2018 AACR Tumor Immunology # A01

Treatment with Imprime PGG, a soluble yeast β-glucan PAMP, induces trafficking of activated Ly6G^{high} cells to secondary lymphoid organs and tumor and facilitates immune activation within the tumor microenvironment

Adria Bykowski Jonas[1], Anissa SH Chan[1], Nadine C Ottoson[1], Xiaohong Qiu[1], Keith Gorden[1], Mark Uhlik[1], Jeremy Graff[1], Nandita Bose[1]

Biothera Pharmaceuticals, Inc., Eagan, MN55121

Abstract

Imprime PGG (Imprime), an intravenously-administered, soluble, yeast β-1,3/1,6 glucan is currently in clinical development with tumor-targeting, anti-angiogenic and checkpoint inhibitor antibodies. The fundamental mechanistic rationale for these different therapeutic combinations is that Imprime, a pathogen associated molecular pattern (PAMP), primes innate immune effector functions to drive a coordinated anti-cancer immune response. Previous preclinical mechanistic work has shown that Imprime activates the functionalities of monocytes, macrophages, and dendritic cells (cells that principally influence the TME) eliciting a tumor microenvironment (TME) that activates T cell-dependent anti-tumor immunity.

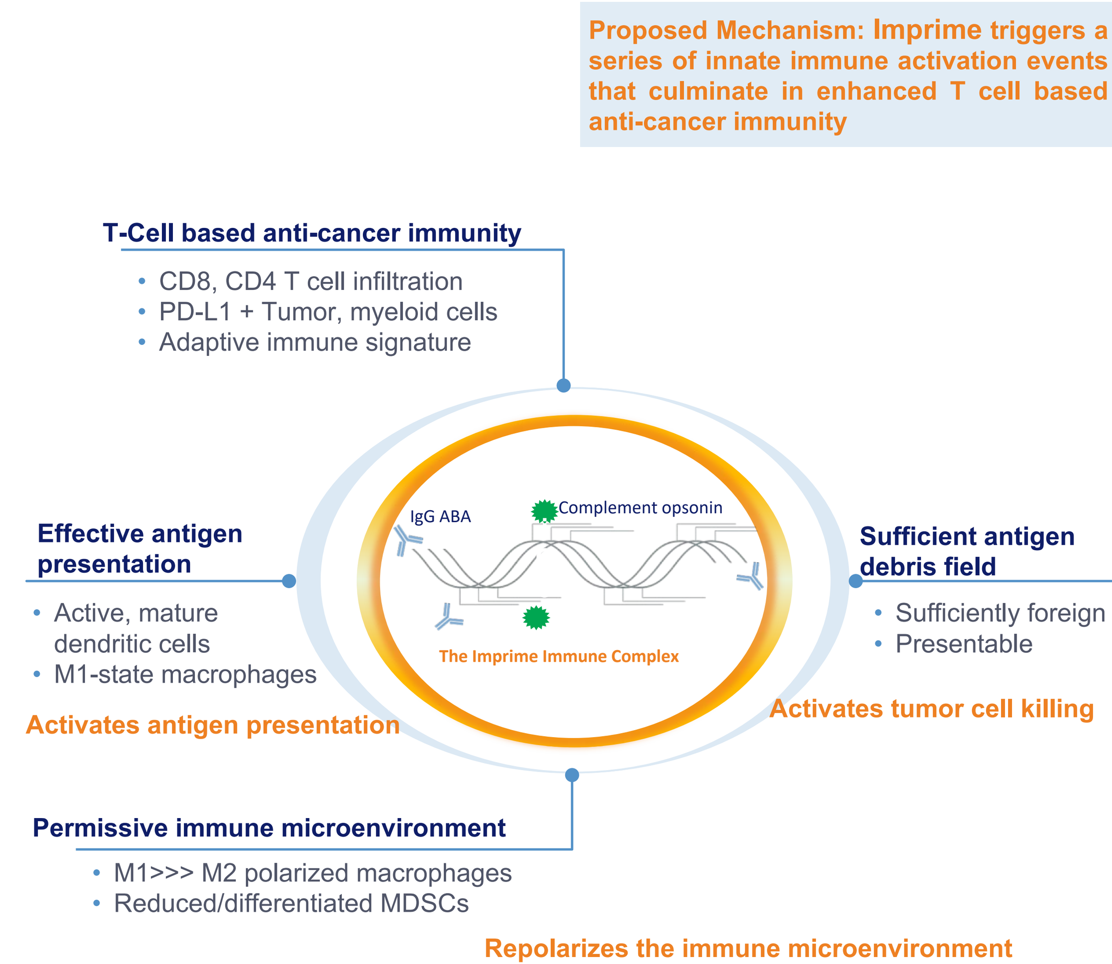
Studies have shown that mature neutrophils and immature myeloid-derived suppressor cells (PMN-MDSC) accumulate, like other myeloid cells, in the tumor and play a role in establishing the immunosuppressive TME and impair T-cell immunity. Herein, we provide evidence that Imprime treatment re-orients neutrophil function, enlisting these cells in anti-cancer immunity. To date, exploration of the effect of Imprime on neutrophils has evaluated direct neutrophil-mediated tumor killing. In this study, we further explored the impact of Imprime treatment on mature neutrophils and immature PMN-MDSC (i.e the Ly6Ghigh population) in both tumor-free and tumor-bearing mice.

In tumor-free C57BL/6 mice, Imprime mobilized Ly6Ghigh cells into the blood, spleen, and skin draining lymph nodes (sdLNs). The increase of these cells in the sdLN coincided with increased levels of CCL2, CCL3, CCL4, CXCL1 and CXCL10, chemokines important for the mobilization of myeloid cells. In the H1299 lung cancer xenograft model, the addition of Imprime to bevacizumab enhanced CD86 expression on Ly6Ghigh cells in both spleen and tumor tissues when compared to bevacizumab alone, indicating that these cells could play a role in T-cell activation. We therefore assessed the effect of Imprime in MC-38, syngeneic tumor model, shown to be sensitive to Imprime in combination with immune checkpoint inhibitor antibodies (i.e. anti-PD1 and PD-L1). Consistent with our earlier findings, Imprime and Imprime + anti-PD-1 treatments significantly increased the percentage of Ly6Ghigh cells in the spleen and the tumor compared to the vehicle alone. The Ly6Ghigh cells in both the spleen and TME showed increased expression of CD86, MHCII and PD-L1. The enriched PMN-MDSC (~60-70% Ly6Ghigh) from the spleens of Imprime-treated mice were significantly less suppressive to CD3/CD28-mediated proliferation of CD3+ splenocytes. Collectively, these data show that neutrophils have a role in Imprime-mediated remodeling of the immunosuppressive TME, driving a more conducive environment to T-cell immunity. The effects of Imprime on peripheral and tumor-associated neutrophils in cancer patients are currently being explored in Phase 2 clinical trials employing the combination of Imprime and anti-PD1 antibody, pembrolizumab.

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



Proposed Mechanism: Imprime triggers a series of innate immune activation events that culminate in enhanced T cell based anti-cancer immunity

Results

Systemic administration of Imprime induces chemokine production and mobilizes Ly6G^{hi} cells in tumor-free C57BL/6 mice

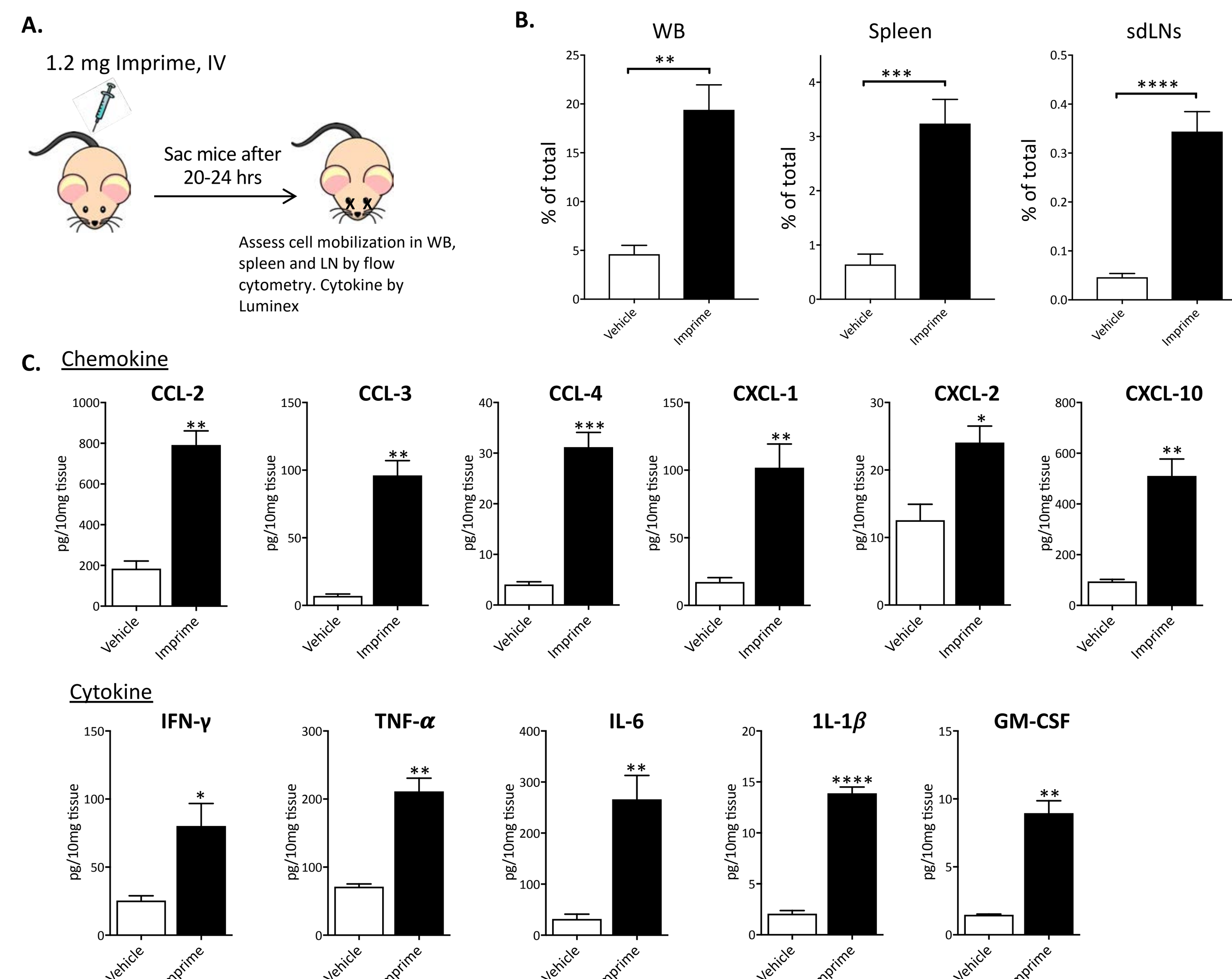


Figure 2. Imprime mobilizes Ly6G^{hi} neutrophils into the blood and secondary lymphoid organs. **(A)** Naïve C57BL/6 mice were injected with Imprime IV or vehicle (PBS) and after 20-24 hours whole blood (WB), spleen and skin-draining lymph nodes (sdLNs) were harvested. Mobilization of Ly6G^{hi} neutrophils 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).

Imprime, in combination with bevacizumab increases the expression of co-stimulatory marker CD86 on Ly6G hi cells in the tumor

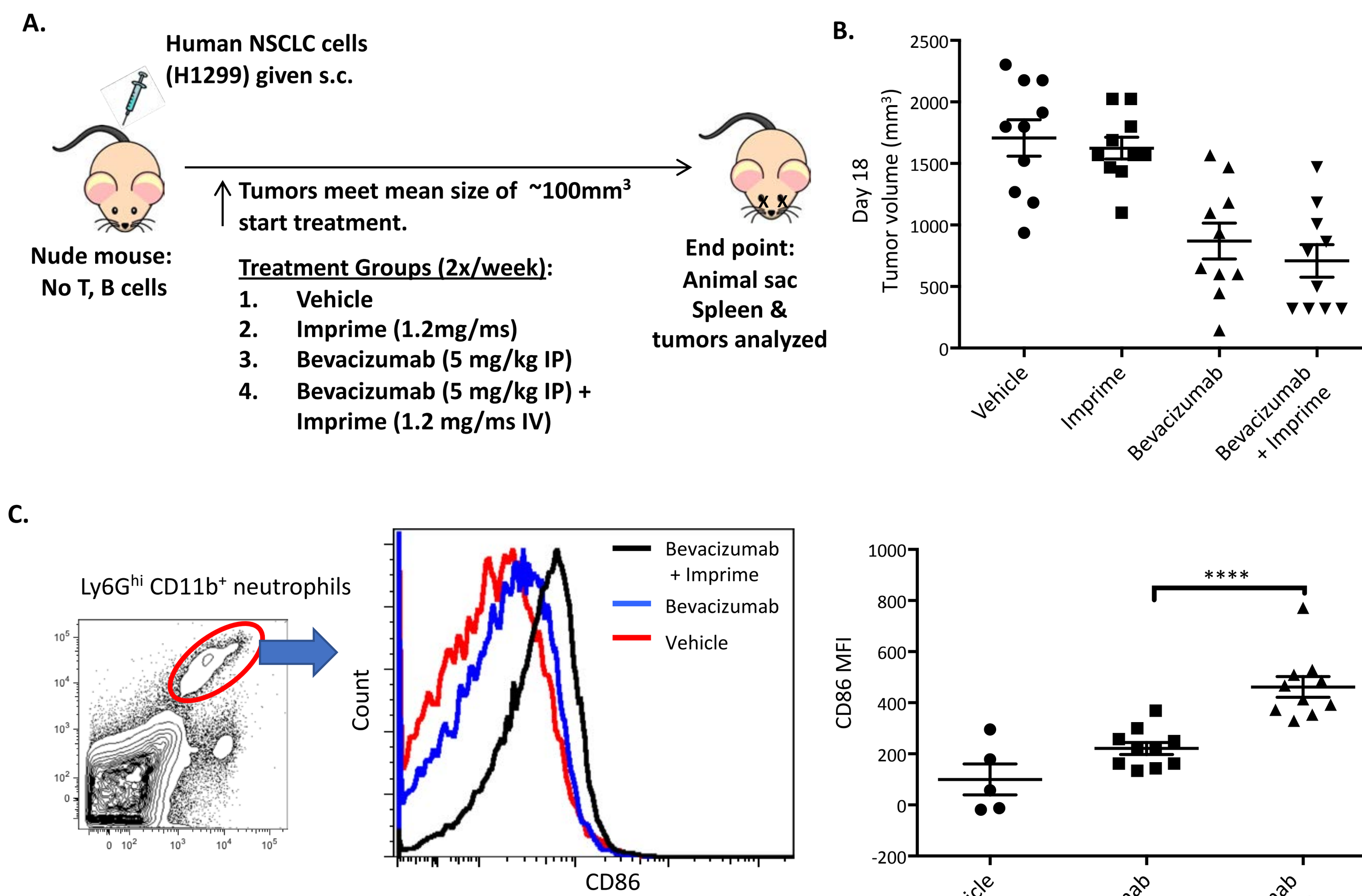


Figure 3. **(A)** Outline of *in vivo* H1299 xenograft mouse model. **(B)** Graph depicting tumor volume of individual mice at day 18 post challenge. **(C)** Gating of tumor neutrophils (Ly6G^{hi} CD11b^{+}) (left panel), representative histograms of CD86 expression of tumor neutrophils from individual mouse (middle panel) and the graphical representation of CD86 expression of the indicated treatment groups. All data shown as mean ± SEM and statistical analysis was done using unpaired t test. ****p<0.0001.

Imprime activates Ly6Ghi cells and enhances efficacy of anti-PD1 antibody in MC38, a syngeneic tumor model

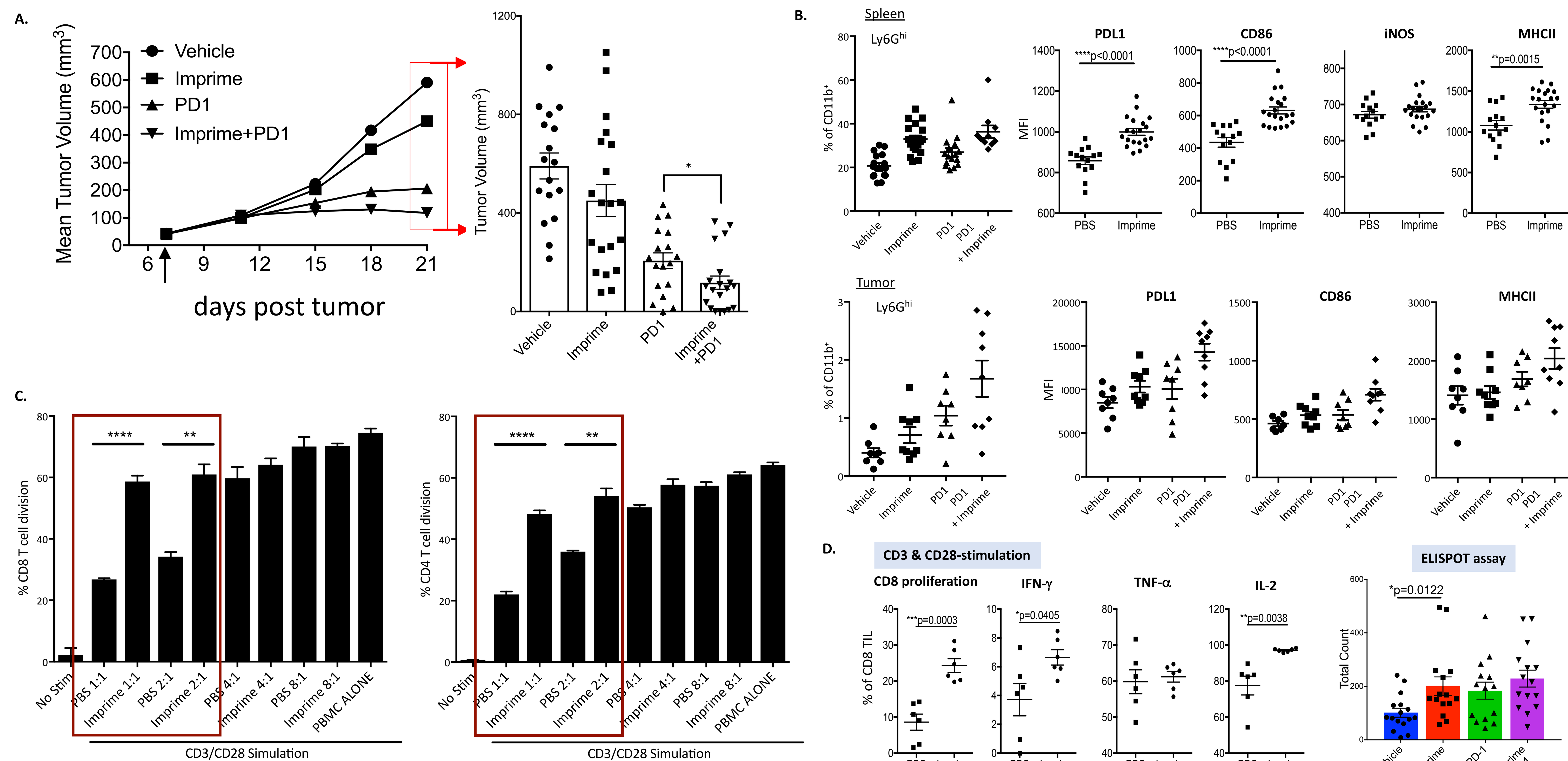


Figure 4. Imprime activates Ly6Ghi cells and synergizes with anti-PD-1 antibody to reduce MC38 tumor growth. C57BL/6 mice were injected with MC38 s.c. When tumors were ~50mm^3, mice were treated with PBS (Vehicle), Imprime, anti-PD-1 mAb (clone RMP1-14), or anti-PD-1 mAb + Imprime. **(A)** Tumor growth kinetics. Mean tumor volume is shown without error bars (left) and tumor volumes on d21 post tumor inoculation (right). Each symbol represents a single mouse and data is shown as mean +/- SEM. **(B)** Spleens and tumors were harvested at the end of treatment. Single cell suspension were analyzed for the activation markers by flow cytometry (combined 3 experiments). **(C)** Suppression assay were performed with MDSCs, predominantly containing Ly6Ghi cells (isolated using Miltenyi Biotec's cell isolation kit) co-incubated with cell-trace violet (CTV)-labeled naïve CD3 splenocytes at the indicated ratio in the presence of 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. **(D)** T-cell functionality was assessed by proliferation assay and Elispot. Splenocytes were labeled with CTV, stimulated with plate bound α-CD3 mAb + α-CD28 mAb in BFA for 6 hrs at 37 ^{0}C. Cell proliferation was measured by CTV dilution and activation markers were measured by flow cytometry. Data are representative of 3 experiments. 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.

Systemic administration of Imprime in Phase I healthy volunteer trial results in the expansion of neutrophils in the blood

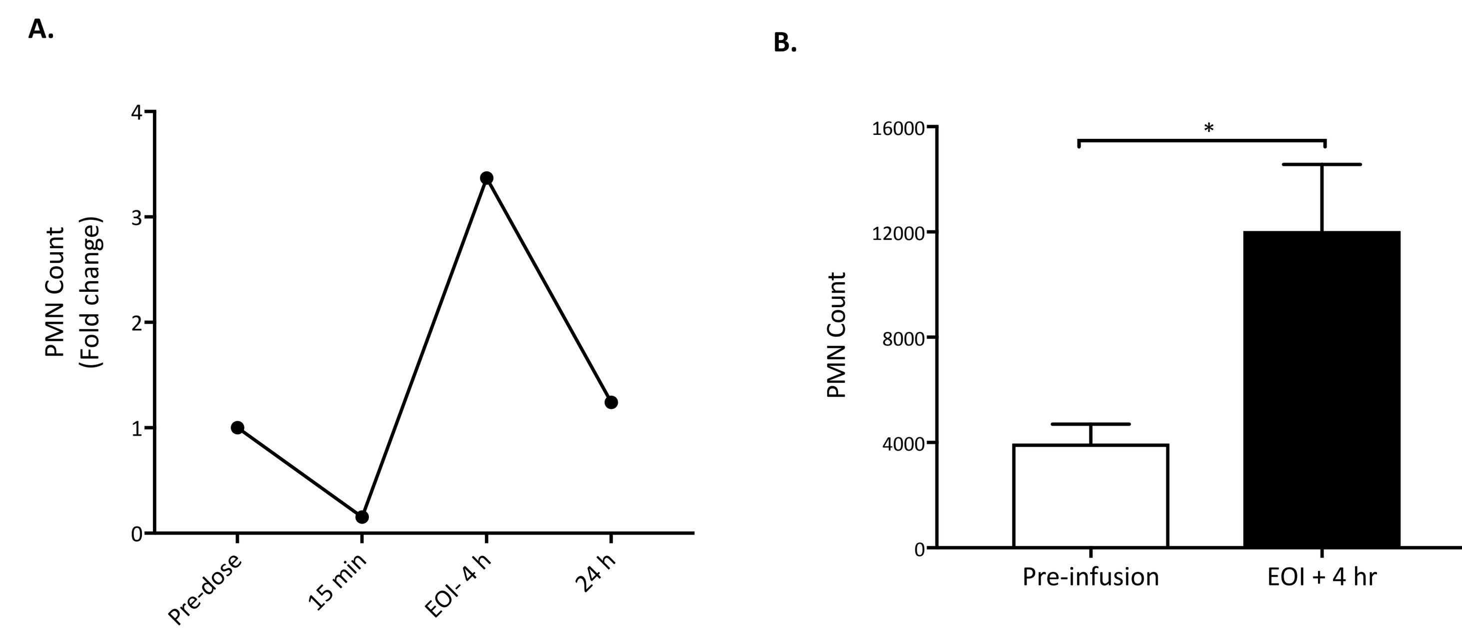


Figure 5. Imprime treatment elicits mobilization of neutrophils *in vivo* in human subjects. Healthy volunteers were given 4mg/Kg Imprime IV and blood was collected at various time point post end of infusion (EOI) to assay for cell count. **(A)** Automated CBC with differential counts were performed to enumerate the changes of neutrophils count and fold change over the pre-dose value was calculated. Representative data of a one donor was shown. **(B)** PMN count are shown as mean ± SEM (N=4). *p<0.05.

Summary

- Systemic treatment of Imprime results in the phenotypic activation and enhancement of functions of Ly6G^{hi} cells
- Ly6G^{hi} cells could potentially have a role in Imprime-mediated remodeling of the immunosuppressive TME, driving a more conducive environment to T-cell immunity.
- Systemic treatment of Imprime results in expansion of neutrophils as measured in the peripheral blood of healthy volunteers. Evaluation of peripheral blood neutrophils and those infiltrating the tumor microenvironment in Imprime-treated cancer patients (multiple Ph2 trials) are underway

Acknowledgements

All experiments funded by Biothera Pharmaceuticals Inc. No external funding was received to support the work.

BIOTHERA pharmaceuticals