Co-targeting of PD-1 and CTLA-4 Inhibitory Pathways with Bispecific DART® and TRIDENT™ Molecules

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Abstract

Introduction: Immunotherapy with the combination of monoclonal antibodies (mAbs) that block PD-1 (nivolumab) and CTLA-4 (ipilimumab) has shown clinical benefit beyond that observed with either mAb alone. We have developed PD-1 x CTLA-4 bispecific proteins aimed at inducing antitumor immunity through simultaneous blockade of both checkpoint molecules. Two proteins, a tetravalent PD-1 x CTLA-4 bispecific DART® protein (bivalent for both PD-1 and CTLA-4) and a trivalent PD-1 x CTLA-4 TRIDENT™ protein (bivalent for PD-1 but monovalent for CTLA-4) were engineered; the TRIDENT protein was designed to promote CTLA-4 blockade through avidity when bound to PD-1 on cells coexpressing both receptors, thus favoring PD-1 over CTLA-4 blockade in cells that do not coexpress both molecules.

Methods: Proteins were engineered from humanized PD-1 and CTLA-4 mAb sequences and demonstrated favorable biophysical properties. Binding assays were performed by ELISA or flow cytometry. Coligation of PD-1 and CTLA-4 was assessed in an enzyme-fragment complementation assay (DiscoverX). T-cell activation was tested in reporter cells, staphylococcus enterotoxin B-stimulated PBMCs or MLR assays.

Results: Both DART and TRIDENT proteins showed equivalent potency in binding immobilized recombinant PD-1 or PD-1-expressing cell lines, inhibition of PD-1 interaction with PD-L1 or PD-L2 as well as reversal of PD-1/PD-L1 mediated T-cell signal inhibition. In all assays, both formats showed activities that were comparable to those of the precursor PD-1 mAb. With respect to CTLA-4, the DART protein showed a minor potency loss in binding to CTLA-4 expressing cells, inhibition of CTLA-4/B7 interaction and reversal of T-cell signal inhibition compared to the precursor mAb. The TRIDENT protein, however, showed substantial lower potency than the DART protein in all CTLA-4 assays, consistent with the monovalent nature of the CTLA-4 arm. Importantly, in cells that coexpress both receptors, DART and TRIDENT proteins show comparable co-engagement of PD-1 and CTLA-4, as shown by enzyme-fragment complementation, suggesting that anchoring through PD-1 compensates for the decreased CTLA-4 avidity of the TRIDENT molecule when both target receptors are expressed. Similarly, in T-cell coactivation assays, both DART and TRIDENT proteins showed a comparable increase in IFN-γ response that recapitulated that observed with the combination of the individual parental mAbs.

Conclusions: Both PD-1 x CTLA-4 DART and TRIDENT bispecific molecules block PD-1 and CTLA-4 pathways, with the TRIDENT protein demonstrating a PD-1-biased binding preference, consistent with its design intended to reduce CTLA-4 blockade in the absence of PD-1 coexpression. Both molecules showed comparable T-cell activation activity. Further development of bispecific PD-1 x CTLA-4 molecules for cancer treatment is warranted.

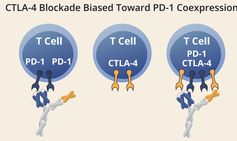
Introduction

PD-1 x CTLA-4 Dual Checkpoint Targeting Strategy

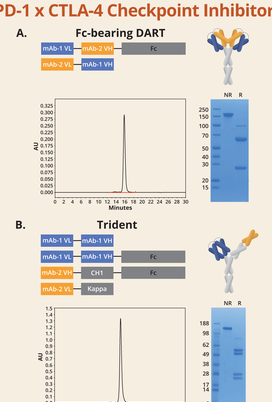
- PD-1 and CTLA-4 are clinically validated T-cell co-inhibitory molecules with complementary mechanisms of action
- Coordinated PD-1 and CTLA-4 blockade has shown combinatorial antitumor activity in the clinic
  - PD-1 blockade reverses T-cell inhibition at tumor sites
  - CTLA-4 blockade results in polyclonal activation/expansion
- Increased antitumor activity with anti-CTLA-4 addition is associated with increased adverse event profile
- Challenge: Maintain PD-1 ligand blockade with tunable CTLA-4 binding blockade

PD-1 x CTLA-4 DART
Bivalent PD-1 & CTLA-4 arms
Equivalent PD-1 & CTLA-4 Blockade

PD-1 x CTLA-4 TRIDENT
Bivalent PD-1 & monovalent CTLA-4 arms
CTLA-4 Blockade Biased Toward PD-1 Coexpression



Bispecific PD-1 x CTLA-4 Checkpoint Inhibitor Molecules

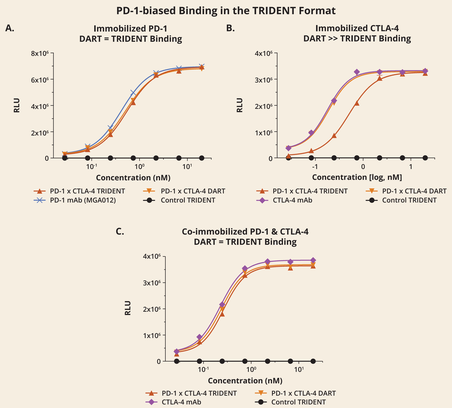


PD-1 mAb (proprietary mAb, MGA012) binding affinity ~1 nM; CTLA-4 mAb binding affinity ~5 nM.

A. PD-1 x CTLA-4 DART is an Fc-bearing (IgG4) DART protein bispecific and bivalent for PD-1 and CTLA-4. Size-exclusion chromatography (bottom left) and SDS-PAGE (bottom right).
B. PD-1 x CTLA-4 TRIDENT is designed with the same mAb sequences as in the DART molecule, but contains monovalent CTLA-4 binding arm. Size-exclusion chromatography (bottom left) and SDS-PAGE (bottom right).

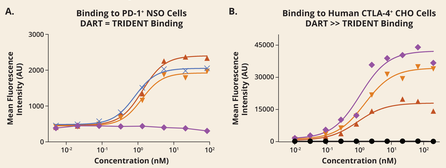
Results

PD-1 x CTLA-4 DART and TRIDENT Binding Properties



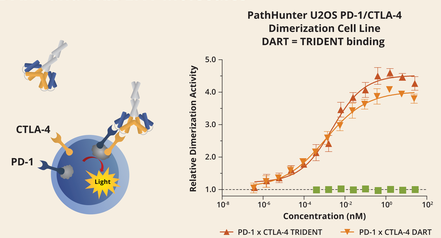
A-B. ELISA-based binding of PD-1 x CTLA-4 DART and TRIDENT proteins to plate-immobilized recombinant human PD-1 **(A)** or human CTLA-4 **(B)** proteins detected by anti-human IgG HRP.
C. Binding of PD-1 x CTLA-4 DART and TRIDENT molecules to co-immobilized PD-1 and CTLA-4 detected by ELISA.

PD-1 x CTLA-4 DART and TRIDENT Molecules Bind to Cell-surface Expressed PD-1 or CTLA-4



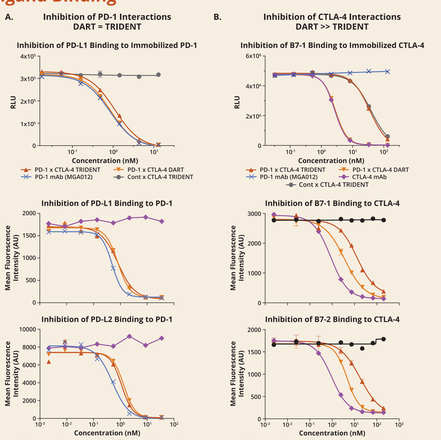
Flow cytometric analyses of PD-1 x CTLA-4 DART or TRIDENT binding to cells expressing PD-1 **(A)** or CTLA-4 **(B)** detected using anti-human IgG.

Bispecific Co-engagement of PD-1 and CTLA-4 by DART and TRIDENT Molecules



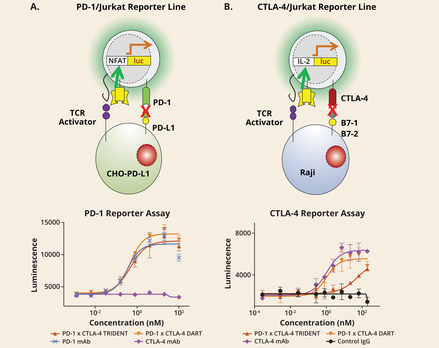
Indicated molecules were evaluated using enzyme fragment complementation assay employing PathHunter® U2OS PD-1/CTLA-4 dimerization cell Line (DiscoverX).

PD-1 x CTLA-4 DART and TRIDENT Molecules Inhibit Ligand Binding: Differential Inhibition of CTLA-4 Ligand Binding



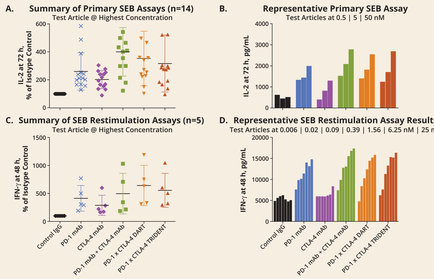
A. Inhibition of PD-L1 ligand binding by ELISA and Flow cytometric analysis of soluble PD-L1 or PD-L2 binding to NS0-PD-1+ cells in the presence of titrating concentrations of the indicated mAbs, PD-1 x CTLA-4 DART and TRIDENT molecules.
B. Inhibition of B7-1 ligand binding by ELISA and Flow cytometric analyses of soluble B7-1-his or B7-2-his binding to CTLA-4+ Jurkat cells in the presence of titrating concentrations of the indicated mAbs, PD-1 x CTLA-4 DART and TRIDENT molecules.

PD-1 x CTLA-4 DART and TRIDENT Molecules Reverse Inhibition of TCR Signaling: Differential Release of CTLA-4 Inhibition



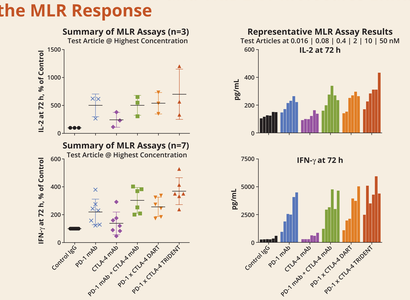
A. The indicated molecules were tested in the Promega PD-1/PD-L1 assay system employing Jurkat-PD-1+ cells (transduced with an NF-AT luciferase reporter) cultured with a CHO-PD-L1 cell line that expresses a TCR activator. The luminescence represents the release of PD-1-mediated suppression of the NF-AT-driven luciferase gene upon TCR stimulation.
B. The indicated molecules were tested in the Promega CTLA-4/B7-1&B7-2 assay system employing Jurkat-CTLA-4+ cells (transduced with an IL-2 promoter-driven luciferase reporter) cultured with a Raji cell line that expresses a TCR activator. The luminescence represents the release of CTLA-4-mediated suppression of the IL-2 promoter-driven luciferase gene upon TCR stimulation.

PD-1 x CTLA-4 DART and TRIDENT Molecules Augment T-cell Response to SEB Stimulation



A-B. Human PBMCs from healthy donors were stimulated with 500 ng/mL SEB for 72 h in the presence of indicated molecules. IL-2 levels were measured in supernatants. Molarity refers to the concentration of individual components, whether used alone or in combination (e.g., 50 nM PD-1 mAb + 50 nM CTLA-4 mAb proteins were compared to 50 nM PD-1 x CTLA-4 bispecifics).
C-D. Human PBMCs from healthy donors were stimulated with SEB for 48 h, washed, and restimulated with 0.5 ng/mL SEB. IFN-γ secretion was determined by ELISA. Molarity refers to the concentration of individual components, whether used alone or in combination.

PD-1 x CTLA-4 DART and TRIDENT Molecules Enhance the MLR Response



Mixed lymphocyte reaction (MLR) between monocyte derived DCs generated by culturing with GM-CSF and IL-4 for 7 days and freshly isolated CD4+ T cells from different donors. After 72 h, IL-2 and IFN-γ in the culture supernatants were measured by ELISA.

Conclusions

- PD-1 x CTLA-4 DART and TRIDENT molecules bind to and inhibit ligand interaction with their targets
  - TRIDENT protein with a monovalent CTLA-4 binding arm shows biased binding favoring PD-1 interaction
- DART and TRIDENT molecules co-engage their targets in an equivalent fashion when PD-1 and CTLA-4 are coexpressed on the same cell
- DART and TRIDENT molecules augment T-cell activation (cytokine release) to levels comparable to those observed with the individual PD-1 and CTLA-4 mAbs
- Further development (nonhuman primate toxicology) of bispecific PD-1 x CTLA-4 molecules is in progress

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