### Introduction

The combination of monoclonal antibodies (mAbs) that targets the immune checkpoint molecules CTLA-4 and PD-1 has shown clinical benefit beyond that of anti-PD-1 alone. This finding has prompted exploration 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 enhanced T-cell responses compared to single anti-PD-1 mAbs, consistent with that of an Fc-bearing molecule.

Studies demonstrated a prolonged circulating half-life through simultaneous blockade of non-redundant receptor/ligand axes, and functional activity in reactivation of prior superantigen-stimulated T cells or in antigen-specific recall assays.

### 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.**
- **Lead mAbs were humanized and engineered as PD-1 x LAG-3 DART proteins that demonstrated favorable functional properties were selected for humanization. Lead PD-1 and LAG-3 mAbs demonstrating favorable functional properties were selected for humanization.**
- **MGD013 is a tetravalent bispecific Fc-bearing DART protein that targets the immune checkpoint PD-1 and LAG-3. It is 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).**

### Results

- **Combination mAb blockade of PD-1 and LAG-3 in animal models resulted in enhanced anti-tumor immunity than with either mAb alone.**
- **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.**
- **MGD013 enhances antigen-specific cytokine secretion in a tetanus-toxoid model.**
- **MGD013 is a tetravalent bispecific Fc-bearing DART protein 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 consistent to nivolumab (anti-PD-1) or 25F7* (anti-LAG-3)**
  - **Enhances T-cell responses compared to individual mAbs or combination blockade**
  - **Demonstrates a PK profile comparable to that of nivolumab in cynomolgus monkeys.**

### Conclusions

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