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Imprime PGG, a systemically administered PAMP, mobilizes monocytes in the periphery, facilitates their trafficking to the tumor site and polarizes the tumor microenvironment (TME) to an immuno-active state

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Abstract

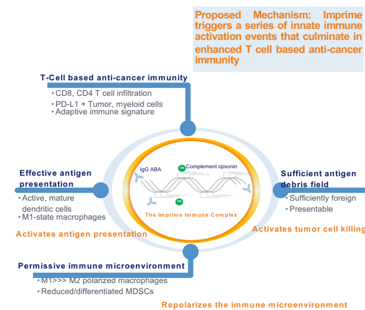
Imprime PGG (Imprime), in combination with both tumor-targeting and anti-angiogenic antibodies, has shown promising efficacy in multiple phase 2 clinical trials. Imprime is currently being tested in combination with the anti-PD-1 checkpoint inhibitor (CPI), pembrolizumab (Pembro) in a phase 2 trial in metastatic melanoma and triple-negative breast cancer (TNBC). Mechanistic and translational studies for anti-angiogenics have shown that harnessing the immune activating ability of myeloid cells in the TME is critical for anti-tumor efficacy. A recent translational study showed that the frequency of classical monocytes in patients could be a predictive factor for clinical response to Pembro. Pre-clinical studies evaluating the anti-tumor mechanism of Imprime in combination with the different therapeutic agents have consistently shown that Imprime modulates the myeloid compartment of the TME, triggering a coordinated anti-tumor immune response. In human *ex vivo* studies Imprime has been shown to enhance maturation of monocyte-derived dendritic cells (DC) and polarize monocyte-derived macrophages to an anti-tumor, M1-like orientation. Consistently, *in vivo* studies also demonstrated that Imprime enhances the differentiation of monocytes into an activated DC phenotype and re-polarizes the tumor associated macrophages (TAM) to an M1 phenotype and functionality. We therefore interrogated the impact of Imprime treatment on production of relevant cytokines/chemokines, monocyte mobilization into the periphery and secondary lymphoid organs, and finally trafficking to the tumor site. Additionally, many of these findings were also confirmed in a human phase 1 healthy volunteer trial.

In tumor-free C57BL/6 mice, Imprime mobilized Ly6C^{hi} monocytes into the blood, spleen, and skin draining lymph nodes (sdLNs). The increase in monocytes in the sdLN was concomitant with increased levels of chemokines CCL2, CCL3, CCL4, CXCL1 and CXCL10, several of which are important for mobilization of myeloid cells from the bone marrow to periphery and secondary lymphoid organs. In the MC38 tumor model, Imprime alone and Imprime + anti-PD-1 treatment significantly increased the percentage of Ly6C^{hi} monocytes in the TME compared to anti-PD-1 treatment alone. Consequently, the proportion of monocytes differentiating to M1 macrophages (Ly6C^{hi} MHC class II^{hi}) was also higher in the Imprime + anti-PD-1 treatment group. In mice depleted of extra tumoral monocytes by clodronate liposomes, the percentage of monocytes in the tumor of Imprime-treated mice was significantly reduced, indicating that Imprime treatment specifically allows trafficking of monocytes from the periphery to the tumor. In a xenograft H1299 lung cancer model, treatment with Imprime and the anti-VEGFR2 antibody, DC101, elicited an M1-polarized TME and suppressed tumor growth more effectively than either agent alone, and clodronate liposome abrogated this anti-tumor effect. Lastly, in a phase 1 healthy volunteer trial Imprime administration by IV infusion resulted in the systemic production of several chemokines including CCL2, CCL3, CCL4, IL-8 and CXCL10, and the expansion of monocytes in the blood within ~4 hrs post infusion.

These results collectively demonstrate that Imprime-mediated modulation of the monocyte population may be a critical mechanism underlying the therapeutic synergy between Imprime and the combination agents.

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.



Results

Fig 2. Systemic administration of Imprime in tumor-free C57BL/6 mice results in monocyte mobilization

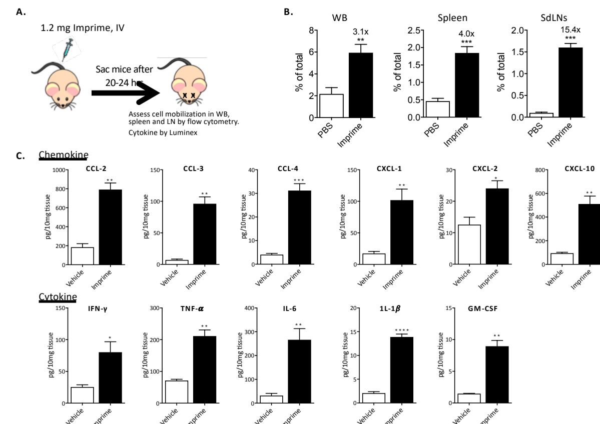


Figure 2. Imprime mobilizes monocytes into the blood and secondary lymphoid organs. (A) Naïve C57BL/6 mice were injected with Imprime IV or vehicle (PBS) and after 20-24 hours whole blood (WB), spleen and skin-draining lymph nodes (sdLNs) were harvested. Mobilization of Ly6C^{hi} monocytes of total CD45^{+} cells were analyzed by flow cytometry (B) and cell lysates of sdLNs were analyzed for cytokine and chemokine using the Luminex platform (C). Each treatment group was comprised of n=3 or 4 mice and data shown were representative of > 4 experiments. All data shown as mean ± SEM and statistical analysis was done using unpaired t test. (*p<0.05, **p<0.01,***p<0.001, and ****p<0.0001).

Fig 3. Imprime synergizes with anti-PD-1 antibody therapy in the murine MC38 tumor model

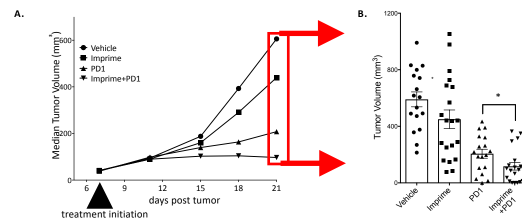


Figure 3. Imprime synergizes with anti-PD-1 antibody to enhance efficacy in MC38 tumor model. C57BL/6 mice were injected with MC38 s.c. When tumors were ~50mm^3, mice were treated with PBS (Vehicle), Imprime, anti-PD-1 mAb (clone RMP1-14), or anti-PD-1 mAb + Imprime. (A) Tumor growth kinetics. Mean tumor volume is shown without error bars. (B) Tumor volumes at d21 post tumor inoculation. Each symbol represents a single mouse and data is shown as mean ± SEM. (*p<0.05, **p<0.01,***p<0.001, and ****p<0.0001).

Fig 4. Imprime treatment modulates the functionality of TAMs *in vivo*

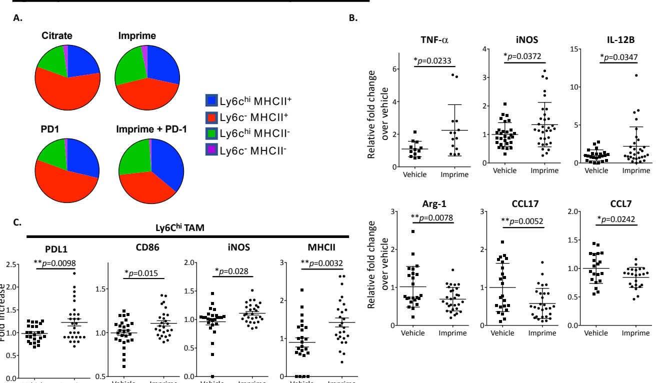


Figure 4. Imprime treatment elicits activation and function of TAMs in the tumor. MC38 syngeneic mouse model was described as in Figure 3. The cellular composition and activation markers of CD11b^{+} myeloid cells were analyzed by flow cytometry (combined of 3 experiments) (A&C). Tissue was collected for RNA analysis (B). Data are shown as mean ± SEM. Results are representative of 1 to 3 experiments.

Acknowledgements

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Fig 5. Clodronate liposomes selectively deplete Ly6C^{lo} cells from the periphery and spleen and inhibits the recruitment of Ly6C^{hi} monocytes into the tumor of Imprime-treated mice

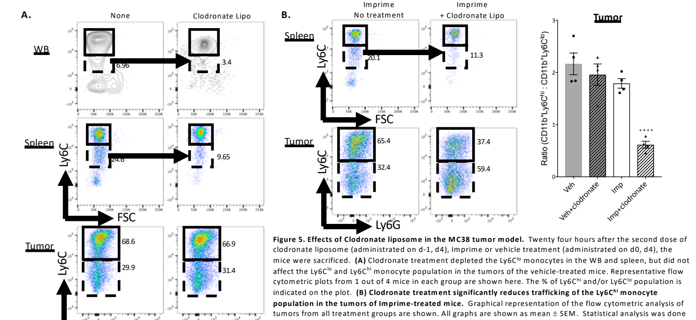

Figure 5. Effects of Clodronate liposome in the MC38 tumor model. Twenty four hours after the second dose of clodronate liposome (administrated on d-1, d4), Imprime or vehicle treatment (administrated on d0, d4), the mice were sacrificed. (A) Clodronate treatment depleted the Ly6C^{lo} monocytes in the WB and spleen, but did not affect the Ly6C^{lo} and Ly6C^{hi} monocyte population in the tumors of the vehicle-treated mice. Representative flow cytometric plots from 1 out of 4 mice in each group are shown here. The % of Ly6C^{lo} and/or Ly6C^{hi} population is indicated on the plot. (B) Clodronate treatment significantly reduces trafficking of the Ly6C^{hi} monocyte population in the tumors of Imprime-treated mice. Graphical representation of the flow cytometric analysis of tumors from all treatment groups are shown. All graphs are shown as mean ± SEM. Statistical analysis was done using unpaired t test. (*p<0.05, **p<0.01,***p<0.001, and ****p<0.0001).

Fig 6. Imprime polarizes TAM to a more M1 like phenotype in xenograft tumor models and clodronate liposomes abrogate the synergistic effect of the anti-angiogenic antibody, DC101

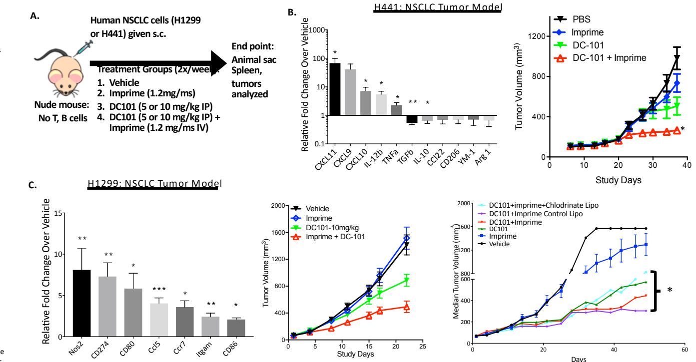

Figure 6. Imprime treatment induces M1 polarization of TAM in non-small cell lung cancer (NSCLC) xenograft models and enhances efficacy of anti-angiogenic agent, DC101 (murine ramucirumab). (A) H441 and H1299 NSCLC xenograft tumors (reached ~100-150mm^3) bearing nude mice were administered DC101 (5 or 10 mg/kg twice weekly IP for up to six weeks) and/or Imprime (1.2 mg/mouse IV twice weekly for up to six weeks). (B) In the H441 model, changes in the mRNA expression of M1/M2 markers of Imprime vs. vehicle-treated tumor cell suspension are shown on the left and the tumor growth in the different treatment groups is shown in the right panel. (C) In the H1299 model, changes in the mRNA expression of M1/M2 markers of Imprime vs. vehicle-treated tumor cell suspension are shown on the left and the tumor growth in the different treatment groups is shown in the middle panel. The right panel shows the abrogation of the enhanced efficacy imparted by Imprime after depleting macrophages by administering clodronate liposomes. Clodronate or control liposomes (100 µl/mouse) were administered on days 1, 3, 6, 10, 17, 34, 31, 38, and 45). (*p<0.05, **p<0.01,***p<0.001, and ****p<0.0001).

Fig 7. Systemic administration of Imprime in Phase I healthy volunteer trial results in the systemic production of chemokines and the expansion of monocytes in the blood

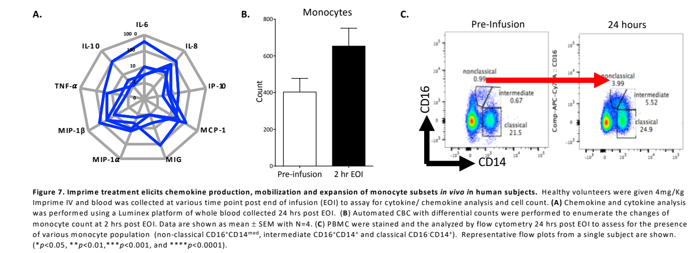

Figure 7. Imprime treatment elicits chemokine production, mobilization and expansion of monocyte subsets *in vivo* in human subjects. Healthy volunteers were given 4mg/Kg Imprime IV and blood was collected at various time point post end of infusion (EOI) to assay for cytokine/ chemokine analysis and cell count. (A) Chemokine and cytokine analysis was performed using a Luminex platform of whole blood collected 24 hrs post EOI. (B) Automated CBC with differential counts were performed to enumerate the changes of monocyte count at 2 hrs post EOI. Data are shown as mean ± SEM with N=4. (C) PBMC were stained and the analyzed by flow cytometry 24 hrs post EOI to assess for the presence of various monocyte population (non-classical CD16^{+}CD14^{med}, intermediate CD16^{+}CD14^{+} and classical CD16^{-}CD14^{+}). Representative flow plots from a single subject are shown. (*p<0.05, **p<0.01,***p<0.001, and ****p<0.0001).

Summary

Imprime treatment modulates the monocyte population in both pre-clinical and clinical studies:

- Imprime elicits the cytokines/chemokines required for mobilization of the myeloid cells
- Imprime treatment mobilizes monocytes into the peripheral blood and secondary lymphoid organs
- Imprime treatment facilitates trafficking of the monocytes to the tumor site
- The monocytes trafficked to the tumor site have an M1-phenotype and functionality
- Ly6C^{lo} (non-classical monocytes) could have a potential role in allowing trafficking of Ly6c^{hi} (classical monocytes)
- The appearance of non-classical monocytes and expansion of the total monocyte population is also seen in healthy volunteers dosed with Imprime

Imprime-mediated modulation of the classical and non-classical monocyte population plays a critical role in the anti-tumor mechanism of Imprime in combination with anti-angiogenic and immunotherapeutic agents.