Blockade of the PD-1/PD-L1 Pathway with the Anti-PD-1 mAb, MGA012, Enhances the Biological Activity of the B7-H3 x CD3 Bispecific DART® Molecule, MGD009 (Orlotamab)

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Abstract

Background: While checkpoint inhibitors have dramatically improved disease outcomes for patients with certain types of tumors, a significant proportion of patients do not benefit from these agents. Moreover, checkpoint inhibitors are most effective in immunogenic tumors with high mutational burden and pre-existing T-cell infiltration, an indication of an ongoing but thwarted immune response. Combinations with agents that have complementary mechanisms of actions, such as T-cell recruiting agents, may provide expanded benefit to patients with resistance or limited response to checkpoint inhibitor treatment. Orlotamab is a clinical stage B7-H3 x CD3 bispecific DART molecule designed to redirect T cells to lyse B7-H3-positive tumor cells. Preclinical studies demonstrated that orlotamab mediates potent anti-tumor activity associated with T-cell activation, expansion and infiltration into tumor sites. Notably, orlotamab activity is also associated with upregulation of PD-1 on T cells and PD-L1 on both tumor and T cells. To address whether the antitumor activity of orlotamab could be further enhanced by coordinating blockade of the PD-1/PD-L1 pathway, we have performed in vitro/in vivo combination studies of orlotamab with MGA012, a clinical-stage anti-PD-1 mAb, also known as INCMGA00012.

Methods: T-cell receptor (TCR)-mediated signaling was evaluated using a PD-1/PD-L1 dependent co-culture reporter system in the presence of orlotamab ± MGA012. In vitro redirected T-cell killing assays were performed using JIMT-1/Luc as target cells and T cells as effectors. In vivo studies were conducted in human PBMC-reconstituted xenografts in MHC class I-null NSG™ mice. Flow cytometry and cytokine multiplex assays were used to evaluate surface/intracellular markers and cytokine levels.

Results: Blockade of the PD-1/PD-L1 checkpoint axis with MGA012 enhanced B7-H3 expression-dependent, orlotamab-induced NFAT signaling beyond that observed with orlotamab alone in a co-culture reporter assay. MGA012 augmented orlotamab-mediated tumor cell lysis of B7-H3+ve tumor cells in redirected T-cell killing assays. In vivo anti-tumor activity of orlotamab was further enhanced by the addition of MGA012 in a human PBMC-reconstituted mouse xenograft model. Mechanism of action studies revealed that orlotamab and MGA012 co-operate to augment granzyme A/B, perforin expression, T-cell activation and expansion beyond that achieved with orlotamab alone and in a B7-H3-dependent manner. Significantly, MGA012 further increased the fraction of central and effector memory T-cells induced by orlotamab.

Conclusions: The combination of orlotamab with MGA012 exerts enhanced cellular signaling and T-cell responses in vitro and increased anti-tumor activity in vivo beyond that achieved with orlotamab alone. These proof-of-principle studies provides rationale for clinically testing this combination approach. *ClinicalTrials.gov: NCT03406949*

Introduction

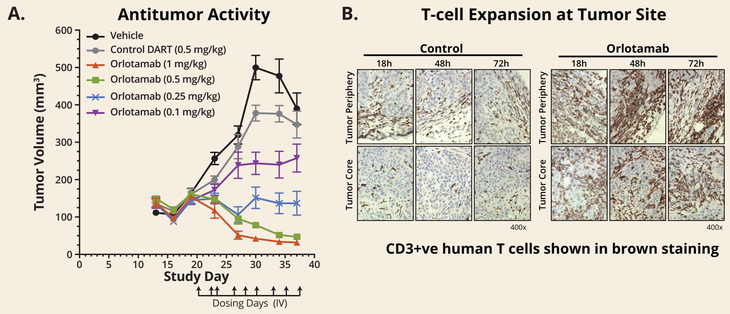
Orlotamab (B7-H3 x CD3 Bispecific DART Molecule)

Anti-B7-H3 x anti-CD3

IgG1(ala,ala) Fc domain

- **Orlotamab:** Humanized, Fc-bearing B7-H3 x CD3 DART molecule
  - Fc engineered for no/reduced binding to FcγR and C1q
  - Retains binding to FcRn and exhibits IgG1-like half-life
- **Intended Function/MoA:** Redirected T-cell killing
  - Recruitment and activation of T cells, irrespective of TCR specificity and MHC restriction
  - Expansion of T cells at tumor site
- **Target:**
  - A member of the B7 family of immune regulators
  - B7-H3 expression in tumors correlated with disease severity and poor outcome
- **Indications:** NSCLC, H&N, bladder cancers, melanoma, mesothelioma, and others
- **Development:**
  - Phase 1 monotherapy & combination therapy with MGA012 (ongoing). *See poster P305*

Orlotamab: Anti-tumor Activity and T-cell Recruitment/ Expansion



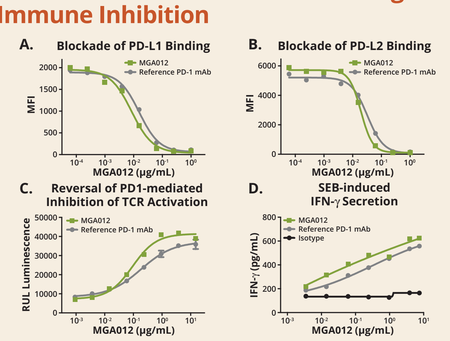
A. NSG β2m$^{-/-}$ mice (n=7/8/group) were intraperitoneally injected with human PBMCs (1 x 10^7) on day-1 followed by intradermal injection of Detroit-562 cells (H&N SCC, 5 x 10^6) on day 0. Tumor cells were allowed to establish themselves in mice to a mean tumor volume of 150 mm^3. On day 20, tumor-bearing mice, after randomization, were treated IV with orlotamab or control DART at indicated concentrations at intervals shown for a total 9 doses. Tumor volumes were measured at indicated times through day 37 and plotted as group mean ± SEM. **B.** IHC analyses of CD3+ve human T cells in xenograft specimens collected following orlotamab treatment.

MGA012/INCMGA00012 (Anti-PD-1 mAb)

Anti-PD-1

- **MGA012:** Humanized, anti-PD-1 mAb
  - Hinge stabilized IgG4
- **Development:**
  - Monotherapy dose expansion ongoing (licensed to Incyte). *See poster P669*
  - Combination therapy with multiple reagents initiated

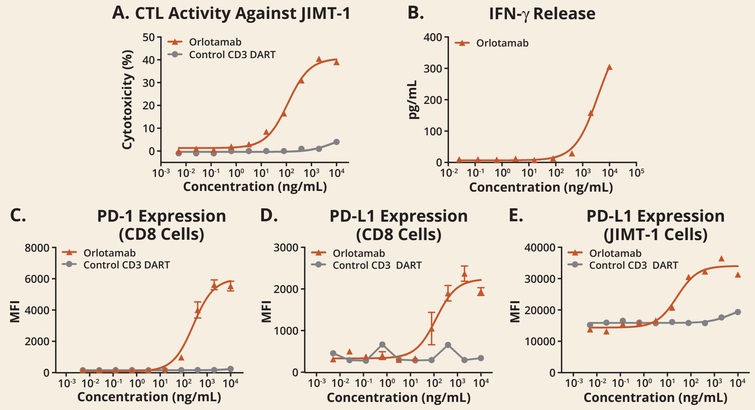
MGA012 Blocks PD-L1/PD-L2 Binding and Reverses PD-1-mediated Immune Inhibition



A–B. Blockade of soluble PD-L1 **(A)** or PD-L2 **(B)** binding to NS0-PD-1$^+$ cells in the presence of titrating concentrations of MGA012 as indicated. **C.** Promega's PD-1/PD-L1 reporter assay to release NFAT blockade within a co-culture reporter assay system. Shown is one representative evaluation of MGA012 to enhance luciferase expression by releasing the inhibitory PD-1/PD-L1 axis. **D.** Evaluation of MGA012 effect on SEB-stimulated IFN-γ release in human PBMCs. Human PBMCs were pre-stimulated with 0.5 ng/mL SEB for 48 hrs and re-stimulated for 48 hrs in the presence or absence of MGA012 at the indicated concentrations. IFN-γ in the supernatant was measured by ELISA.

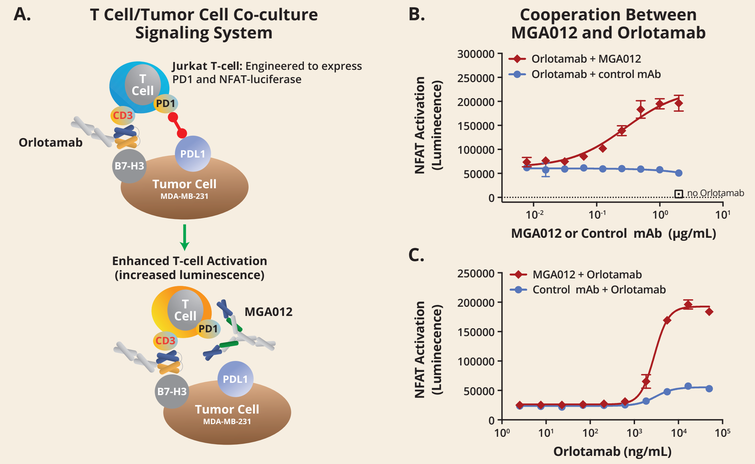
Results

Orlotamab-mediated Cytolysis of B7-H3-expressing Cells is Associated with Up-regulation of PD-1 and PD-L1



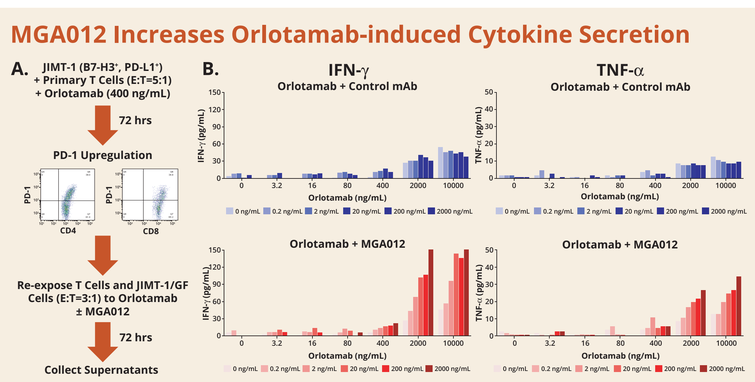
JIMT-1 (B7-H3$^+$, PD-L1$^+$) breast carcinoma cells were mixed with freshly isolated human T cells at E:T ratio = 3:1 in the presence of increasing concentrations of orlotamab or control CD3 DART. **A.** The level of JIMT-1 target cell cytotoxicity mediated by orlotamab determined by evaluation of LDH release at 48 hrs. **B.** The level of released IFN-γ induced by orlotamab at 48 hrs measured by ELISA. **C.** The surface expression level of PD-1 on the effector T-cell population at 48 hrs measured by flow cytometry. **D–E.** The surface expression level of PD-L1 on the effector T-cell population at 48 hrs **(D)** and PD-L1 on the tumor target cells at 24 hrs **(E)** by flow cytometry.

MGA012 Enhances Orlotamab-induced Signal Transduction



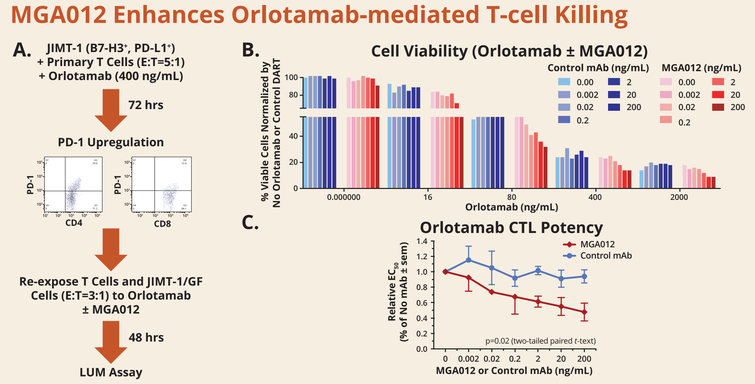
A. Schematic representation of T cell/ tumor cell co-culture signaling model system designed to evaluate the combination activity of MGA012 and orlotamab. **B–C.** The cooperative effect of a fixed orlotamab concentration (1 μg/mL) with various concentrations of MGA012 (red line) or control mAb (blue line) as indicated **(B)** or a fixed concentration of MGA012 (1.87 μg/mL, red line) or the negative mAb control (blue line) with various concentrations of orlotamab at indicated concentrations following 24 hours of co-culture with the Jurkat reporter line.

MGA012 Increases Orlotamab-induced Cytokine Secretion



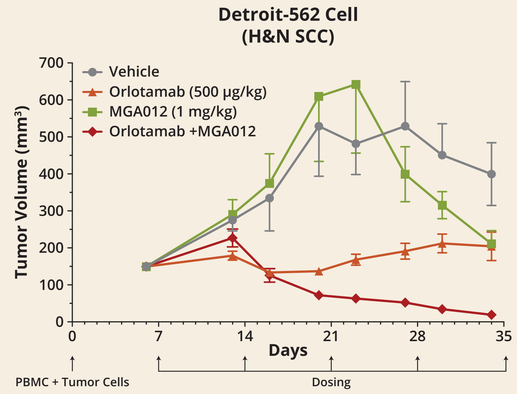
A. Flow chart showing orlotamab-mediated PD-1 upregulation on T-cells following treatment, with T-cells then re-exposed in second round assay to evaluate anti-PD1 combo effect of MGA012 with orlotamab in T-cell cytokine release. **B.** Orlotamab mediated T-cell cytokine release, exemplified by IFN-γ and TNF-α, was evaluated in the presence of MGA012 or control mAb and JIMT-1/GF breast cancer cells (stably transfected with constitutive luciferase reporter gene) mixed with pre-activated T cells at E/T ratio of 3:1. Supernatants collected after 72 hr incubation were subject to ELISA assay to determine IFN-γ and TNF-α levels.

MGA012 Enhances Orlotamab-mediated T-cell Killing



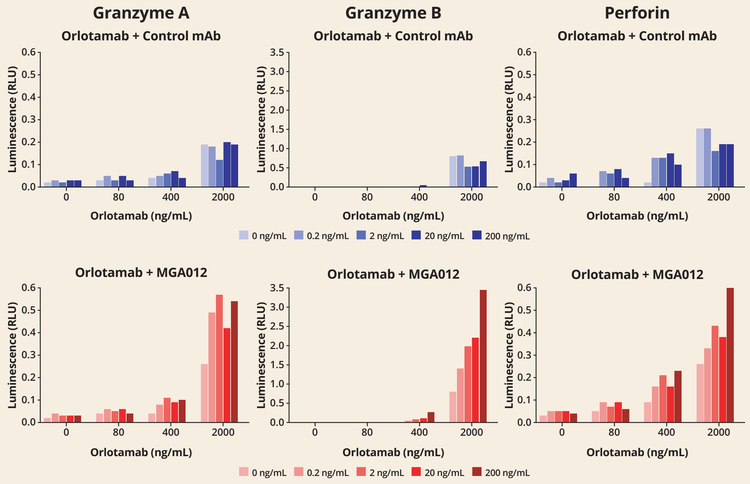
A. Flow chart showing orlotamab-mediated PD-1 upregulation on T-cells following treatment, with T-cells then re-exposed in second round CTL assay to evaluate anti-PD1 combo effect of MGA012 with orlotamab in T-cell cytolytic killing of tumor target cells. **B.** Orlotamab mediated cytolysis of B7-H3 target cells was assessed in the presence of MGA012 or control mAb and JIMT-1/GF breast cancer cells (stably transfected with constitutive luciferase reporter gene) mixed with pre-activated T-cells at E/T ratio of 3:1. Cell viability was determined by evaluation of luciferase levels at 48 hrs. **C.** Orlotamab potency curve over MGA012 concentration. Relative EC_{50} values were obtained by normalization of EC_{50} values of orlotamab + MGD012 or + control mAb-cotreatment against the EC_{50} values of orlotamab treatment alone. Shown is EC_{50} mean ± SEM of 4 independent donors.

MGA012 Enhances Orlotamab-mediated Anti-Tumor Activity



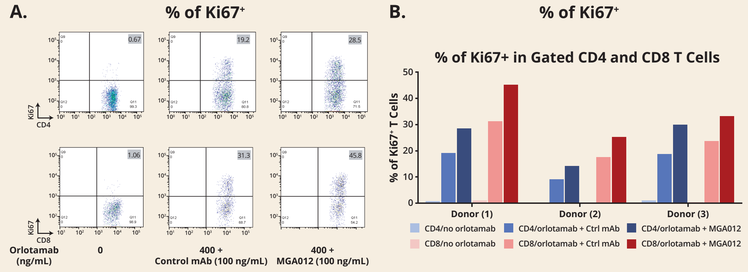
Tumor growth in NSG MHC-1$^{-/-}$ mice of B7-H3-expressing Detroit-562 tumor cells (5.0 x 10^6 injected intradermally) mixed with human PBMCs (1.0 x 10^6 engrafted intraperitoneally). Tumor cells were allowed to establish themselves in mice to a mean tumor volume of 150 mm^3 for 7 days. Mice were treated once weekly for 5 weeks with either vehicle (gray line), MGA012 (green line, 1 mg/kg), orlotamab (orange line, 0.5 mg/kg), or MGA012 + orlotamab (red line, 1 mg/kg + 0.5 mg/kg). Mean tumor volume was measured every 3–7 days.

Orlotamab and MGA012 Cooperate to Augment Granzyme A/B and Perforin Expression



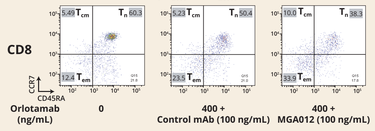
Orlotamab-mediated T-cell granzyme A/B and perforin expression were evaluated in the presence of various MGA012 or control mAb concentrations as indicated and JIMT-1/GF breast cancer cells mixed with freshly isolated T cells at E/T ratio of 3:1. Supernatants collected after 72 hr incubation were subject to Luminex® Multiplex assay to determine granzyme A/B and perforin levels.

Orlotamab and MGA012 Cooperate to Enhance T-cell Proliferation



Orlotamab-mediated T-cell proliferation were evaluated in the presence of 100 ng/mL of MGA012 or control mAb and JIMT-1/GF breast cancer cells mixed with freshly isolated T-cells at E/T ratio of 3:1. T-cells were collected after 72 hr incubation and were subject to flow cytometry staining and analysis to determine % Ki67$^+$ cells in gated CD4 and CD8 T-cell subsets. **A.** A representative flow cytometry plot showing % Ki67$^+$ cells increase in both CD4 and CD8 subsets of T cells following co-treatment with orlotamab and MGA012. **B.** Bar graphs shown are percentage of Ki67$^+$ cells in CD4 (blue) and CD8 (red) cells from 3 independent donors following co-treatment with orlotamab and MGA012 or orlotamab and control mAb.

MGA012 Increases the Fraction of Memory T Cells Induced by Orlotamab



B. % of CD8 T_{em} ($CD45RA^-CCR7^-$)

Donor #	CD8+ Tem (%)		
	No Treatment	Orlotamab + Control mAb	Orlotamab + MGA012
A	12	24	34
B	37	43	57
C	28	33	43

Percentage of CD8 T_{cm} and T_{em} cells were examined following co-culture of freshly isolated T cells with JIMT-1 cells at E/T ratio of 3:1 under suboptimal orlotamab stimulation condition (400 ng/mL) and in the presence of 100 ng/mL of MGA012 or control mAb. T cells were collected after 3–5 day incubation and were subject to flow cytometry staining and analysis to determine T_{cm} ($CCR7^+CD45RA^-$) and T_{em} ($CCR7^-CD45RA^-$) in gated CD8 T-cell subset. **A.** A representative flow cytometry plot showing % Tcm and % Tem increase in CD8 subset following co-treatment with orlotamab and MGA012. **B.** Percentage of Tem cells in gated CD8 subset from 3 independent donors following co-treatment with orlotamab and MGA012 or orlotamab and control mAb.

Conclusions

- MGA012 enhances orlotamab-induced cell signaling and T-cell responses
- MGA012 cooperates with orlotamab to enhance anti-tumor activity in vivo

These proof-of-principle studies provide rationale for the clinical evaluation of orlotamab and MGA012 combination therapy.