Imprime PGG modulates the functionality of immunosuppressive myeloid components of the tumor microenvironment and drives enhanced anti-tumor efficacy in combination with anti-PD1 antibody

Poster # 1052

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Abstract

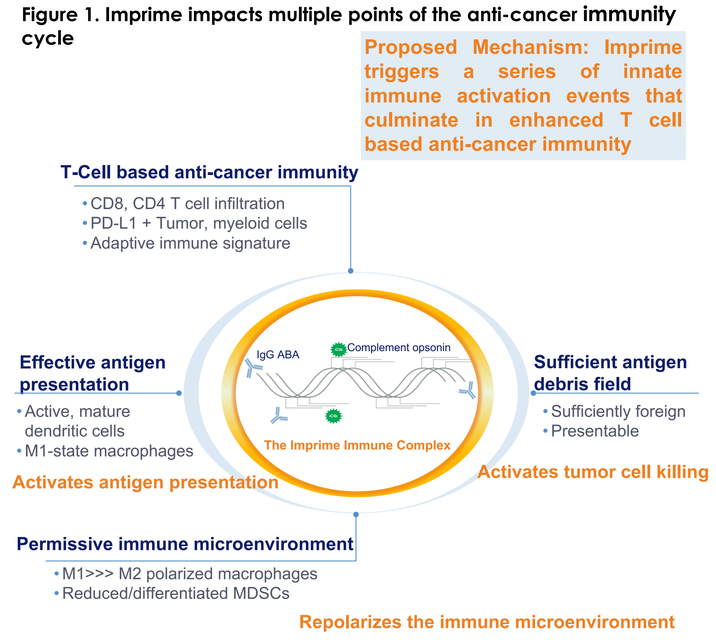
Checkpoint inhibitor therapies (CPI) have shown great promise, albeit only in a limited percentage of patients. One of the key mechanisms behind the limited efficacy of CPI therapy is immune resistance mediated by immunosuppressive myeloid cells at the tumor microenvironment (TME), namely M2 macrophages and myeloid-derived suppressor cells (MDSCs). Multiple therapeutic interventions are being developed to target these cell types with the intention of reshaping the TME and enhancing the effector functions of the cytotoxic T cells. Imprime PGG (Imprime), a novel yeast derived β-glucan PAMP is being clinically developed as an innate immune modulator in combination with anti-PD1 antibody.

In *ex vivo* human and *in vivo* xenograft and syngeneic tumor models, Imprime has been shown to modulate the immunosuppressive tumor-associated macrophages (TAMs) and MDSCs in the spleen as well as TME to an M1 phenotype. **The objective of this study was to evaluate Imprime's ability to modulate the functionality of these suppressive myeloid cells**. We chose MC38 colon carcinoma model because it is highly immunogenic and anti-PD-1 antibody alone has significant efficacy which can be further increased by the combination with Imprime. TAMs from Imprime-treated MC38 tumors produced increased levels of TNF-a in response to LPS stimulation. Furthermore, in comparison to the TAMs enriched from tumors treated with anti-PD1 alone, the TAMs enriched from those treated with Imprime and anti-PD1 antibody showed lower expression of indoleamine 2,3-dioxygenase as well as reduced ability to suppress the proliferation of CD3/CD28-stimulated CD3+ splenocytes. Strikingly, the MDSCs enriched from the spleens of Imprime-treated mice also showed decreased suppressive functionality. Along with the anti-tumor T-cell immunity-enhancing changes in myeloid cells, Imprime treatment resulted in increased effector functionality of the tumor-infiltrating CD8 T cells and splenocytes. Collectively, these data show that Imprime is able to remold the immunosuppressive TME to a more functionally active one, thereby enabling the clinical effectiveness of CPI therapy.

Background

- Imprime PGG, a yeast-derived pharmaceutical-grade soluble 1,3/1,6 β-glucan is being developed for the treatment of cancer in conjunction with tumor targeting and immunomodulatory antibodies (Abs).
  - Imprime has shown promising results in multiple Phase 2 clinical trials in non-small cell lung cancer (NSCLC) and chronic lymphocytic leukemia (CLL), and is currently in Phase 2 clinical trials in combination with anti-PD-1 in TNBC, Melanoma and HNSCC.
- β-glucans are conserved microbial structures found in the cell wall of unicellular and multicellular pathogens. They are considered pathogen-associated molecular patterns (PAMPs) recognized by the pattern recognition receptors (PRR) including Dectin-1, and Complement Receptor 3 (CR3). Imprime forms an immune complex with endogenous serum immunoglobulin IgG or IgM anti-beta-glucan antibodies (ABA) before being recognized by these PRRs.

Figure 1. Imprime impacts multiple points of the anti-cancer immunity cycle



Results

Imprime synergizes with anti-PD-1 antibody therapy in the murine MC38 tumor model

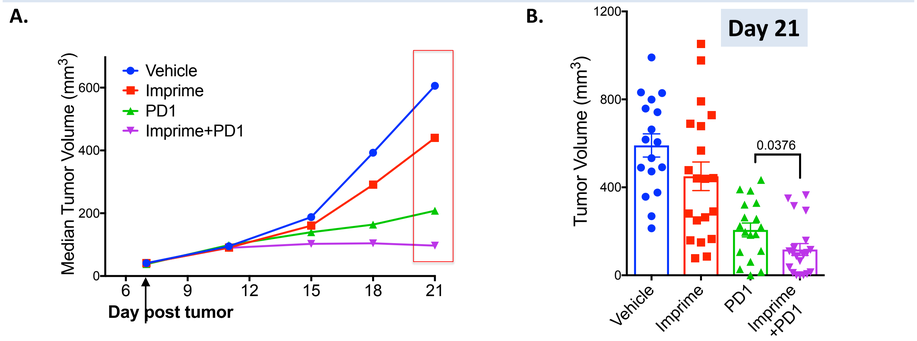


Figure 2. Imprime synergizes with anti-PD-1 antibody to reduce MC38 tumor growth. C57BL/6 mice were injected with MC38 s.c. When tumors were ~50mm^3, mice were treated with PBS (Vehicle), Imprime, anti-PD-1 mAb (clone RMP1-14), or anti-PD-1 mAb + Imprime. **(A)** Tumor growth kinetics. Mean tumor volume is shown without error bars. **(B)** Tumor volumes on d21 post tumor inoculation. Each symbol represents a single mouse and data is shown as mean +/- SEM.

Systemic Imprime administration alters the Immunosuppressive myeloid cells and enhances T cell functionality in the spleen

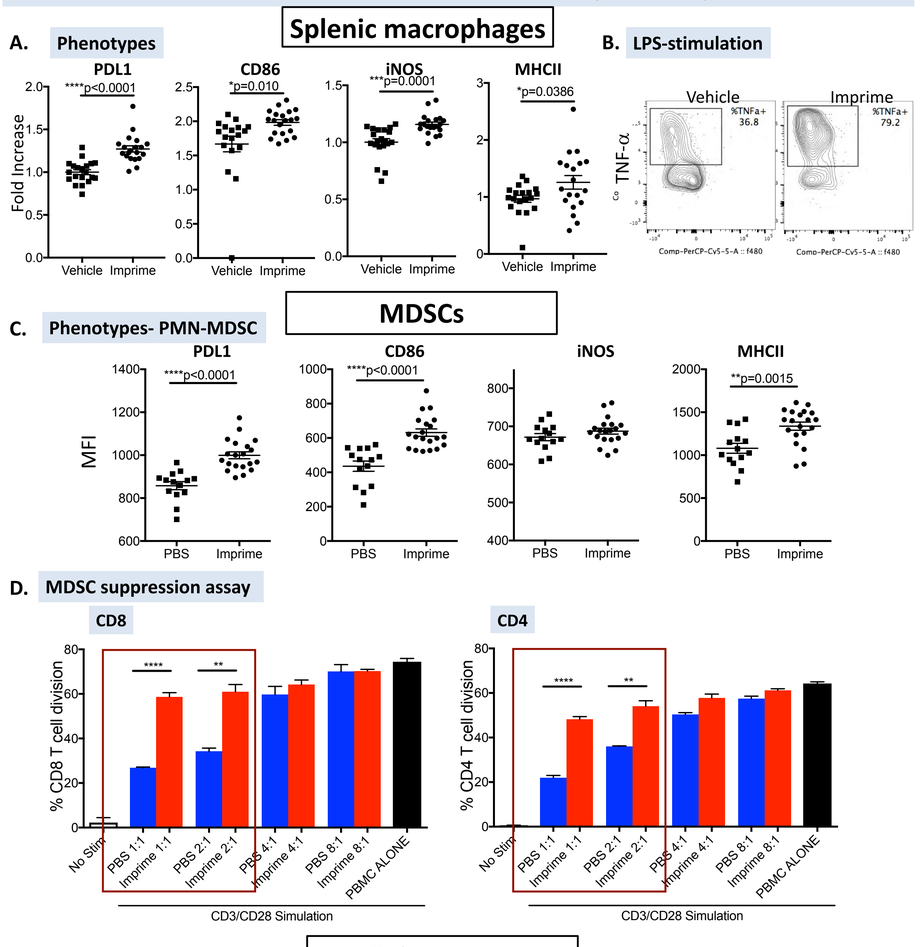


Figure 3. Imprime treatment elicits activation and function of myeloid cells in the spleen and enhances the function of T cells *in vivo*. MC38 tumor model was described as in Figure 2. Spleens were harvested at the end of treatment. Single cell suspension were analyzed for the activation markers by flow cytometry (combined 3 experiments) **(A** and **C)**, and stimulated with 100ng/ml LPS and BFA for 3hrs at 37°C and then subsequently analyzed for TNF-α production by intracellular flow cytometry **(B). (D)** MDSC suppression assay were performed with MDSC (isolated using Miltenyi Biotec's cell isolation kit) co-incubated with cell-trace violet (CTV)-labeled naive CD3 splenocytes at the indicated ratio in the presence of α-CD3 mAb + α-CD28 mAb for 3 days, cell proliferation was then measured by CTV dilution with gating on CD4 and CD8 population. **(E)** Splenocytes were labeled with CTV, stimulated with plate bound α-CD3 mAb for 48hrs and then stimulated with α-CD3 + α-CD28 mAbs in BFA for 6 hrs at 37°C. Cell proliferation was measured by CTV dilution and activation markers were measured by flow cytometry. Data are representative of 3 experiments. **(F)** The total count of IFNγ and IL2 producing splenocytes (including both single and dual cytokine producing cells) in response to co-incubation with MC38 cells was determined using the murine IFNγ/ IL2 double-color enzymatic ELISPOT assay kit (Cellular Technology Limited). Shown here is data from three combined experiments. Each symbol represents a single mouse.

Imprime treatment modulates the functionality of TAMs in the TME

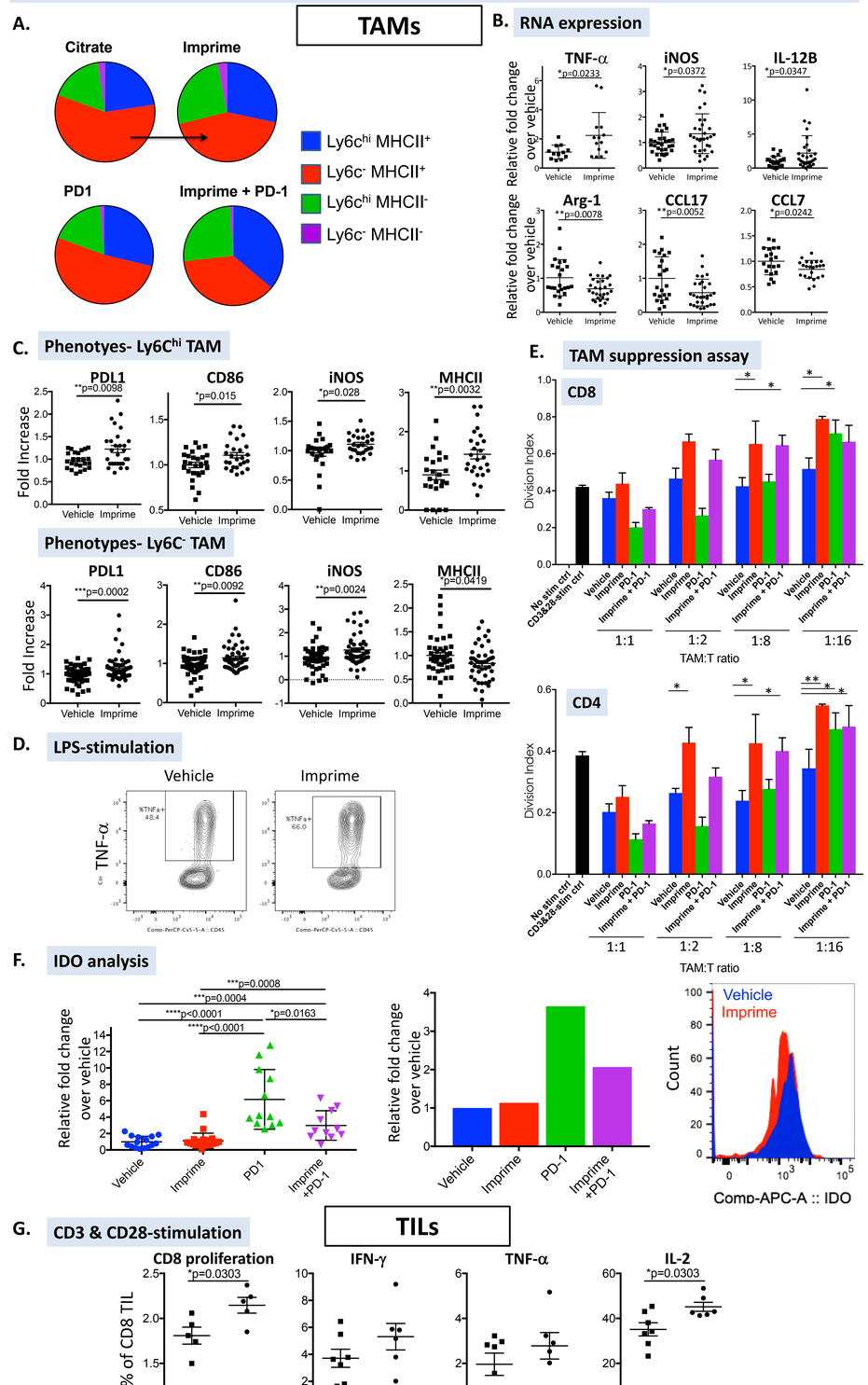


Figure 4. Imprime treatment elicits activation and function of myeloid cells in the tumor and enhances the function of T cells *in vivo*. MC38 syngeneic mouse model was described as in Figure 2. The cellular composition of CD11b$^+$ myeloid cells were analyzed by flow cytometry **(A).** Tissue was collected for RNA analysis (**B**), single cell suspensions were analyzed for the activation markers by flow cytometry (combined 3 experiments) (**C**), and stimulated with 100ng/ml LPS and BFA for 3hrs at 37°C and then subsequently analyzed for TNF-α production by intracellular flow cytometry (**D**). **(E)** TAM suppression assay was performed similarly as described for MDSC suppression assay, except for using enriched CD11b$^+$ TAM. **(F)** For IDO analysis, RNA was isolated from both tissue (left) and enriched CD11b$^+$ TAM (center) for transcriptional study with Quantigene Multiplex Assay-41 (Thermo Fisher), and single cell suspensions were analyzed for surface IDO by flow cytometry (right). Data are shown as mean +/- SEM. Results are presentation of 1 to 3 experiments. **(G)** Single cell suspension was labeled with CTV, stimulated with plate bound α-CD3 mAb for 48hrs and then stimulated with α-CD3 + α-CD28 mAbs in BFA for 6 hrs at 37°C. Cell proliferation was measured by CTV dilution and activation markers were measured by flow cytometry and gated on CD8$^+$ cells.

Summary

Here we have shown that the systemic treatment of Imprime results in:

- The activation and enhancement of functions of the myeloid cells, including splenic macrophages, MDSCs in spleen and TAMs in tumor.
- The activation and enhancement of the function of CD8 T cells in spleen and tumor.

These findings collectively demonstrate that Imprime is able to repolarize the immunosuppressive TME to a more functionally active one, thereby potentially enhancing the efficacy of CPI therapy.

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