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Imprime PGG, a novel innate immune therapeutic in phase 2 clinical development, induces mobilization of monocytes and focalized recruitment of innate immune cells to tumor sites

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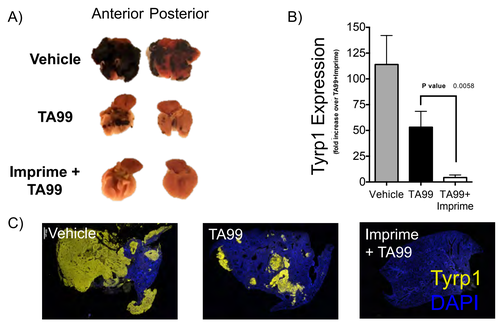
Abstract

Immune checkpoint inhibitors (CPI) have shown compelling clinical efficacy in multiple tumor types, though only in a minority of treated patients. Significant research and clinical development are focused on expanding CPI efficacy. Imprime PGG is a novel, IV administered 1,3/1,6 β-glucan PAMP (pathogen-associated molecular pattern) that activates innate immune effector cells to enhance tumor killing, to repolarize the suppressive myeloid cells of the tumor microenvironment and to activate the antigen presentation capability of dendritic cells, macrophages and monocytes. In multiple preclinical models, Imprime enhances the anti-tumor efficacy of CPIs. Imprime is now in multiple phase 2 clinical studies in combination with the CPI, pembrolizumab. We sought to understand more precisely how Imprime activates the innate immune system to enable a concerted innate and adaptive anti-cancer immune response.

Using multispectral fluorescence IHC we now show that Imprime induces focalized recruitment of innate immune cells to tumor bearing tissue. In the B16F10 experimental metastasis model, Imprime dosed in combination with the tumor-targeting antibody TA-99 can nearly completely repress the outgrowth of pulmonary metastases across a 19 day time course. At 24h post-Imprime treatment, the presence of Ly6G+ neutrophils was evident throughout the lung tissue. At later time points (72h and beyond) the formation of immune cell clusters was readily evident in lungs from Imprime treated mice and rarer in control mice or mice treated only with TA-99. These immune cell clusters were predominately localized to arterioles near B16 tumor sites and comprised of multiple immune cell subtypes including macs, B cells, T cells as well as a monocyte population that are CD11b+, Ly6G- and F4/80- and strongly positive for MHCII.

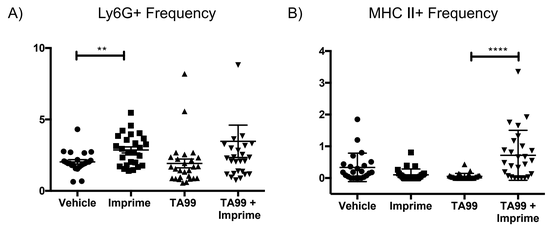
Consistent with these preclinical findings, IV administration of Imprime to healthy human volunteers increased neutrophil and monocyte mobilization into peripheral blood 2-3 fold 4h post infusion. Imprime treatment also resulted in a significantly increased subset of CD16+ monocytes that are known to have higher antigen presentation capability and express higher levels of the activation markers CD86, PD-L1, and HLA-DR (MHCII). Furthermore, RNA expression profiling of whole blood from Imprime-treated volunteers shows increased expression of the CCL3, CCL4, IL-1b and TNF-a, functional mediators produced by these monocyte populations. Together, these data show that Imprime drives the concerted activation of multiple innate immune subtypes and promotes the appearance of unique monocyte populations that may be critical for an Imprime-induced anti-cancer immune response.

Fig 1. Imprime PGG synergizes with tumor-targeting antibodies to reduce B16 lung metastases



C57BL/6 mice were given 1e5 B16F10 melanoma cells i.v. The anti-Tyrp1 monoclonal antibody TA99 was given i.p. at 50ug/mouse at D1,3,5,7,10 after tumor challenge and Imprime was given i.v. at 1.2mg/mouse at D1,3,7,10,14. A) Mice were euthanized D21 and lungs removed. B) The left lobe from each set of lungs was homogenized and analyzed via qRT-PCR for tumor burden (Tyrp1) and normalized to 18S. C) IHC of FFPE lung tissue (inferior lobe) stained with anti-tyrp1 and DAPI, demonstrating almost total clearance of lung metastases in the TA99 + Imprime group.

Fig 4. Imprime treatment results in rapid increase in Neutrophils and MHCII+ cells proximal to tumor lesions



B16 tumor cells were injected i.v. and 10 days later mice were treated with Imprime, TA99 alone and in combination. 24hrs later lungs were harvested and FFPE processed. IHC analysis of the myeloid cells within B16 tumor containing images are shown for A) Ly6G+ PMNs and B) MHC II+ cells.

Fig 2. Immunohistochemistry IHC Analysis workflow

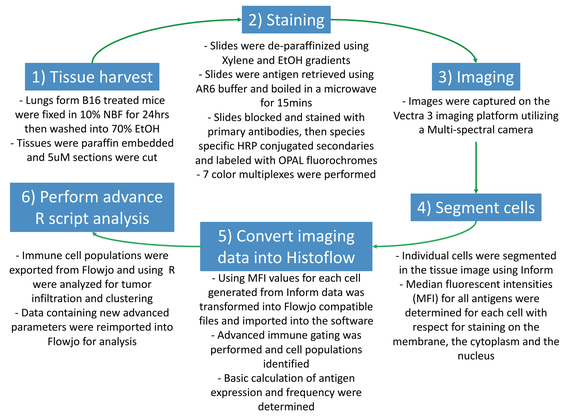
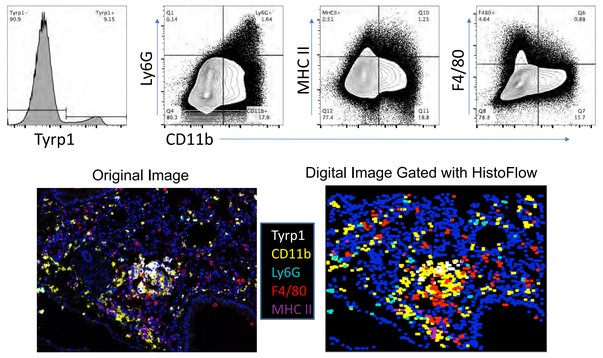
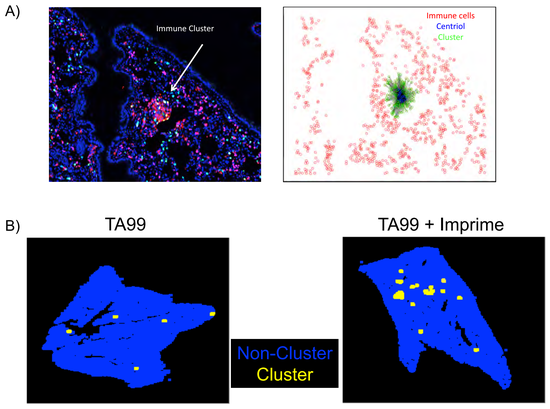


Fig 3. HistoFlow to analyze IHC



Representative example of how 7 color multispectral tissue images can be transformed into individual cells and gated using flow cytometry immune cell gating.

Fig 5. Immune cell clustering Algorithm



A) Immune clusters are objectively defined using R based algorithms by first identifying centriols. Centriols are defined as cells that posses a user-defined amount of cell neighbors within a given distance. Then using the centriols the surrounding cells within the defined distance of a centriol are define as an individual immune cell cluster. B) Representation of immune clusters in the context of the entire tissue specimen (not restricted to an individual microscope field).

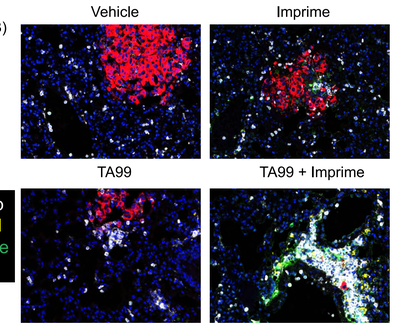
Fig 6. Imprime synergizes with TA99 to induce immune cell clusters containing large proportion of MHCII+ cells

A)

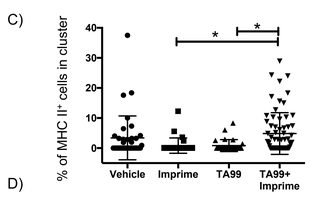
	# of clusters
PBS	37
Imprime	29
TA99	29
TA99+Imprime	68

	Avg cluster size
PBS	60
Imprime	52
TA99	63
TA99+Imprime	79

B)



C)

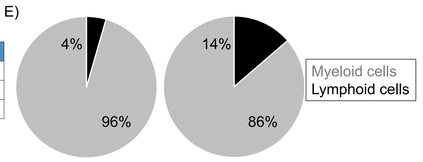


D)

	# of clusters
TA99	11
TA99+Imprime	17
% increase	55

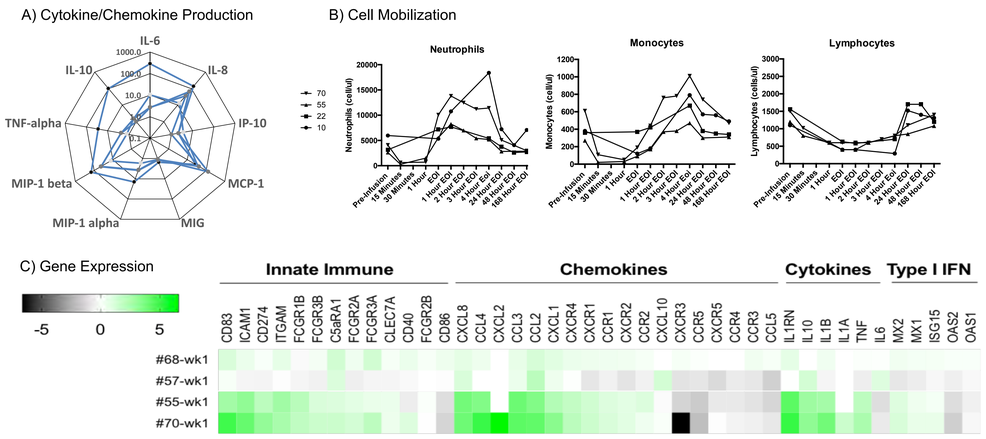
	avg cluster size
TA99	116
TA99+Imprime	161
% increase	40

E)



A) Tables demonstrating the increased number and size of myeloid clusters in the TA99+Imprime animals 24 hours post treatment (clusters defined as cells expressing at least one of the following surface markers (CD11b,F4/80,MHCII,Ly6G) with 30 neighbors within 30um). B) Representative images from B16 tumors stained with anti-Tyrp1, Imprime, MHC II and CD11b. C) MHC II+ cells are increased in myeloid clusters in the TA99+Imprime group. D) 20 days post treatment (treatment schedule described in Fig1) also resulted in increased frequency and size of clusters (clusters are composed of T cells, B cells, CD11b cells) with a greater proportion of the clusters containing lymphoid cells (E).

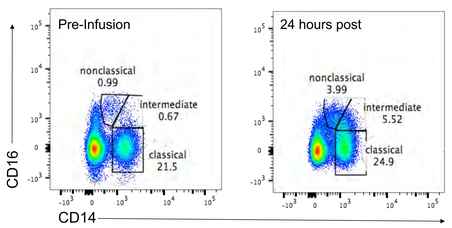
Fig 7. Administration of Imprime in healthy donors imparts distinct immunopharmacodynamic responses *in vivo*



Whole blood or serum was drawn from healthy volunteers at various time points before and after a single dose (cohort 1) or multiple weekly doses (cohorts 2 and 3) of Imprime infusion. A) Cytokine/chemokine measurement in serum was performed using Novex magnetic multiplex assay (Life Technologies) the Luminex XMAP technology. Fold over pre-dose values are plotted. B) Cell mobilization was measured by complete blood cell counts, plus differentials. Neutrophil, monocyte and lymphocyte numbers are represented. C) Gene expression in blood of subjects dosed with Imprime. Whole blood was collected in PaxGene blood collection tubes (BD Biosciences) at various time points pre and post-Imprime administration. RNA were isolated and assayed by Quantigeneplex (Affymetrix). Relative fold changes over pre-dose levels were log 2 transformed. Data presented are from subjects who were treated with Imprime only with no pre-medications.

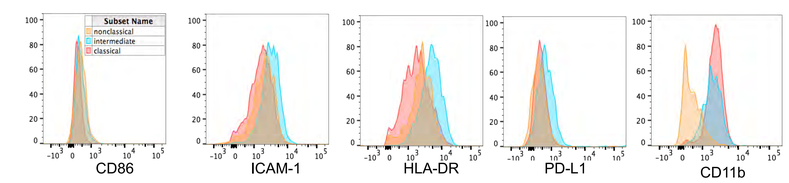
Fig 8. Imprime increases the presence of nonclassical/intermediate monocytes *in vivo*

A) Increase in percent of CD16+ Monocytes



A) Human PBMC were stained with indicated markers and analyzed by flow cytometry before and 24-hours post Imprime infusion. B) Each of the monocyte populations were assessed for expression of activation markers CD86, ICAM-1, HLA-DR, PD-L1 and CD11b.

B) Activation marker expression of CD16+ monocytes 24 hr after Imprime



Conclusions

- Imprime PGG is a PAMP that acts to inspire a coordinated attack of the immune system against tumors.
- In our pre-clinical models Imprime in conjunction with the tumor targeting antibody TA99 induces:
  - Reduced tumor burden
  - A rapid influx of neutrophils and MHCII+ cells to tumor sites
  - Profound myeloid cell clustering (24hr)
  - Persistent clusters (D20) containing increased percentages of lymphocytes
- In our human studies we demonstrate similar changes induced by Imprime:
  - Innate-centric cytokine and chemokines
  - Innate cell mobilization
  - Enhanced MHCII expression on a rapidly mobilized monocyte population
- These data support Imprime's role in coordinating the activation of innate immune cells (mobilization, recruitment to tumors, upregulation of antigen presentation markers) and their crosstalk to the adaptive immune system, thus providing further rational for Imprime as a combination with immune checkpoint inhibitors.

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