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 action, such as T-cell recruiting agents, may provide expanded benefit to patients with resistance or limited response to checkpoint inhibitor treatment. Oroltamab 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 and in vivo combination studies of orlotamab with MGA012, a clinical-stage anti-PD-1 mAb, also known as INCMA00012.

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 NCI-1 nude 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+ 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 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 extends 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 provide rationale for clinically testing this combination approach.

Introduction

Oroltamab (B7-H3 x CD3 Bispecific DART® Molecule)

- Oroltamab: Humanized, Fc-bearing B7-H3 x CD3 DART molecule
- Fc engineered for reduced binding to FcR and CD16
- Retains binding to FcγRIII 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 found 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 (on-going)
  - See poster P301

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

A. Anti-tumor Activity

- CD8+ T-cells were treated with or without orlotamab for 24 hours, stained with viability dye, CD8 and activated marker, then analyzed by flow cytometry.

B. T-cell Expansion at Tumor Site

- Tumor cells were treated with or without orlotamab for 24 hours, stained with viability dye, CD3 and activated marker, then analyzed by flow cytometry.

Oroltamab + MGA012/INCMA00012 (Anti-PD-1 mAb)

- Anti-PD-1
  - MGA012: Humanized, anti-PD-1 mAb
  - Hinge stabilized IgG4
  - Development:
    - Monotherapy dose expansion ongoing (licensed to Immyle) See poster P302
    - Combination therapy with multiple reagents initiated

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

A. Blockade of soluble PD-1 (100 ng/mL) using MGA012

- A mouse model of PD-1/PD-L1 blockade was used to evaluate the effects of MGA012 on PD-1-mediated immune inhibition.

- Results:
  - MGA012 significantly reduced PD-1 expression on CD8+ T-cells.

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

A. CTL Activity Against JIMT-1

- CTLs were treated with MGA012 or control mAb, then co-cultured with JIMT-1 cells at different ratios over 72 hours.

- Results:
  - MGA012 significantly increased the cytotoxicity of CTLs against JIMT-1 cells.

B. IFN-γ Release

- CTLs were treated with MGA012 or control mAb, then co-cultured with JIMT-1 cells at different ratios over 72 hours.

- Results:
  - MGA012 significantly increased IFN-γ release from CTLs.

C. PD-1 Expression (CD8 Cells)

- CTLs were treated with MGA012 or control mAb, then co-cultured with JIMT-1 cells at different ratios over 72 hours.

- Results:
  - MGA012 significantly increased PD-1 expression on CD8+ T-cells.

D. PD-L1 Expression (CD8 Cells)

- CTLs were treated with MGA012 or control mAb, then co-cultured with JIMT-1 cells at different ratios over 72 hours.

- Results:
  - MGA012 significantly increased PD-L1 expression on CD8+ T-cells.

E. PD-L1 Expression (JIMT-1 Cells)

- JIMT-1 cells were treated with MGA012 or control mAb, then co-cultured with CTLs at different ratios over 72 hours.

- Results:
  - MGA012 significantly increased PD-L1 expression on JIMT-1 cells.

Results

MGA012 Enhances Oroltamab-mediated Anti-Tumor Activity

Detroit-S62 Cell (H&N SCC)

- The level of JIMT-1 target cell cytotoxicity mediated by orlotamab determined by evaluation of LDH release at 48 hrs.

- Orlotamab potency curve over MGA012 concentration. Relative EC50 values were obtained by normalization of EC50 values of orlotamab + MGA012 to that of orlotamab alone.

Oroltamab + MGA012 Cooperate to Augment Granzyme A/B and Perforin Expression

- A representative flow cytometry plot showing % CD4+ orlotamab + MGA012 versus CD4+ orlotamab + control mAb.

- A representative flow cytometry plot showing % CD8+ orlotamab + MGA012 versus CD8+ orlotamab + control mAb.

Oroltamab + MGA012 Cooperate to Enhance T-cell Proliferation

- A representative flow cytometry plot showing % Ki67+ in gated CD4 and CD8 T cells.

MGA012 Increases the Fraction of Memory T Cells Induced by Oroltamab

- A representative flow cytometry plot showing % CD38 high or MGA012 versus CD38 low.

Conclusions

- MGA012 enhances orlotamab-mediated 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.

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