MGD019, a PD-1 x CTLA-4 Tetravalent Bispecific DART® Protein, Provides Optimal Dual Checkpoint Blockade

Abstract B027

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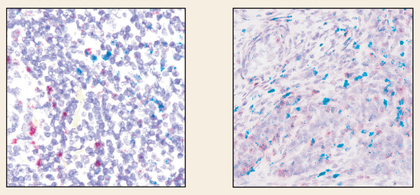
Abstract

- Combinatorial blockade of PD-1 and CTLA-4 has shown clinical benefit beyond that observed with individual mAbs, albeit with increased toxicity. Co-expression of the checkpoint receptors creates an opportunity for selective, enhanced blockade of dual PD-1/CTLA-4 expressing cells through use of a bispecific inhibitor targeting both pathways simultaneously
- Consistent with previous reports, multiplex in-situ hybridization (ISH) demonstrated enrichment of PD-1/CTLA-4-dual positive cells in neoplastic lesions relative to normal tissues, where distinct populations expressed CTLA-4 or PD-1. PD-1 x CTLA-4 DART proteins were designed as either bivalent or tetravalent bispecific engagers with a human IgG4 backbone; in vitro characterization revealed that both 1x1 and 2x2 bispecific PD-1 x CTLA-4 formats block each checkpoint molecule, with enhanced blockade of CTLA-4 over that achieved with anti-CTLA-4 mAb on dual expressing cells. As expected, 1x1 format molecule was less efficient in blocking PD-1 in cell models lacking CTLA-4 expression, while 2x2 molecule demonstrated full PD-1 blockade. Likewise, the 2x2 format molecule effectively blocks CTLA-4 ligand interactions on PD-1^{neg}CTLA-4^{pos} cells, while the 1x1 design is significantly less efficient. The tetravalent bispecific molecule was designated as MGD019 and selected for clinical development
- In primary cell assays in which both PD-1 and CTLA-4 contribute to inhibit T-cell activation, MGD019 enhances antigen-driven in vitro T-cell activation to a level comparable to the combinatorial PD-1 plus CTLA-4 blockade. Tumor microenvironment models that recapitulate vascular or stromal compartments confirmed MGD019 induces in vitro immune response profiles comparable to those observed with replicas of ipilimumab plus nivolumab. Unlike ipilimumab, however, MGD019 does not reduce the number of peripheral blood Tregs ex vivo
- MGD019 was well tolerated in cynomolgus monkeys, with no mortality or significant adverse findings up to 100 mg/kg QWx4. T-cell proliferation in the periphery and expansion in lymphoid organs was observed, with increases in ICOS+ CD4 cells and memory T cells, findings attributable to the CTLA-4 blocking arm, since the anti-PD-1 mAb precursor was devoid of these activities
- In summary, MGD019 offers the convenience of a single molecule administration for dual checkpoint blockade. In addition to providing full blockade on cells expressing PD-1 or CTLA-4 individually, MGD019 exploits dual target avidity resulting in preferential engagement and enhanced blockade on cells that express both checkpoint molecules, a feature that could provide additional benefits given the preeminent co-expression of PD-1 and CTLA-4 by TILs. These data indicate support clinical testing of MGD019 in cancer patients

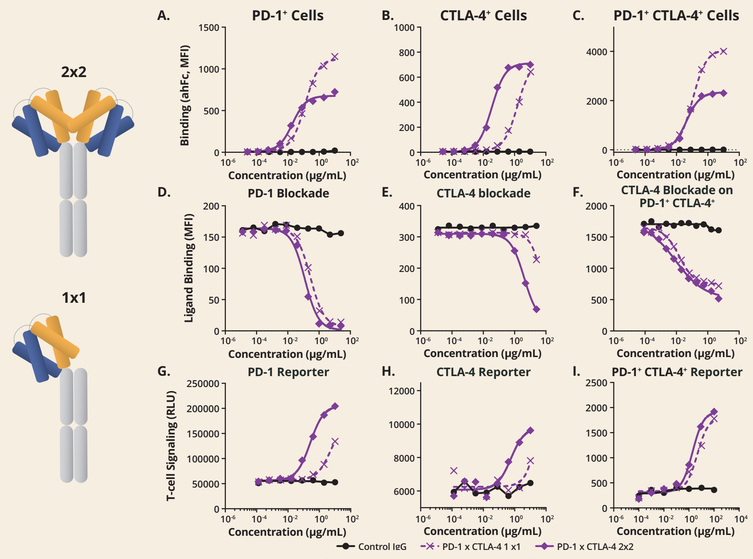
Introduction

T Cells Co-expressing PD-1 and CTLA-4 Are Prevalent Among TILs Compared to Healthy Tissues



Expression of PD-1 (red) and CTLA-4 (blue) mRNA in tissue samples of normal human tonsils **(left)** or ovarian cancer **(right)** visualized by RNAscope™.

Rationale and Format Selection for Dual PD-1 and CTLA-4 Targeting

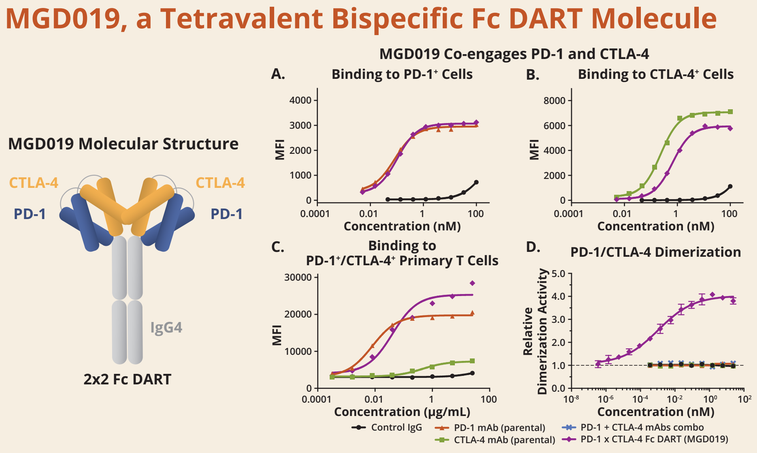


2x2 and 1x1 DART molecules were evaluated for binding to **(A, B, C)**, ligand blockade **(D, E, F)** and restoration of T-cell signaling **(G, H, I)** in PD-1 only, CTLA-4 only or PD-1+CTLA-4 models by flow cytometry and reporter gene activation.

Rationale for PD-1xCTLA-4 Dual Checkpoint Targeting Strategy

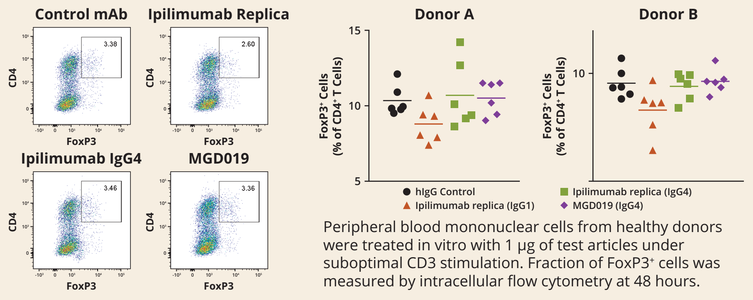
Results

MGD019, a Tetravalent Bispecific Fc DART Molecule



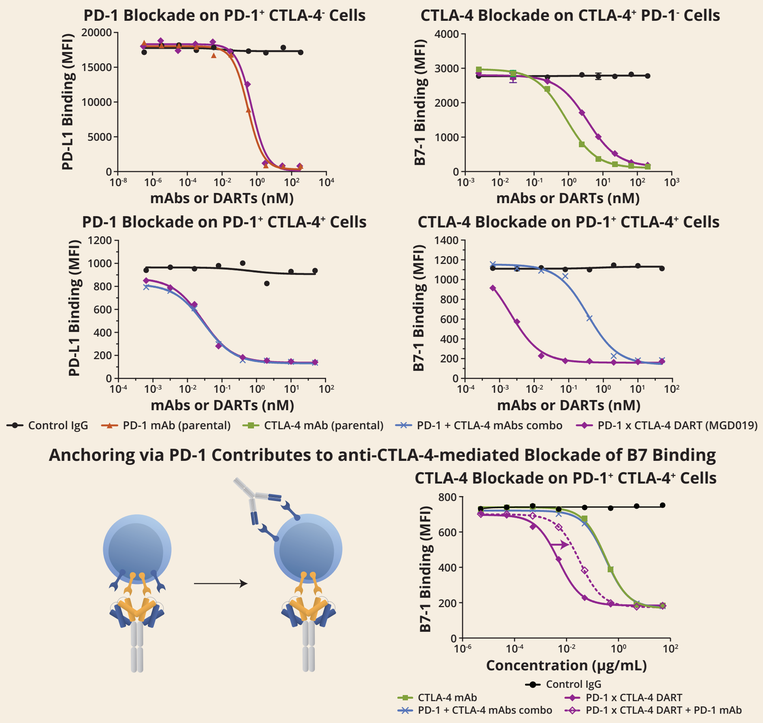
Binding of MGD019 or control molecules to Jurkat/PD-1 **(A)** or Jurkat/CTLA-4 **(B)** cells measured by flow cytometry. **C.** Binding of MGD019 or control molecules to in vitro activated primary human T cells by flow cytometry. **D.** Co-engagement of PD-1 and CTLA-4 on the cell surface by MGD019 measured by DiscoverX™ enzyme complementation assay.

MGD019 Does Not Affect the Circulating Treg Population



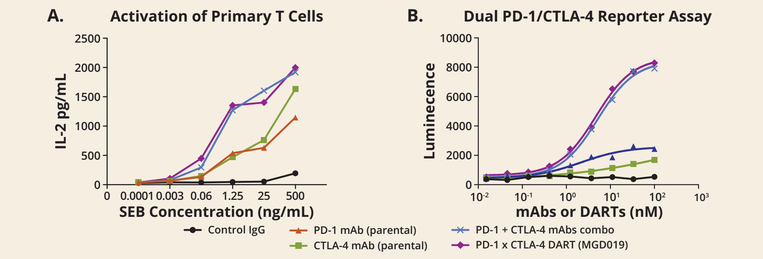
Peripheral blood mononuclear cells from healthy donors were treated in vitro with 1 µg of test articles under suboptimal CD3 stimulation. Fraction of FoxP3+ cells was measured by intracellular flow cytometry at 48 hours.

Enhanced Blockade of CTLA-4 by MGD019 on Dual Expressing Cells



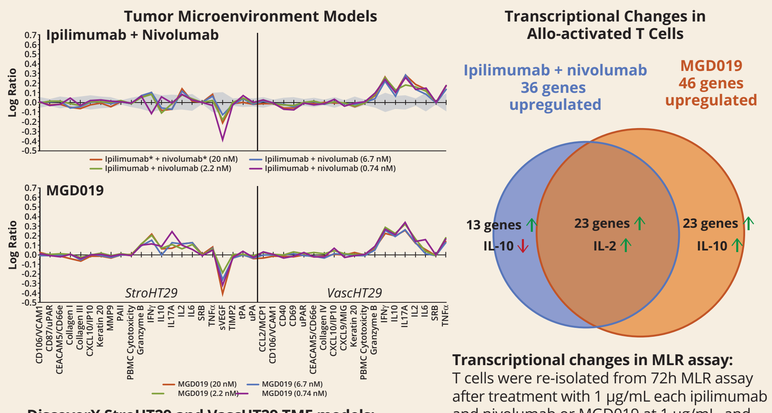
PD-1 or CTLA-4 binding to their respective soluble ligands on Jurkat cells engineered to express PD-1 and/or CTLA-4 was measured by flow cytometry.

MGD019 Enhances T-cell Activation



A. PBMC were activated by SEB in the presence of 10 µg/mL of the indicated molecules. IL-2 secretion was measured at 96h by ELISA. **B.** Expression of IL-2 reporter cassette in Jurkat cells co-expressing PD-1 and CTLA-4 (Promega) co-incubated with APC in the presence of the indicated molecules.

Characterization of MGD019-mediated Enhancement of T-cell Activation



DiscoverX StroHT29 and VascHT29 TME models:
HT-29 colorectal cells were cultured with stromal cells and PBMCs to recapitulate a tumor microenviroment (DiscoverX). Protein expression was measured following treatment with mAbs or MGD019. *Replicas of nivolumab and ipilimumab were constructed on the basis of published sequences.

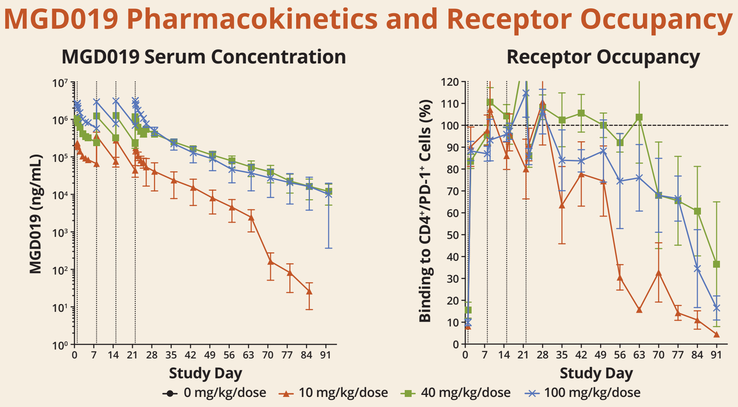
Transcriptional changes in MLR assay:
T cells were re-isolated from 72h MLR assay after treatment with 1 µg/mL each ipilimumab and nivolumab or MGD019 at 1 µg/mL, and RNAseq was performed using Illumina HiSeq platform. Gene alignment, filtering for 750 immune- related genes and differential expression analysis were done using Strand NGS software. Genes were clustered based on upregulation in both donors.

MGD019 GLP Toxicology Study

Finding	PD-1xCTLA-4 (MGD019)			aPD-1 (parental)
	10 mg/kg	40 mg/kg	100 mg/kg	≥100 mg/kg
Adverse clinical signs	-	-	-	-
Body weight loss	-	-	-	-
Increased spleen weight	+	++	++	-
Lymphoid hyperplasia in spleen	-	+	++	-
GI tract inflammation	-	-	-	-
Circulating cytokines	-	-	-	-
T-cell proliferation (Ki67+)	+	++	++	+/-

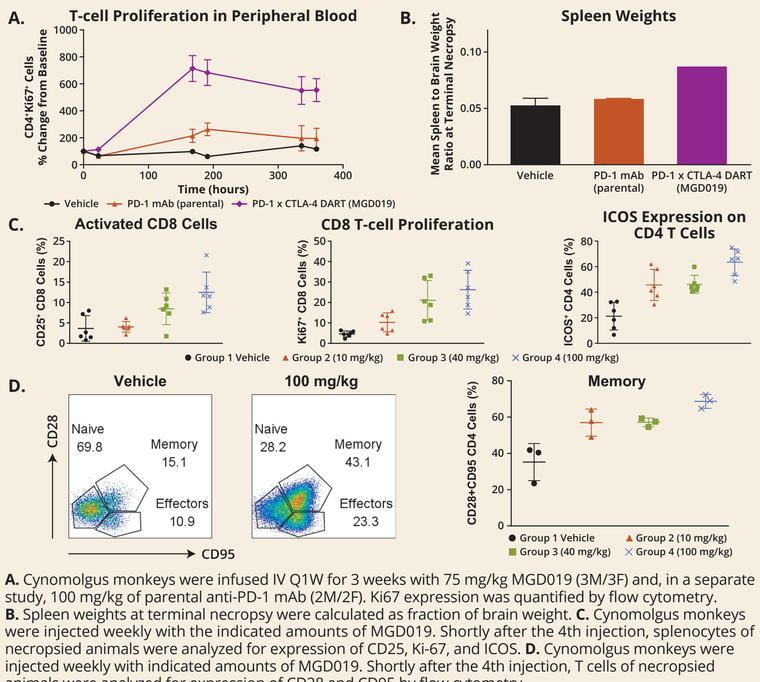
- MGD019 was well-tolerated compared to prior reported PD-1+CTLA-4 mAb combination[1]

MGD019 Pharmacokinetics and Receptor Occupancy



Cynomolgus monkeys (3F/3M) were infused with 10, 40 or 100 mg/kg/dose MGD019 at Day 1, 8, 15, and 22. Serum concentration was measured by ELISA **(right)** and receptor occupancy was measured by flow cytometry **(left)**.

MGD019 Supports Homeostatic T-cell Proliferation and Memory T-cell Expansion in Cynomolgus Monkeys



A. Cynomolgus monkeys were infused IV Q1W for 3 weeks with 75 mg/kg MGD019 (3M/3F) and, in a separate study, 100 mg/kg of parental anti-PD-1 mAb (2M/2F). Ki67 expression was quantified by flow cytometry. **B.** Spleen weights at terminal necropsy were calculated as fraction of brain weight. **C.** Cynomolgus monkeys were injected weekly with the indicated amounts of MGD019. Shortly after the 4th injection, splenocytes of necropsied animals were analyzed for expression of CD25, Ki-67, and ICOS. **D.** Cynomolgus monkeys were injected weekly with indicated amounts of MGD019. Shortly after the 4th injection, T cells of necropsied animals were analyzed for expression of CD28 and CD95 by flow cytometry.

Phase I Study

A Phase 1, First-in-Human, Open-Label, Dose Escalation and Cohort Expansion Study of MGD019: Key Study Objectives

Primary Objective:

- Characterize safety, including tolerability, dose limiting toxicities (DLTs), and maximum tolerated dose (MTD) or maximum administered dose (MAD) if no MTD is defined, of MGD019 given intravenously to patients with advanced solid tumors

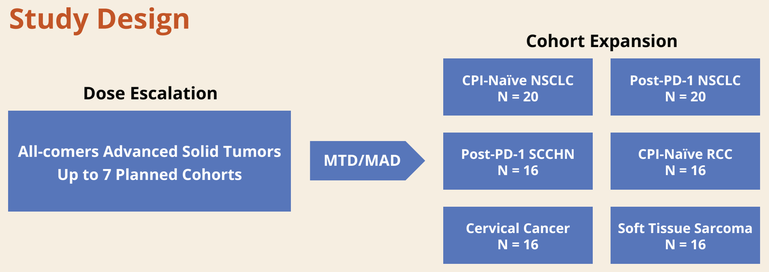
Secondary Objectives:

- Characterize PK and immunogenicity
- Describe preliminary evidence of antitumor activity using conventional RECIST v1.1 and immune-related RECIST

Exploratory Objectives:

- Explore relationship between PK, pharmacodynamics, and antitumor activity; immune-regulatory effects of MGD019, including measures of immune cell activation/exhaustion in peripheral blood and/or tumor biopsy specimens; relationships among PD-1, PD-L1, and CTLA-4 expression on tumor cells and immune cell infiltrates within biopsy specimens (including CD4+ and CD8+ T cells) and antitumor activity; relationship between gene profiles and antitumor activity; relationship between tumor mutational burden and antitumor activity

Study Design



Entry Criteria

Key Inclusion Criteria

- Dose escalation: Patients with histologically proven, unresectable, locally advanced or metastatic solid tumors for whom no approved therapy with demonstrated clinical benefit is available or patients who are intolerant to standard therapy
- Cohort expansion phase: Disease-specific prior therapy requirements to be specified
- ECOG performance status of 0–1
- Life expectancy ≥12 weeks
- Measurable disease as per RECIST 1.1 for the purpose of response assessment must either (a) not reside in a field that has been subjected to prior radiotherapy or (b) have demonstrated clear evidence of radiographic progression since the completion of prior radiotherapy and prior to study enrollment
- All patients must have an identified formalin-fixed, paraffin embedded tumor specimen for immunohistochemical evaluation of pharmacodynamic markers of interest
- Acceptable laboratory parameters and adequate organ reserve

Key Exclusion Criteria

- In patients who have previously received an immune checkpoint inhibitor, toxicities related to the CPI must have resolved to ≤ Grade 1 or baseline. Patients with well controlled immune endocrinopathies secondary to prior checkpoint therapy are eligible
- Patients with symptomatic CNS metastases. Patients with history of CNS metastasis must have been treated, must be asymptomatic, and must not have concurrent treatment for the CNS disease, progression of CNS metastases on MRI or CT for at least 14 days after last day of prior therapy for the CNS metastases, or concurrent leptomeningeal disease or cord compression
- Patients who experienced the following Grade 3 CPI-related AEs are ineligible: ocular AE, changes in liver function tests that met the criteria for Hy's law, neurologic toxicity, colitis, renal toxicity, pneumonitis
- Patients with prior therapy with a combination of monoclonal antibodies against PD-1/PD-L1 and CTLA-4 will be excluded in Cohort Expansion
- Patients with any history of known or suspected autoimmune disease with certain exceptions
- History of prior allogeneic bone marrow, stem cell, or solid organ transplantation
- History of trauma, major surgical procedure, systemic antineoplastic therapy, or investigational therapy within 4 weeks and treatment with radiation therapy within 2 weeks prior to initiation of study drug administration

Conclusions

- T cells co-expressing PD-1 and CTLA-4 are more prevalent in tumors compared to healthy tissues
- MGD019 binds to and blocks its targets with increased activity on dual PD-1/CTLA-4-expressing cells
- MGD019 does not deplete peripheral blood Tregs in vitro
- In cynomolgus monkeys, MGD019 demonstrates IgG4-like PK and an acceptable safety profile similar to that observed with PD-1 blockade alone, while demonstrating biological effects of CTLA-4 antagonism

References

1. Selby M. et al. Preclinical Development of Ipilimumab and Nivolumab Combination Immunotherapy: Mouse Tumor Models, In Vitro Functional Studies, and Cynomolgus Macaque Toxicology. *PLoS One*. 2016 Sep 9;11(9):e0161779.

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