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Imprime PGG, a novel phase 2 immunotherapeutic, enhances the anti-tumor activity of checkpoint inhibitors (CPI) and suppresses CPI-induced Indoleamine 2,3-dioxygenase (IDO) expression

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Abstract

Background and Objective: Although checkpoint inhibitors (CPI) have shown unprecedented efficacy in cancer treatment, a significant fraction of patients eventually develop resistance to CPI. Therefore, there is a growing need to identify resistance mechanisms as well as rational combination strategies to combat this resistance. Imprime PGG (Imprime), a novel yeast derived β-glucan pathogen-associated molecular pattern (PAMP), is being developed as a combination agent with CPI in patient populations who have failed single-agent CPI therapy. In pre-clinical mechanistic studies, Imprime has been shown to reprogram the immunosuppressive myeloid cells in the microenvironment and enhance the effector functions of tumor infiltrating T cells. The objective of this study was to focus on IDO, one of the critical resistance mechanisms in the microenvironment that hinders T cell antitumor immunity.

Methods: The anti-tumor efficacy of Imprime in combination with anti-PD-1 was evaluated in the murine colon cancer model MC38. Transcriptional changes in the tumor were assessed by QuantiGene Multiplex platform. IDO1 gene expression in IFN-g-stimulated human whole blood post Imprime treatment was assessed by qRT-PCR. Tryptophan and kyneurinine levels were measured in the serum by LC/MS.

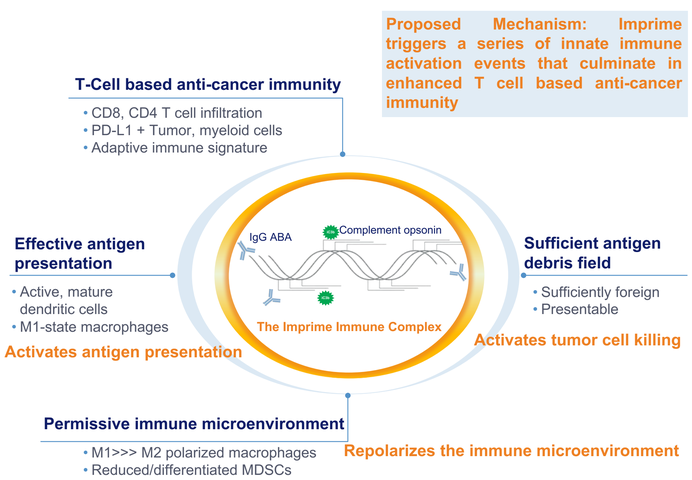
Results: In the MC38 model, Imprime in combination with anti-PD-1 resulted in significantly reduced tumor growth as compared to anti-PD-1 monotherapy. Consistent with our previous results, transcriptional analyses of tumor tissues showed that Imprime alone induced a M1 skewing gene expression profile by modulating several genes including iNOS, TNF, CXCL10, Arg1, and CCL17. Anti-PD-1 treatment alone upregulated several genes affecting T cell functionality, such as IFN-g, PD-L1 and GzmB. Interestingly, anti-PD-1 treatment also resulted in increased expression of several immunosuppressive genes, such as IL10, Arg1, and most notably, IDO1. Furthermore, IDO1 expression was inversely correlated with tumor volume, suggesting IDO1 upregulation is a counter-regulatory mechanism induced in the tumor and/or myeloid cells in response to enhanced IFN-g production by anti-PD-1-treated tumor-infiltrating T-cells. Interestingly, this anti-PD-1 mediated IDO1 induction was dampened significantly by the addition of Imprime to anti-PD-1. Flow cytometry showed that Imprime treatment affected IDO expression in the Ly6C^{hi} monocytes and macrophages, but not in tumor cells. In human whole blood and isolated monocytes, IFN-g treatment increased the transcriptional level of IDO1 and the ratio of tryptophan to kyneurinine, but Imprime treatment significantly inhibited this IFN-g-induced IDO1 increase.

Conclusion: These results collectively demonstrate that Imprime treatment can enhance efficacy of anti-PD-1 treatment and may do so by restricting compensatory immunosuppressive mechanisms mediated by myeloid cells.

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) with additional studies ongoing.
- 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 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 CR3 and FcgRIIA on innate immune cells.

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



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Results

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

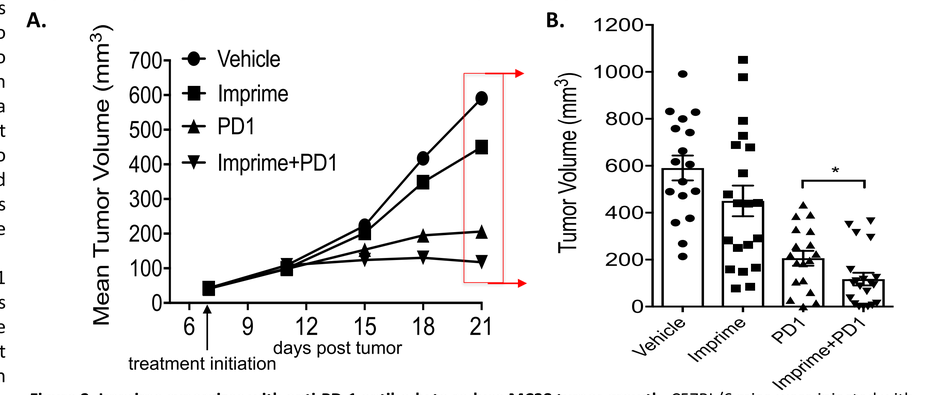

Figure 2. Imprime synergizes with anti-PD-1 antibody to reduce MC38 tumor growth. C57BL/6 mice were injected with MC38 s.c. When tumors were ~50mm^3, mice were treated with PBS (Vehicle), Imprime, anti-PD-1 mAb (clone RMP1-14), or anti-PD-1 mAb + Imprime. **(A)** Tumor growth kinetics. Mean tumor volume 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 Immunosupressive M2 phenotype of myeloid cells and enhances T cell functionality in the tumor and the spleen

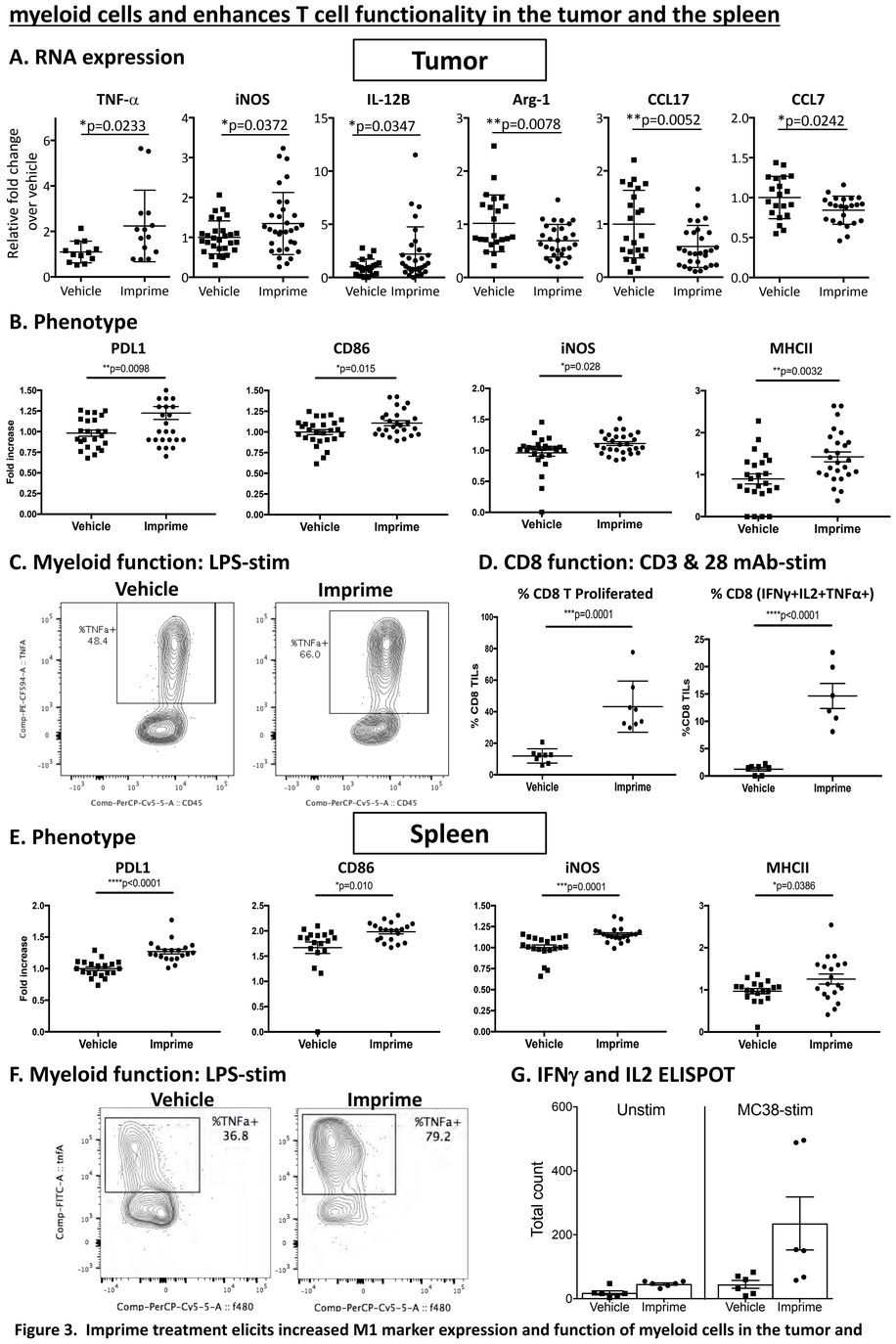

Figure 3. Imprime treatment elicits increased M1 marker expression and function of myeloid cells in the tumor and spleen, and enhances the function of T cells *in vivo*. MC38 syngeneic mouse model was described as in Figure 2. After one week of treatment tumors and spleens were harvested. Tissue was collected for (**A**) RNA analysis, and single cell suspensions were analyzed for the activation markers by flow cytometry (combined 3 experiments) (**B** and **E**), 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 (**C** and **F**). (**D**) Tumor-derived cells were stained with CellTrace Violet (CTV), stimulated with plate bound anti-CD3 mAb for 48hr and then stimulated with CD3 and CD28 mAbs in BFA for 6 hrs at 37◦C. Cell proliferation was measured by CTV dilution and activation markers were measured by flow. Data are representative of 3 experiments. (**G**) 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 representative data of two independent assays.

Imprime/PD-1 combination therapy reduces CPI-induced IDO1 mRNA enhancement in mouse tumor tissue and TAM, and Imprime reduces IDO1 in mouse tumor cells

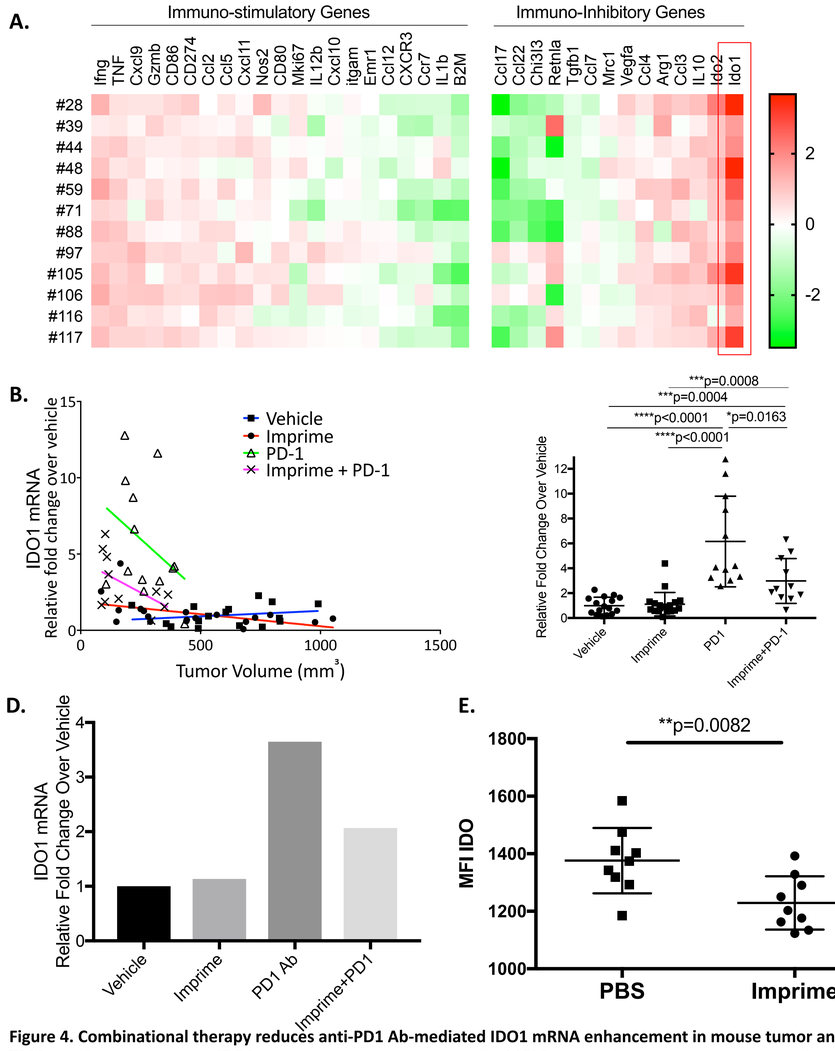

Figure 4. Combinational therapy reduces anti-PD1 Ab-mediated IDO1 mRNA enhancement in mouse tumor and enriched TAM, and Imprime reduces IDO1 levels in tumor suspension cells. MC38 syngeneic mouse study was described as in Figure 2. After various treatments, mouse tumors were harvested and homogenized. RNA was isolated from both enriched CD11b+ TAM (wk1) and tumor tissue (wk2) for transcriptional study with Quantigene Multilex Assay-41 (Thermo Fisher). **(A)** Transcriptional profile in anti-PD-1 Ab treated mouse tumor**.** Heatmap displays log 2 of fold changes over vehicle-treated tumors. Each row indicates an individual animal within the anti-PD-1 Ab treated group**. (B)** Correlation between IDO1 mRNA levels and tumor volumes. **(C)** IDO1 mRNA levels were analyzed in mouse tumors under various treatments. Each symbol represents a single mouse and data is shown as mean +/- SEM. **(D)** IDO1 mRNA levels was analyzed in enriched TAM cells combined from 3-8 mice. **(E)** Single cell suspensions from the tumor were analyzed for IDO by flow cytometry at wk1.

Imprime-treated human monocytes have reduced IDO expression and display M1-like characteristics

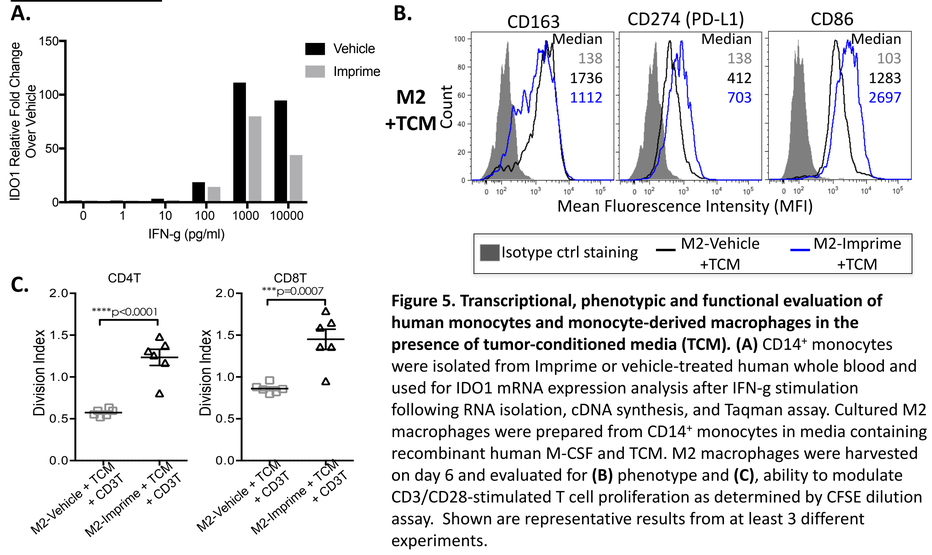

Figure 5. Transcriptional, phenotypic and functional evaluation of human monocytes and monocyte-derived macrophages in the presence of tumor-conditioned media (TCM). (A) CD14$^+$ monocytes were isolated from Imprime or vehicle-treated human whole blood and used for IDO1 mRNA expression analysis after IFN-g stimulation following RNA isolation, cDNA synthesis, and Taqman assay. Cultured M2 macrophages were prepared from CD14$^+$ monocytes in media containing recombinant human M-CSF and TCM. M2 macrophages were harvested on day 6 and evaluated for **(B)** phenotype and **(C)**, ability to modulate CD3/CD28-stimulated T cell proliferation as determined by CFSE dilution assay. Shown are representative results from at least 3 different experiments.

Conclusion

Immunotherapy can up-regulate immunosuppressive pathways, such as IDO as contributor to compensatory mechanisms to thwart T-cell anti-tumor immunity. Imprime, by programming monocytes to an M1 orientation can not only subvert the existing immunosuppressive forces restraining T-cell immunity, but also ameliorate some of the immunotherapy treatment-related emerging rebound immunosuppressive pathways. Thus, Imprime's unique mechanistic feature of remolding the suppressive tumor microenvironment could potentially sensitize, as well as sustain the efficacy of other immunotherapeutic modalities.