# Preclinical Characterization of MGD013, a PD-1 x LAG-3 Bispecific DART® Molecule

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## Abstract

**Background:** Monoclonal antibodies (mAbs) that target the immune checkpoints, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1), have shown enhanced clinical antitumor activity when given in combination, triggering interest in determining whether additional checkpoint inhibitor combinations may afford enhanced clinical benefit. Lymphocyte-activation gene 3 (LAG-3) is another immune checkpoint expressed on activated T cells and tumor infiltrating lymphocytes (TILs). Recognizing the therapeutic potential of dual checkpoint blockade, we have engineered MGD013, a IgG4κ bispecific DART molecule, to bind PD-1 and LAG-3 concomitantly or independently and disrupt these nonredundant inhibitory pathways to further restore exhausted T-cell function.

**Methods:** Proprietary PD-1 and LAG-3 mAbs were generated and selected based on binding characteristics, biophysical properties, the ability to block their respective receptor/ligand axes and to synergize in T-cell stimulation assays. Humanized sequences were incorporated into a tetravalent bispecific DART format and benchmarked against combinations of replicas of the approved PD-1 mAb (nivolumab) and BMS-986016 anti-LAG-3 mAb (25F7), which is currently under clinical evaluation. MGD013 biological activity was evaluated in various primary cell-based immune assays. Safety was assessed in cynomolgus monkey toxicology studies performed at MPI (Mattawan, MI) under Institutional Animal Care and Use Committee-approved protocols.

**Results:** MGD013 bound with high affinity to human and cynomolgus monkey PD-1- and LAG-3-expressing cells and blocked PD-1/PD-L1, PD-1/PD-L2 and LAG-3/HLA (MHC-II) interactions, with resultant signaling blockade. Functional characterization revealed enhanced cytokine secretion in response to antigen stimulation that was greater than that of the combination of individual equimolar amounts of PD-1 and LAG-3 mAbs. MGD013 was well tolerated in a repeated-dose (Q1Wx4) cynomolgus monkey toxicology study. Except for the occurrence of watery feces in a few animals, no MGD013-related adverse findings were noted, including hematological or clinical chemistry changes, serum cytokine levels or gross and microscopic histological findings, establishing a no-observed-adverse-effect level (NOAEL) of 100 mg/kg.

**Conclusion:** MGD013 is a bispecific DART molecule capable of simultaneously blocking the PD-1 and LAG-3 pathways, resulting in enhanced T-cell activation compared to single or combination mAb blockade. MGD013 has demonstrated a favorable preclinical safety and toxicological profile and is currently initiating clinical testing [NCT03219268].

## Introduction

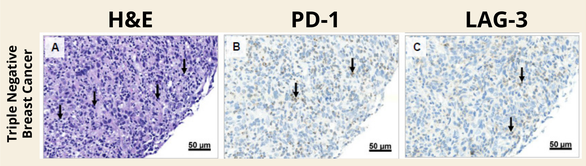
### Rationale

- PD-1 and LAG-3 are two coinhibitory molecules that deliver negative signals upon interaction with ligands expressed on tumor cells and/or antigen presenting cells (PD-L1, PD-L2, or MHC-II).

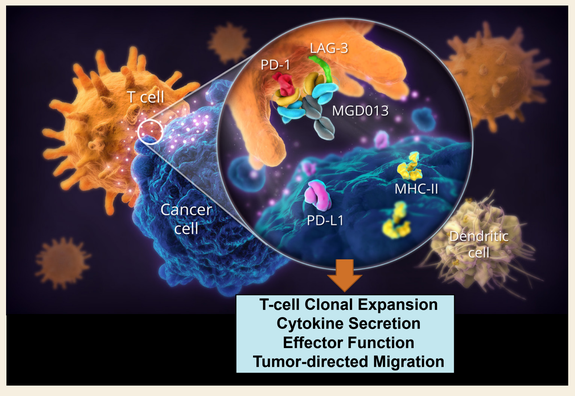
**PD-1 and LAG-3 are Expressed on TILs**

| Tumor MicroArray (TMA) Spot Count for Particular Indication | Expression of Checkpoint(s) Across All TMAs* | | | Overlapping Expression** | |
|---|---|---|---|---|---|
| | LAG-3⁺ Total | PD-1⁺ Total | LAG-3⁺PD-1⁺ Total | LAG-3⁺ PD-1⁺ | PD-1⁺ LAG-3⁺ |
| Lung Squamous Cell Carcinoma | 18/36<br>50% | 15/36<br>42% | 10/36<br>28% | 10/15<br>67% | 10/18<br>56% |
| Lung Adenocarcinoma | 19/33<br>58% | 18/33<br>55% | 15/33<br>46% | 15/18<br>83% | 15/19<br>79% |
| Triple Negative Breast Cancer | 16/29<br>55% | 12/29<br>41% | 10/29<br>35% | 10/12<br>83% | 10/16<br>63% |

*Spot Counts indicate the number of TMA positive for LAG-3, PD-1, or LAG-3 + PD-1 expression divided by the total TMAs
**Spot Counts indicate the number of TMA with overlapping checkpoint expression of PD-1 and LAG-3




- Combination mAb blockade of PD-1 and LAG-3 in animal models resulted in enhanced antitumor immunity than either mAb alone and is actively being tested clinically.
- **MGD013 is a checkpoint inhibitor DART molecule currently under clinical evaluation that has been designed to restore T-cell effector function and enhance antitumor activity by simultaneously targeting PD-1 andLAG-3.**

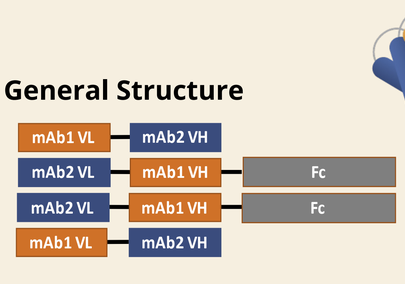


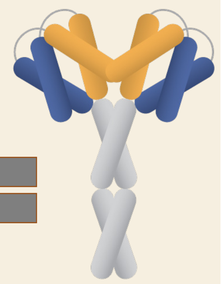

## Results

### Structure of MGD013

- High affinity binding to PD-1 and LAG-3 that compares favorably to nivolumab* (anti-PD-1) or 25F7* (anti-LAG-3)

**General Structure**



**MGD013 (PD1 x LAG3) hinge stabilized IgG4 tetravalent, bispecific DART molecule**

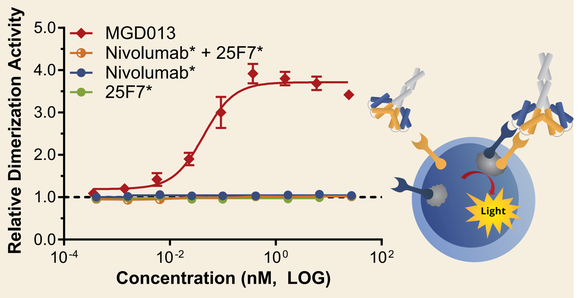
* Replicas of nivolumab and 25F7 mAbs produced at MacroGenics based on published sequences

### MGD013 Binds PD-1 and LAG-3 and Blocks Ligand Interactions



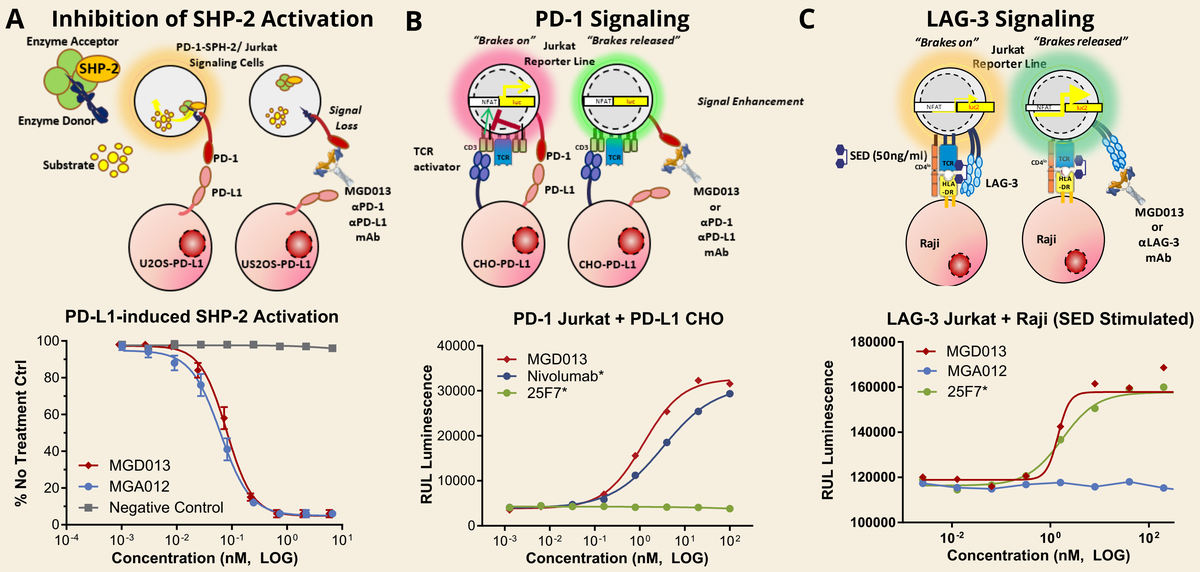

Binding of MGD013 to NS0-PD-1⁺ **(A)** and NS0-LAG-3⁺ **(B)** engineered cells was assessed by FACS analysis. Inhibition of soluble PD-L1 binding to NS0-PD-1⁺ cells **(C)** or soluble LAG-3 binding to class II⁺ Daudi cells **(D)**, respectively, was assessed by FACS analysis. Similar data were obtained for soluble PD-L2 binding to NS0-PD-1⁺ cells (data not shown).

### MGD013 Co-engages PD-1 & LAG-3




Indicated molecules were evaluated using enzyme fragment complementation assay employing PathHunter® U2OS PD-1/LAG-3 dimerization cell line (DiscoverX).

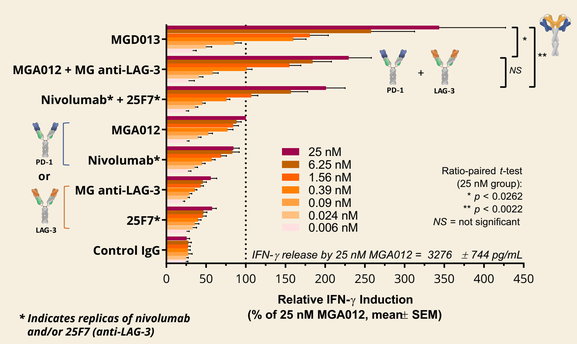
### MGD013 Disrupts PD1- & LAG-3- Mediated T-cell Inhibitory Signaling




MGD013 demonstrates a dose dependent blockade of the PD-1/PD-L1 axis comparable to anti-PD-1 mAbs as evaluated in PD-1 reporter models obtained from DiscoverX's PathHunter® Enzyme Fragment Complementation Assay to inhibit SHP-2 activation **(A)** or Promega's PD-1/PD-L1 Blockade Biosassay to release NF-AT blockade **(B)**. Similarly, MGD013 demonstrates a dose dependent blockade of the LAG-3/MHC-class II axis comparable to a replica of BMS's 25F7 [anti-LAG-3 mAb] evaluated in Promega's LAG-3/MHC-class II Blockade Bioassay to release NF-AT blockade **(C)**.

### MGD013 Enhances Antigen-driven T-cell Cytokine Function In Vitro

**Enhancement of T-cell response following SEB stimulation**

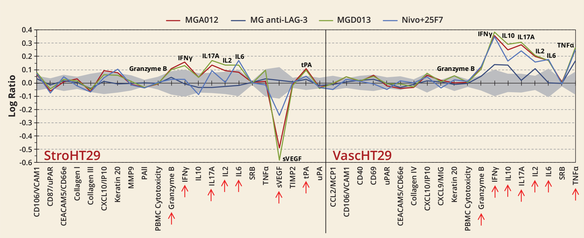



** Indicates replicas of nivolumab and/or 25F7 (anti-LAG-3)*

- MGA012 + MG anti-LAG-3 combination comparable to benchmark antibody combination
- MGD013 enhanced IFN-γ secretion beyond that observed with antibody combinations

### MGD013 Enhances Immune Responses in TME Models

**Compares favorably to PD-1 + LAG-3 mAb combination**




MGD013, MGA012 (PD-1 mAb), MG's LAG-3 mAb were evaluated and compared against the combination of nivolumab* + 25F7* for their ability to capture immune activation under the mimicry of the tumor microenvironment (TME). HT-29 colorectal cells were cultured with fibroblasts and PBMCs to recapitulate a stromal microenviroment (left panel) or with endothelial cells and PBMCs to recapitulate a vascular microenvironment (right panel). Immune profiling of checkpoint targets including adhesion molecules, cytotoxic granules, and cytokines were measured (DiscoverX).

### Well Tolerated in Cynomolgus Monkeys

| | |
|---|---|
| **Pilot Toxicology Study Design** | ▪ 1-hour IV infusion at 100 (1 male) or 150mg/kg (2 males)<br>▪ QW x 2 |
| **GLP Toxicology Study Design** | ▪ 1-hour IV infusion at 10, 40 or 100 mg/kg (5 animals/sex/group)<br>▪ QW x 4; 10-week recovery |
| **Observations** | ▪ Well tolerated. Drug-related changes were limted to watery feces at ≥ 40 mg/kg with no impact to body weight.<br>▪ Nonadverse increase in incidence of mononuclear cell infiltrates.<br>▪ No cytokine release |
| **Conclusion** | ▪ NOAEL = 100 mg/kg; MTD > 150 mg/kg |

## Conclusions

- MGD013 was engineered as a tetravalent bispecific DART molecule in a human hinge-stabilized IgG4 backbone.
- MGD013 is capable of simultaneously binding PD-1 and LAG-3.
- MGD013 blocks PD-1/PD-L1/PD-L2 and LAG-3/MHC-Class II interactions and resultant inhibitory signal with potency comparable to MGA012 (anti-PD-1), and replicas of nivolumab or 25F7 (anti-LAG-3).
- MGD013 enhances T-cell responses compared to individual mAbs or combination mAb blockade.
- MGD013 was well-tolerated and demonstrates favorable pharmacokinetics in cynomolgus monkeys.

**Clinical testing of MGD013 in several cancer indications is ongoing [NCT03219268].**

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