A PD-1 x CTLA-4 Bispecific DART® Protein with Optimal Dual Checkpoint Blockade and Favorable Tolerability in Nonhuman Primates

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Abstract

PD-1 x CTLA-4 DART Molecule In Vitro:

MGD019, the PD-1 x CTLA-4 DART molecule, demonstrated binding to immobilized programmed cell death protein 1 (PD-1) and PD-1-expressing cells lines, inhibition of PD-1 interaction with PD-L1 or PD-L2, as well as reversal of PD-1-mediated T-cell signal inhibition in gene-reporter assays comparable to that supported by a replica of nivolumab. Similarly, binding, ligand blocking and rescue of CTLA-4-mediated T-cell suppression was comparable to that supported by a replica of ipilimumab. MGD019 demonstrated activation properties comparable to the combination of the replicas of ipilimumab and nivolumab in a variety of human primary T-cell assays and showed enhanced B7-ligand binding blockade over that mediated by the ipilimumab replica on PD-1/CTLA-4 double-positive cells.

PD-1 x CTLA-4 DART Molecule In Vivo:

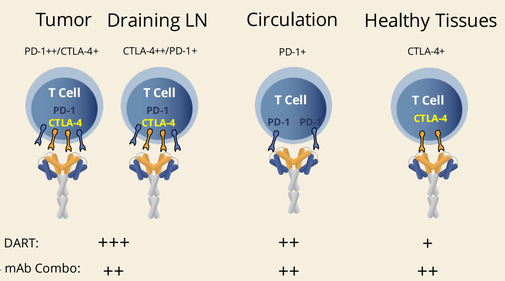
In cynomolgus monkeys, MGD019 exhibited a PK profile consistent with that of an IgG4 and was well tolerated with no mortality or significant adverse findings up to 75 mg/kg QWx3, the highest dose tested. T-cell expansion in the peripheral blood and lymphoid organs was observed, which was attributable to the CTLA-4 blocking arm, since no such finding was observed with similar or higher doses of the anti-PD-1 constituent of the bispecific molecule.

Significance:

The favorable safety and tolerability profile of the MGD019 combined with its enhanced activity on PD-1/CTLA-4 double-positive cells suggest a potential for an improved therapeutic window for PD-1/CTLA-4 co-blockade strategies, with the administration of a single molecule providing dosing convenience and ease of incorporation into additional therapeutic regimens.

Introduction

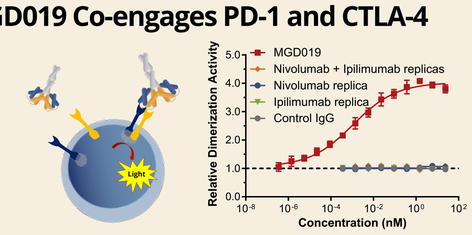
Rationale for PD-1 x CTLA-4 Dual Checkpoint Targeting Strategy



MGD019 is designed to block both PD1 and CTLA4 checkpoint inhibition pathways with enhanced activity on PD1$^+$CTLA4$^+$ double-positive cells.

MGD019 (PD1 x CTLA-4)
hinge stabilized
IgG4 tetravalent bispecific
DART molecule

MGD019 Co-engages PD-1 and CTLA-4

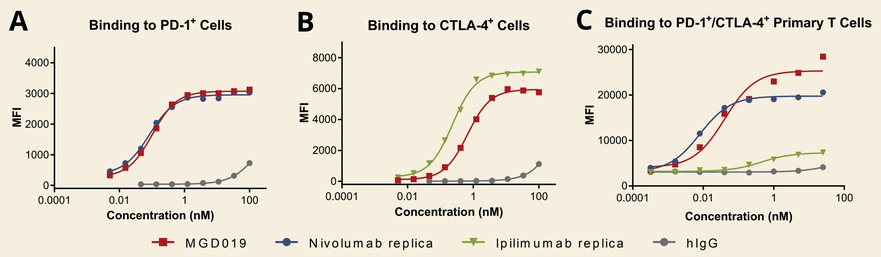


Molecules were evaluated using enzyme fragment complementation assay employing PathHunter® U2OS PD-1/CTLA-4 dimerization cell line (DiscoverX).

Results

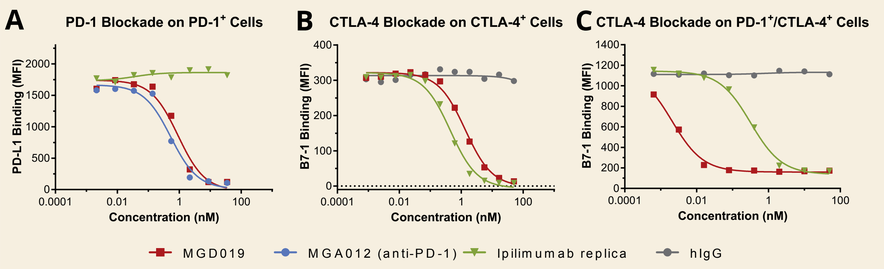
MGD019: Enhanced Response in PD-1$^+$CTLA-4$^+$ Cells

PD-1 and CTLA-4 cell surface binding



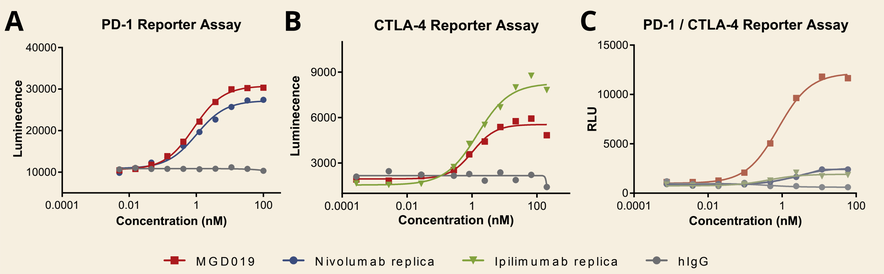
PD-1-expressing Jurkat cells **(A)**, CTLA-4 expressing Jurkat cells **(B)** or In vitro expanded primary T-cells **(C)** were incubated with control IgG, ipilimumab replica, nivolumab replica, or MGD019.

Blockade of ligand binding to CTLA-4



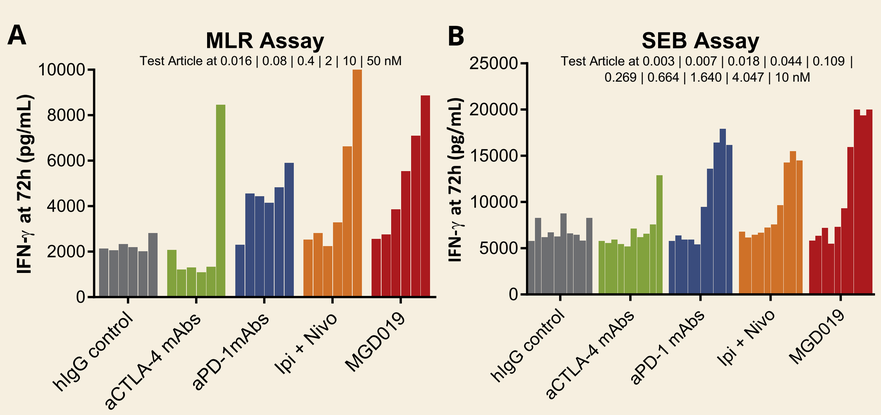
PD-1-expressing Jurkat cells **(A)**, CTLA-4 expressing Jurkat cells **(B)**, or PD-1$^+$/CTLA-4$^+$ dual-expressing Jurkat cells **(C)** were coincubated with control IgG, ipilimumab replica, MGA012 (anti-PD-1), or MGD019.

Restoration of T-cell signaling



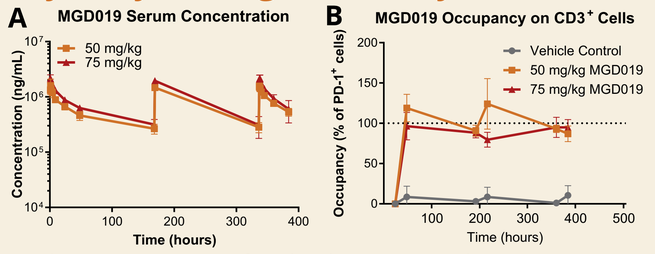
PD-1 **(A)**, CTLA-4 **(B)** and PD-1/CLTA-4 **(C)** cell-based reporter systems (Promega) were evaluated with control IgG, ipilumumab replica, nivolumab replica, or MGD019.

MGD019 is Equipotent to Nivolumab + Ipilimumab Combination in Vitro



(A) Mixed lymphocyte reaction between monocyte derived dendritic cells and freshly isolated CD4+ T cells in the presence MGD019, parental mAbs or combination of replicas of nivolumab and ipilimumab. IFN-γ secretion was measured by ELISA at 72 hours. **(B)** Human PBMCs were stimulated with 500 ng/mL SEB for 72 h. IFN-γ secretion was determined by ELISA. Molarity refers to the concentration of individual components, whether used alone or in combination.

MGD019 Displays Prolonged Exposure and T-cell Occupancy in Cynomolgus Monkeys



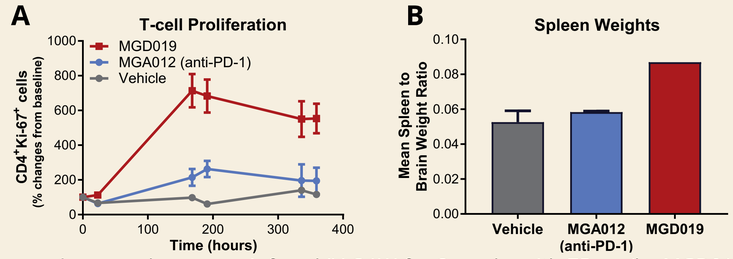
Cynomolgus monkeys (3F/3M) were infused with 50 or 75 mg/kg MGD019 at Day 1, 8, and 15. Serum concentration was measured by ELISA **(A)** and receptor occupancy was measured by flow cytometry **(B)**.

MGD019: Observations in Cynomolgus Monkeys

Finding	Control	50 mg/kg	75 mg/kg
↑ Spleen weight	Not applicable	Yes, both M & F	Yes, both M & F
↑ Thymus weight	Not applicable	Yes, both M & F	Yes, M only
Spleen – minimal to slight lymphoid follicle hypertrophy	0/2	4/6	4/6

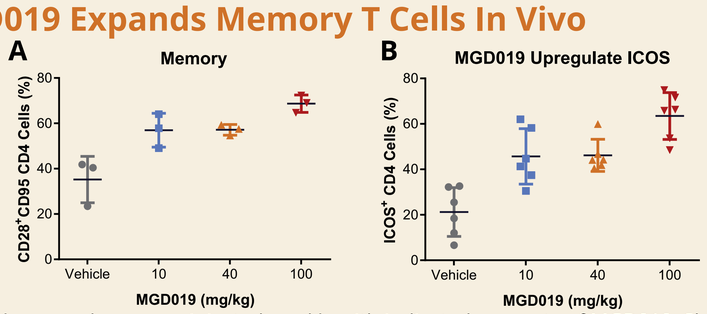
No mortality or significant adverse findings

MGD019 Expands T Cells In Vivo through CTLA-4 Blockade



(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 MGA012 (anti-PD-1, 2M/2F). Ki67 expression was quantified by flow cytometry. **(B)** Cynomolgus monkeys (3M/3F) were infused IV with 100 mg/kg MGD019 (Q1W for 3 weeks) and, in a separate study, 150 mg/kg MGA012 (Q1W for 4 weeks, 3M/3F). Spleen weights at terminal necropsy were calculated as fraction of brain weight.

MGD019 Expands Memory T Cells In Vivo



Cynomolgus monkeys were injected weekly with indicated amounts of MGD019. Shortly after 4th injection, splenocytes of necropsied animals were analyzed for expression of CD95/CD28 **(A)** and ICOS **(B)**.

Conclusions

- MGD019 binds to and blocks its targets with increased activity on dual PD-1/CTLA-4-expressing cells.
- MGD019 enhances T-cell responses in vitro to the level achieved by a combination of replicas of nivolumab and ipilimumab.
- MGD019 was well tolerated in cynomolgus monkeys with a safety profile similar to that observed with PD-1 blockade alone while demonstrating biological effects of CTLA-4 antagonism.

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