Preclinical Characterization of MGA012, A Novel Clinical-stage PD-1 Monoclonal Antibody

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Abstract

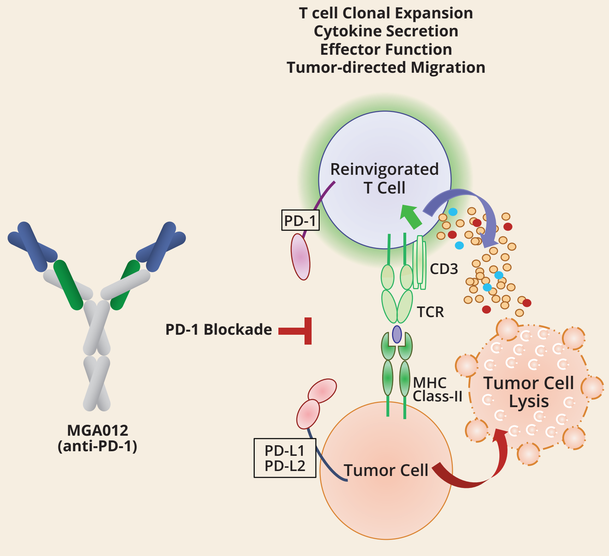
Background: Monoclonal antibodies (mAbs) that target immune checkpoint pathways, such as the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and the programmed cell death protein 1 (PD-1) pathways, have demonstrated broad clinical efficacy against a variety of malignancies as monotherapy or in a combination. MGA012 is a novel anti-PD-1 mAb developed to disrupt the PD-1 interaction with PD-L1/PD-L2 to restore or improve T-cell function as stand-alone therapy or in combination with other immune approaches.

Methods: Murine PD-1 mAbs were generated and benchmarked against replicas of the approved mAbs, nivolumab, and pembrolizumab. Several mAbs with favorable characteristics were further chimerized or humanized. MGA012, a humanized, hinge-stabilized IgG4κ mAb, was selected based on binding and biophysical properties as well as a functional characterization inclusive of enhanced T-cell activation following superantigen restimulation.

Results: MGA012 bound human PD-1 with an affinity equal to or exceeding those of replicas of nivolumab or pembrolizumab. MGA012 bound PD-1-expressing cell lines and chronically-activated T cells, blocked PD-1 interactions with PD-L1/PD-L2, resulting in inhibition of PD-1 signaling, and enhanced antigen-driven cytokine secretion to levels comparable to those observed with nivolumab or pembrolizumab replicas. Furthermore, characterization of MGA012 in *ex vivo* tumor microenvironment immune models showed activation profiles recapitulating the benchmark PD-1 mAbs. MGA012 showed combinatorial activity *in vitro* when added to anti-CTLA-4 or anti-LAG-3 lymphocyte-activation gene 3 (LAG-3) mAbs and enhanced the activity of a T-cell redirecting molecule in a mouse tumor model. MGA012 showed no unexpected cross-reactivity in human tissues, with staining observed primarily in lymphocytes and lymphoid organs. In a repeat-dose (10–150 mg/kg QWx4) study in cynomolgus monkeys, pharmacokinetics (PK) was linear with a beta half-life of 11.2 days (±4.6 SD) and full PD-1 occupancy on circulating T cells at all doses tested. Occupancy of ≥80%, persisting for 4–7 weeks, was also observed in monkeys receiving a single 10 mg/kg dose. MGA012 was well tolerated in cynomolgus monkeys and demonstrated a favorable safety profile with a no-observed-adverse-effect level (NOAEL) of 150 mg/kg.

Conclusion: MGA012 is a novel anti-PD-1 mAb with favorable preclinical characteristics, including PD-1 binding and biophysical properties, PD-1 pathway blockade, the ability to enhance T-cell responses in vitro and in vivo, and a favorable PK and safety profile in cynomolgus monkeys. Clinical trials are ongoing [NCT03059823] or in planning stage to ascertain the safety and preliminary activity of MGA012 alone or in combination with other immune oncology agents, including T-cell redirecting bispecific DART® molecules.

Introduction and Strategy



- A mouse anti-PD-1 mAb panel was subjected to performance-based selection and benchmarked against replicas of nivolumab* and pembrolizumab*:
  - Binding characteristics
  - Ligand-binding and inhibitory signaling blockade
  - Immune response enhancement (cytokine release)
  - Cynomolgus monkey cross-reactivity
- Lead mAbs were humanized and engineered as a hinge-stabilized IgG4κ mAb for further testing

*Replicas of nivolumab and pembrolizumab were generated by MacroGenics based on published sequences.

Results

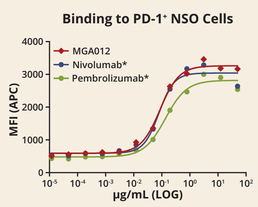
MGA012 Binding Characteristics

Compares favorably to benchmarks

A.

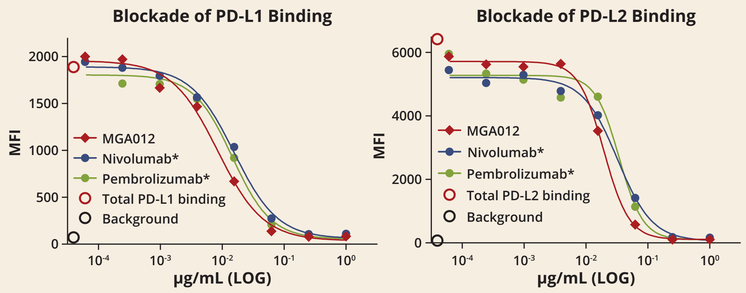
anti-PD-1 mAbs	Soluble Human PD-1		
	K_D nM	k_a $M^{-1}s^{-1}$	k_d s^{-1}
MGA012	0.6	4.3×10^5	2.4×10^{-4}
Nivolumab*	6.1	1.3×10^5	7.9×10^{-4}
Pembrolizumab*	9.6	2.6×10^5	25.0×10^{-4}

B.



(A) Surface plasmon resonance analysis was conducted to measure binding of soluble human PD-1 (6.25, 12.5, 25, 50, and 100 nM) to captured MGA012, nivolumab*, or pembrolizumab*. **(B)** Binding to NSO-PD-1+ cells was detected by flow cytometry.

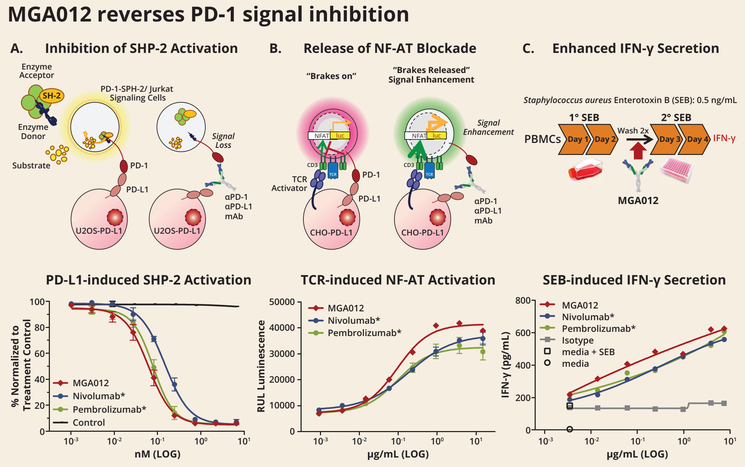
Inhibition of PD-1 Ligand Binding



Blockade of soluble PD-L1 or PD-L2 binding to NS0-PD-1+ cells in the presence of titrating concentrations of the indicated PD-1 mAbs.

Signal Blockade & Functional Activity

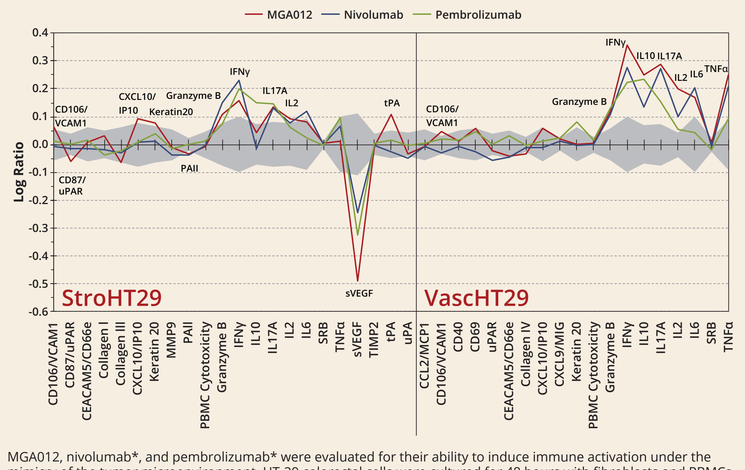
MGA012 reverses PD-1 signal inhibition



MGA012 and replicas of nivolumab and pembrolizumab were 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)** and their ability to enhance IFN-γ secretion in T cells following antigen-driven restimulation of PBMCs with SEB **(C)**.

Immune Activation in the Tumor Microenvironment

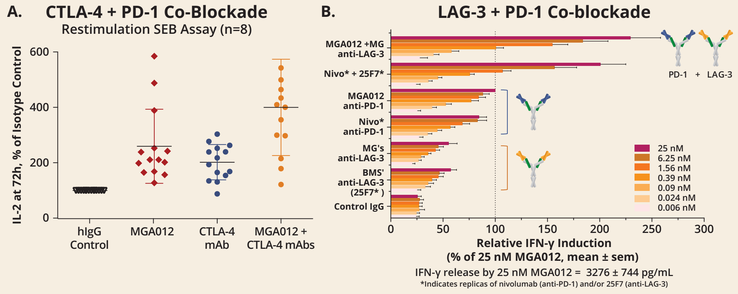
MGA012 induces immune changes consistent with other PD-1 mAb's



MGA012, nivolumab*, and pembrolizumab* were evaluated for their ability to induce immune activation under the mimicry of the tumor microenvironment. HT-29 colorectal cells were cultured for 48 hours 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 and normalized as a log ratio against untreated or isotype controls (DiscoverX).

Combinatorial Activity

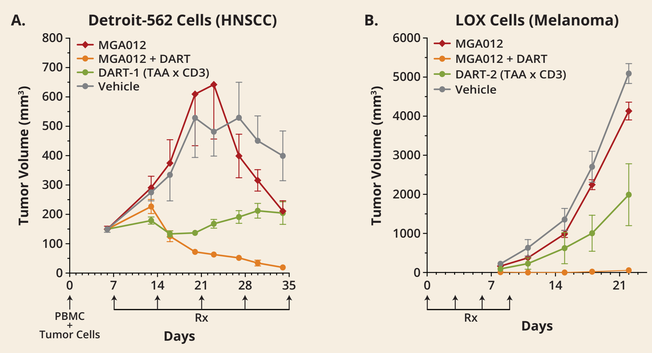
CTLA-4 or LAG-3 blockade enhances MGA012-driven T-cell response



Human PBMCs were cultured with MGA012 the presence of CTLA-4 **(A)** or LAG-3 **(B)** mAbs in SEB-stimulated (500 ng/mL) assays to induce cytokine release. CTLA-4 + PD-1 combinatorial activity was measured by enhanced IL-2 release **(A)**. LAG-3 + PD-1 combinatorial activity was measured as enhanced IFN-γ release **(B)**.

MGA012 Enhances Antitumor Activity In Vivo

Combinatorial activity with CD3-based DART molecules

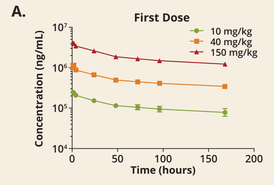


(A) NSG MHC-I$^{-/-}$ mice were injected with Detroit-562 cells (intradermal) + PBMCs (intraperitoneal) and allowed to establish ~150 mm tumors. Anti-tumor activity was induced via redirected T-cell killing against a tumor-associated antigen (TAA) with TAA x CD3 DART molecules. Treatment (Rx) was initiated on Day 7 and continued Q1W for 4 weeks. **(B)** LOX cells + PBMCs were co-mixed and injected subcutaneously (SC) into NSG mice. Rx was initiated at Day 0 and continued Q1W for 3 weeks. In both models, tumor volume was measured twice weekly. The dose of each respective test article in both models is 0.5 mg/kg (MGA012 or DART-1) and 125 ng/mL (DART-2).

MGA012 Evaluation in Cynomolgus Monkeys

Linear PK and full T-cell occupancy at all doses tested

A.

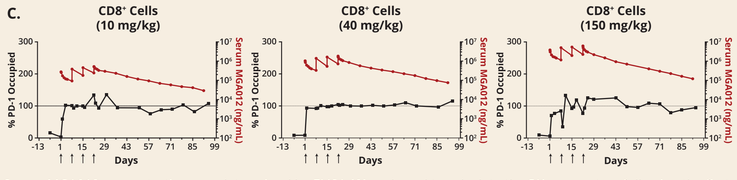


B.

Dose (mg/kg)	C_{max} (mg/mL)	AUC (h•mg/mL)	$t_{½,ß}$ (days)
10	0.24	47.3	10.1
40	1.08	205.7	10.6
150	3.94	745.7	13.0

- $t_{½,ß}$ of 11.2 days (±4.6 SD)
- Well tolerated at all doses tested
- NOAEL 150 mg/kg

C.



Serum MGA012 concentration was determined by ELISA **(A)** and used to calculate the PK parameters following the first dose **(B)**. **(C)** Percent occupied PD-1 receptor on CD8 cells (black line) and serum MGA012 (red line) were determined by flow cytometry and ELISA, respectively. PD-1 was expressed by ~10% of circulating T cells with no evidence of modulation upon administration of MGA012. Similar results were observed with CD4+ T cells.

Conclusions

- MGA012 blocks PD-1/PD-L1 and /PD-L2 interactions, interrupts PD-1 signaling and enhances antigen-induced IFN-γ release with potency comparable to replicas of nivolumab or pembrolizumab
- MGA012 shows combinatorial activity with LAG-3 or CTLA-4 in antigen-driven T-cell stimulation assays (see also Poster 337, 10 Nov 17 and Poster 308, 11 Nov 17)
- MGA012 enhances antitumor activity in combination with CD3-based DART molecules
- MGA012 is well-tolerated and demonstrates favorable PK with full receptor occupancy at doses ≥ 10 mg/kg in cynomolgus monkeys
- A Phase 1 study [NCT03059823] evaluating the safety, tolerability and PK of MGA012 in patients with advanced solid tumors is on-going (see Poster 249, 10 Nov 17)