene alignment and differential expression analysis were done using Strand NGS software. Genes were clustered based on at least 2-fold upregulation in both donors.

Conclusions

- PD-L1 x CD137 DART and TRIDENT bispecific molecules **block PD-L1** and **activate CD137** co-stimulation in PD-L1-dependent fashion
  - Format and valency of each arm dictate properties of bispecific molecules
- PD-L1 x CD137 bispecific molecules are consistently **more potent** in augmenting T-cell activation **than combination** of two mAbs
  - PD-L1 x CD137 bispecific molecules induce enhanced gene expression signature compared to combination of independent mAbs
- PD-L1 x CD137 bispecific molecules enhance T-cell effector function in vitro and in vivo
  - Anti-tumor activity in vivo is primed by redirected T-cell killing as a model of anti-tumor immune response

PD-L1 x CD137 DART Protein 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.

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