In Vitro Potency Assays for Immune Checkpoint Blockade using Human Primary Cells, Murine HuGEMM™ Immune Cells, and Patient-Derived Tumor Organoids

Abstract No.237

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INTRODUCTION

The demand for evaluating the potency of immune checkpoint modulators is steadily growing within immuno-oncology drug development. We aimed to establish a platform to assess the effects of immune checkpoint blockade using human primary immune cells, humanized murine primary immune cells, and co-cultures of tumor cells or patient-derived tumor organoids with immune cells.

METHODS

Antigen-specific T cell recall and activation assay: PBMCs from CMV seropositive and HLA-A02-01+ donors were stimulated with CMVpp65 peptide for one week. CVMpp65-tetramer was used to measure the expansion of CMVpp65-specific CD8$^+$ T cells by flow cytometry.

Mixed lymphocyte reaction (MLR) assay: Isolated dendritic cells from donor 1 and T cells from donor 2 were co-cultured to evaluate the activation of allogenic T cells in the presence of anti-PD-1 antibodies. IL-2 and IFN-γ production was measured at different time points by ELISA.

Tumor cell killing assay: PD-L1$^+$ tumor cells were co-cultured with allogenic T cells from healthy donors. Anti-PD-1 antibody was used to evaluate the effect of checkpoint blockade by measurement of tumor cell killing. Tumor cells were labeled with CFSE. The percentage of dead and live CFSE$^+$ tumor cells were analyzed by flow cytometry.

Dendritic cell (DC) activation assay: Bone marrow from CD40 HuGEMM mice were used for differentiation of DCs. DCs were stimulated with an anti-human CD40 agonistic antibody to evaluate the activation and maturation of DC by flow cytometry.

PD-1 blockade assay for co-culture of tumor organoids and PBMCs: PD-L1$^+$ luciferase engineered tumor organoids were co-cultured with PBMCs from healthy donors for 48 hours. Luciferase activity was measured to indicate the organoid killing mediated by allogenic T cells in the presence or absence of anti-PD-1. Activation of allogenic cells responding to tumor organoids was also evaluated by IFN-γ ELISA.

RESULTS

Fig 1. PD-1 blockade assay for antigen-specific T cell recall and activation. Anti-PD-1 antibody (Keytruda®) treatment enhances the expansion of CMVpp65-specific CD8$^+$ T cells after CMVpp65 peptide stimulation for one week

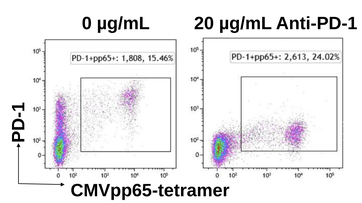


Fig 2. MLR assay to evaluate PD-1 blockade by anti-PD-1 Abs. (A) IL-2; **(B)** IFN-γ in the supernatant were measured by ELISA at different time points

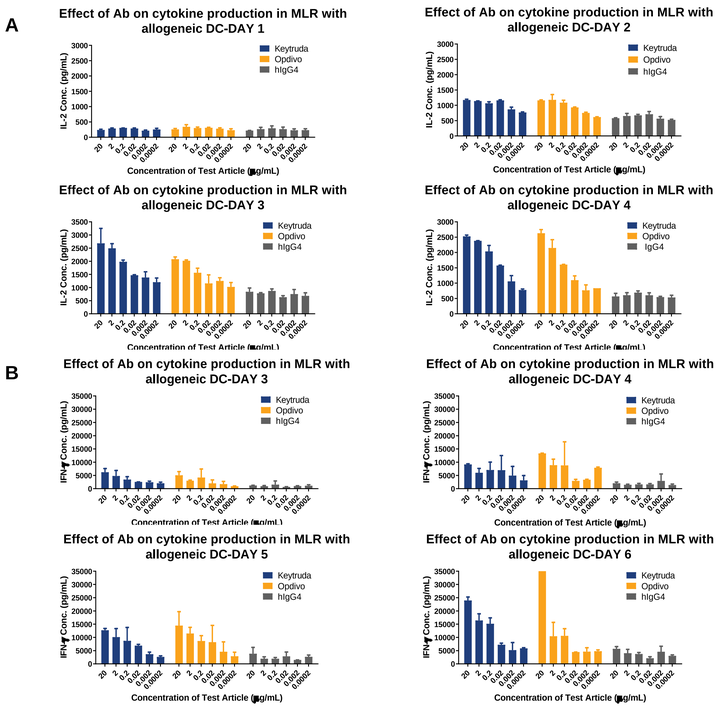


Fig 3. Allogenic T-cell mediated tumor cell killing assay to evaluate the effect of PD-1 blockade. CFSE labelled PD-L1$^+$ HT-3 tumor cells were co-cultured with PBMC in the presence of anti-PD-1 Ab or isotype for 5 days. % dead cells among CFSE+ tumor cells were analyzed with FACS

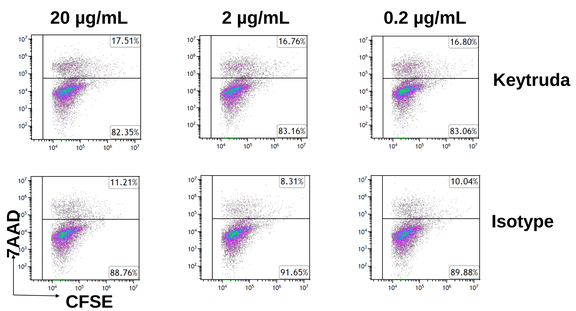


Fig 4. Bone marrow-derived DC (BMDC) activation assay using HuGEMM mice. CD40 HuGEMM mice were used to generate BMDC expressing human CD40. Anti-CD40 agonistic antibody (selicrelumab) or LPS was used to stimulate DC and activation markers MHCII, CD80, and CD86 were measured on CD11c+ cells

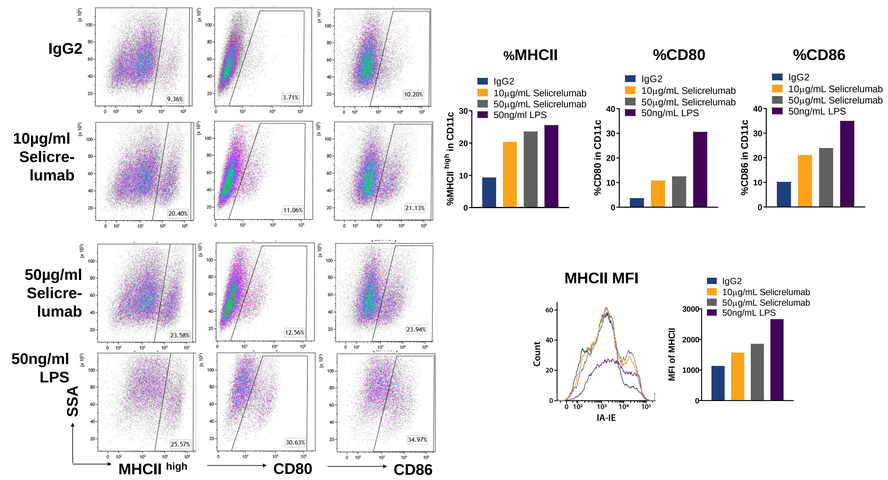
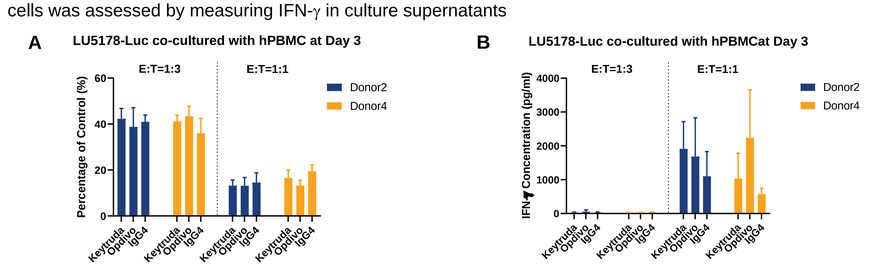


Fig 5. Co-culture assays of tumor organoids and allogenic T cells to evaluate PD-1 blockade. PD-L1$^+$ tumor organoids engineered with luciferase were co-cultured with allogenic T cells from different donors. **(A)** Organoid killing was evaluated by measuring luciferase activity in organoids; **(B)** Activation of allogenic T cells was assessed by measuring IFN-γ in culture supernatants



SUMMARY

- We have established a panel of *in vitro* assays with human tissue/tumor/targets to evaluate the potency of next generation immune checkpoint inhibitors
- Potency assays for immune checkpoint blockade, such as anti-PD-1 antibodies, were validated using T cell activation and mixed lymphocyte reaction (MLR) assays
- Co-culture of tumor cells or tumor organoids with allogenic T cells was established to measure the effect of PD-1 blockade on tumor cell killing mediated by T cells and activation of T cells
- HuGEMM mice express engineered human immune checkpoint targets on immune cells and they serve as an excellent resource of primary immune cells to test drug candidates targeting human checkpoints *in vitro*