# MGD013, a Bispecific PD-1 x LAG-3 Dual-Affinity Re-Targeting (DART®) Protein with T-cell Immunomodulatory Activity for Cancer Treatment

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## Abstract

**Introduction:** The combination of monoclonal antibodies (mAbs) that targets the immune checkpoint molecules CTLA-4 and PD-1 has shown clinical benefit beyond that observed with either mAb alone. This finding has prompted exploring whether such an approach could be applied within the context of additional combinations of checkpoint molecules, such as PD-1 and lymphocyte activation gene-3 (LAG-3). Animal tumor models have validated combining anti-PD-1 with anti-LAG-3 mAbs in eliciting synergistic tumor-eradicating immunity[1]; expression of PD-1 and LAG-3 on exhausted T cells and tumor-infiltrating lymphocytes (TILs) further supports their dual-targeting. We have developed a bispecific DART protein that targets PD-1 and LAG-3, aimed at inducing potent antitumor immunity through simultaneous blockade of non-redundant checkpoint pathways intrinsic to exhausted T cells.

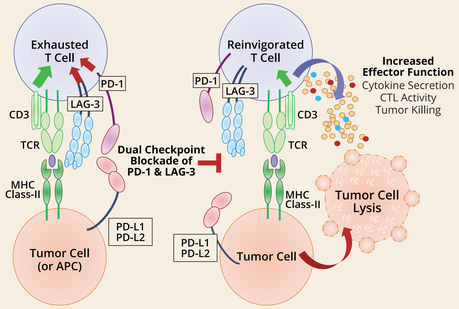
**Methods:** mAbs against PD-1 and LAG-3 were generated and selected for DART conversion based on binding, biophysical and functional blocking against their respective receptor/ligand axes, and functional activity in reactivation of prior superantigen-stimulated T cells or in antigen-specific recall assays.

**Results:** Lead PD-1 and LAG-3 mAbs demonstrating favorable functional properties were selected for humanization. Immunohistochemistry (IHC) confirmed that the lead LAG-3 and PD-1 mAbs display restricted lymphocyte expression in human tissues and overlapping expression in TILs. The humanized mAbs were assembled into MGD013, an Fc-bearing PD-1 x LAG-3 DART protein that demonstrated favorable biophysical and manufacturability properties. MGD013 bound specifically with high affinity to PD-1 and LAG-3, as well as to target-expressing cell lines and chronically-activated T cells. MGD013 blocked PD-1/PD-L1, PD-1/PD-L2, and LAG-3/HLA (MHC-II) interactions and PD-1 signaling. Further functional characterization of MGD013 revealed enhanced cytokine secretion in response to antigenic rechallenge of previously stimulated T cells compared to that observed upon independent blockade of either the PD-1 or LAG-3 pathways alone. Furthermore, under the above experimental conditions, MGD013 mediated greater cytokine secretion than that observed with the combination of equivalent (equimolar) levels of replicas of the approved PD-1 mAb, nivolumab[2], and the LAG-3 mAb, 25F7, which is currently undergoing clinical testing. Finally, cynomolgus monkey pharmacokinetic (PK) studies demonstrated a prolonged circulating half-life consistent with that of an Fc-bearing molecule.

**Conclusion:** MGD013 blocks both PD-1 and LAG-3 pathways, resulting in enhanced T-cell responses compared to single or combination mAb blockade. Together with favorable cynomolgus monkey PK, these studies support further clinical development of MGD013.

## Introduction

- PD-1 and LAG-3 are two co-inhibitory molecules that deliver negative signals upon interaction with ligands expressed on tumor cells (PD-L1) and/or antigen presenting cells (APCs) (PD-L1, PD-L2, or MHC-II molecules)
- PD-1 and LAG-3 are co-expressed on exhausted T cells in chronic viral infections and on TILs




Modified from Freeman, et al., 2012[3]

- Combination mAb blockade of PD-1 and LAG-3 in animal models resulted in enhanced antitumor immunity than with either mAb alone[2]
- MGD013, a checkpoint inhibitor DART molecule, has been designed to restore T-cell effector function and enhance antitumor activity by simultaneously targeting PD-1 and LAG-3

**1.** Wang C. et al. Cancer Immunol Res. 2014 Sep;2(9):846-56. **2.** Woo SR, et al. Cancer Res. 2012 Feb 15;72(4):917-27. **3.** Freeman GJ, et al. Nat Immunol. 2012 Jan 19;13(2):113-5.

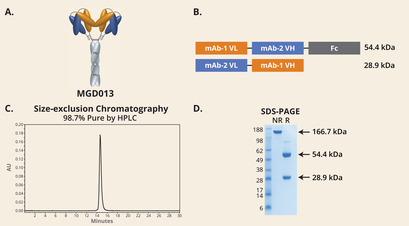
## Strategy

- Mouse anti-human PD-1 and LAG-3 mAb panels generated
- Performance-based selection evaluated against replicas of nivolumab* (anti-PD-1) and 25F7* (anti-LAG-3), including:
  - Cynomolgus monkey cross-reactivity
  - Binding characteristics (surface plasmon resonance [SPR] analysis & ELISA)
  - Binding to PD-1 or LAG-3 transfectants and stimulated human or non-human primate peripheral blood mononuclear cells (PBMCs)
  - Ligand binding blockade
    - Soluble human PD-1/PD-L1, PD-1/PD-L2, or LAG-3/MHC-II binding inhibition
    - PD-1/PD-L1 signaling blockade
  - Enhanced IFN-γ secretion following staphylococcal enterotoxin B (SEB) stimulation
- Lead mAbs were humanized and engineered as PD-1 x LAG-3 DART molecules for further testing

*Replicas of nivolumab (a licensed anti-PD-1 mAb) and 25F7 (a clinical stage anti-LAG-3 mAb) were generated by MacroGenics based on published sequences.

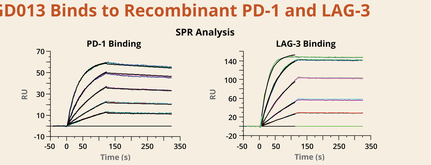
## Results

### MGD013: A Bispecific PD-1 x LAG-3 DART Checkpoint Inhibitor Molecule




MGD013 is a cynomolgus monkey cross-reactive Fc-bearing (IgG4) DART protein comprising bispecificity for two checkpoint molecules, PD-1 (CD279) and LAG-3 (CD223), (A) yielding a homogenous product with an anticipated molecular weight of 166.7 kDa composed of a two-chain protein structure with a molecular weight of 54.4 kDa and 28.9 kDa (B), as shown by size-exclusion chromatography (C) and SDS-PAGE (D).

### MGD013 Binds to Recombinant PD-1 and LAG-3

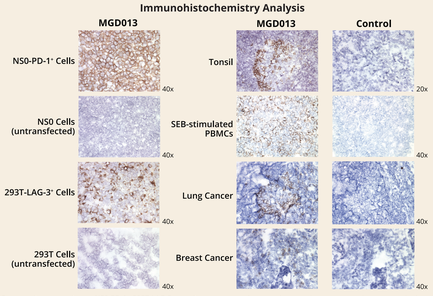



| | Soluble Human PD-1 | | | Soluble Human LAG-3 | | |
|---|---|---|---|---|---|---|
| | $K_D$ (nM) | $k_a$ ($M^{-1}s^{-1}$) | $k_d$ ($s^{-1}$) | $K_D$ (nM) | $k_a$ ($M^{-1}s^{-1}$) | $k_d$ ($s^{-1}$) |
| MGD013 (PD-1 x LAG-3 DART) | 1.0 | $3.1 \times 10^5$ | $3.0 \times 10^{-4}$ | 0.1 | $4.1 \times 10^5$ | $3.9 \times 10^{-5}$ |
| MacroGenics' anti-PD-1 | 0.6 | $4.3 \times 10^5$ | $2.4 \times 10^{-4}$ | No binding | No binding | No binding |
| MacroGenics' anti-LAG-3 | No binding | No binding | No binding | 0.1 | $9.9 \times 10^4$ | $<1.0 \times 10^{-5}$ |
| Nivolumab* (anti-PD-1) | 6.1 | $1.3 \times 10^5$ | $7.9 \times 10^{-4}$ | No binding | No binding | No binding |
| 25F7* (anti-LAG-3) | No binding | No binding | No binding | 1.1 | $5.8 \times 10^5$ | $6.3 \times 10^{-4}$ |

SPR analysis of binding of soluble human PD-1 or LAG-3 to MGD013 captured on $Fab_2$ goat-anti-human Fc-coated surface.

- MGD013 compares favorably to nivolumab* or 25F7*

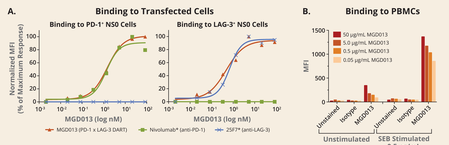
### MGD013 Reacts with Both PD-1 and LAG-3




IHC was performed on frozen NS0-PD-1+, 293T-LAG-3+ transfectants and their respective negative control cell lines, lymphoid tissues (tonsils), SEB-stimulated human PBMCs, and lung or breast tumor, using biotinylated MGD013 and a negative control antibody, followed by streptavidin-HRP and a chromogenic detection system.

- MGD013 binds lymphocyte populations as expected, including lymphoid organs (tonsil) and activated human PBMCs
- MGD013 also binds both lung and breast cancer TILs

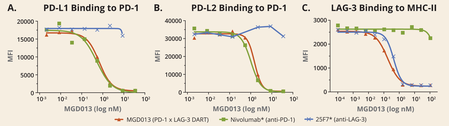
### MGD013 Binds Cell-surface Expressed PD-1 and LAG-3




| | Binding to PD-1+ NS0 Cells | Binding to LAG-3+ NS0 Cells |
|---|---|---|
| | $EC_{50}$, nM (mean ± SEM), n = 4 | |
| MGD013 (PD-1 x LAG-3 DART) | 1.65 ± 0.08 | 0.41 ± 0.07 |
| Nivolumab* (anti-PD-1) | 1.79 ± 0.18 | No binding |
| 25F7* (anti-LAG-3) | No binding | 0.76 ± 0.24 |

**A.** Flow cytometry analysis of $ALEXA_{647}$-labeled MGD013 to NS0 cells transfected with human PD-1 or LAG-3.
**B.** Flow cytometry analysis of $ALEXA_{647}$-labeled MGD013 to human unstimulated or SEB-stimulated human PBMCs.

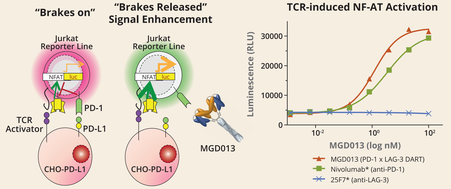
### MGD013 Blocks Ligand Binding to PD-1 and LAG-3




**A & B.** 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 or DART molecule. Shown is a representative experiment of four performed.

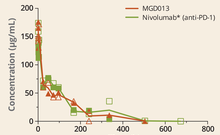
**C.** Flow cytometric analysis of soluble LAG-3 binding to MHC class-II-expressing Daudi cells in the presence of titrating concentrations of the indicated mAbs or DART molecule. Shown is a representative experiment of four performed.

### MGD013 Releases PD-1 Co-inhibitory Signaling




The indicated mAbs and DART molecule 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. Shown is a representative experiment of four performed.

### MGD013 Pharmacokinetics Following a Single Dose Infusion in Cynomolgus Monkeys

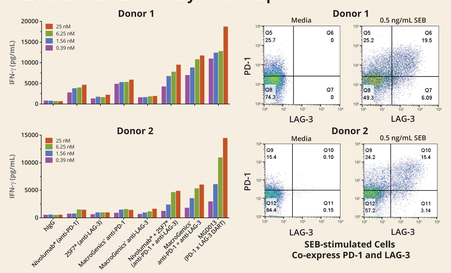



Pharmacokinetic data obtained from the serum of two cynomolgus monkeys (1 female, 1 male) per test article infused IV (1 hour) with a single dose of MGD013 (5 mg/kg) or nivolumab* (10 mg/kg). The solid line represents the mean of both male and female monkeys infused with MGD013 (brown) or nivolumab* (green). Open symbols: females; filled symbols: males.

- MGD013 concentration-time profile comparable to nivolumab*

### MGD013 Enhances T-cell Antigen Receptor-driven Activation In Vitro

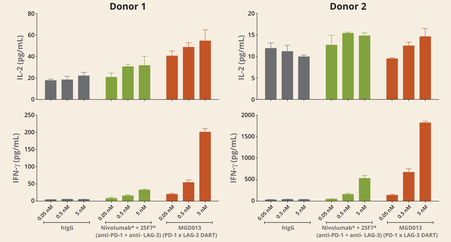
#### MGD013 Enhances Primary T-cell Response




Human PBMCs from two representative donors were stimulated with SEB for 48 hours, washed twice, and restimulated with SEB at 0.5 ng/mL. IFN-γ secretion was determined by ELISA. PD-1 and LAG-3 expression was demonstrated by flow cytometry. Data shown are representative of 12 separate donors tested; MGD013 outperformed nivolumab* + 25F7* combination in all assays performed. Molarity refers to the concentration of individual components, whether used alone or in combination (1 nM = 0.17 µg/mL DART; 1 nM = 0.15 µg/mL mAb).

- MGD013 shows enhanced SEB-induced IFN-γ secretion compared to PD-1 + LAG-3 mAb combination

#### MGD013 Enhances Antigen-specific T-cell Response




CD4 memory T cells (1 x $10^5$ cells/well) were cultured for 7 days with irradiated monocytes (1 x $10^4$ cells/well, 3000 rads) in the presence of 5 µg/mL tetanus toxoid. Shown are two representative donors of five tested. Molarity refers to the concentration of individual components, whether used alone or in combination (1 nM = 0.17 µg/mL DART; 1 nM = 0.15 µg/mL mAb).

- MGD013 enhances antigen-specific cytokine secretion in a tetanus-toxoid recall assay

## Conclusions

- MGD013 is a tetravalent bispecific Fc-bearing DART molecule with a human IgG4 backbone:
  - Capable of simultaneously binding PD-1 and LAG-3
  - Blocks PD-1/PD-L1, PD-1/PD-L2, and LAG-3/MHC-II interactions with potency comparable to nivolumab* (anti-PD-1) or 25F7* (anti-LAG-3)
  - Enhances T-cell responses compared to individual mAb or combination mAb blockade
  - Demonstrates a PK profile comparable to that of nivolumab* in cynomolgus monkeys

**Further clinical development of MGD013 as cancer treatment is warranted**

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