Poster #

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Imprime PGG, a soluble yeast β-glucan PAMP, activates both innate and adaptive immune effector cells resulting in enhanced anti-tumor responses that synergize with anti-PD-1 antibody therapy

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Abstract

Immune checkpoint therapy has made a splash in the cancer world by providing long-term durable responses in patients across multiple cancer indications. However, not all patients respond to checkpoint intervention as this therapeutic relies on an underlying recognition and activation of T cells against the tumor. Furthermore, recent evidence suggest that acquired resistance to CPIs may occur through beta-2 microglobulin mutations and loss of MHC class I-restricted T cell responses, in which case other cytotoxic effector cells like natural killer (NK) cells could play a critical role. Therefore, combination therapies utilizing checkpoints and innate immune modulators such as PAMPs (pathogen-associated molecular patterns) are being evaluated to expand both the magnitude and duration of the response, as well as, the responding patient population. Imprime PGG (Imprime) is a soluble, systemically delivered yeast 1,3/1,6 β-glucan PAMP capable of triggering innate immune cell function leading to a cascade of immune activation and enhanced anti-tumor killing. Imprime is currently being evaluated in combination with anti-PD1 therapy in multiple clinical trials.

Here we employ a combination of experimental techniques, including both flow cytometry and multiplex immunofluorescence (IFC), to examine how Imprime modulates the anti-tumor response in the MC38 murine adenocarcinoma model. C57BL/6 mice were injected with MC38 cells subcutaneously and randomized into 4 treatment groups (vehicle, Imprime, anti-PD1, Imprime+anti-PD1) once tumors reached ~50mm^3. After 2 weeks of treatment, mice were evaluated for inhibition of tumor growth, presence and activation of tumor-infiltrating immune cells by IFC, and the induction of polyfunctional T cells by flow cytometry. Mice treated with the combination of Imprime and anti-PD1 had significantly smaller tumors than all other groups, including those treated with anti-PD1 alone. IFC-based analysis of these tumors showed that these mice had increased frequencies of CD8$^+$GranzymeB$^+$ cells, indicative of an enhanced anti-tumor T cell response. TILs from these tumors also showed increased frequency of IL-2 production after stimulation *ex vivo* with a pool of known MC38 tumor antigens. Interestingly, there was also an increase in a CD3$^-$GranzymeB$^+$ population (also negative for B220, F4/80, Ly6C, Ly6G, MHCII) in the tumor of mice treated with the combination. We classified these as most likely to be NK cells. In peripheral blood, flow cytometric analysis of NK1.1$^+$ NK cells also showed increased GranzymeB expression. To explore the impact of Imprime on NK-mediated tumor killing, we further evaluated *in vivo* killing of MHCI deficient cancer cell targets after intravenous administration of Imprime. In these experiments Imprime was able to enhance the NK cytotoxic killing of the targets providing further evidence that Imprime effects innate effector function.

These data demonstrate a unique synergy between anti-PD1 and Imprime treatment. The enhanced cytotoxic T cell response coupled to enhanced effector NK function represents an exciting novel mechanism that may provide enhanced therapeutic benefit to patients in our ongoing clinical trials.

Structure of Imprime PGG

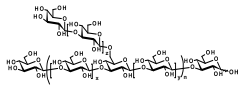
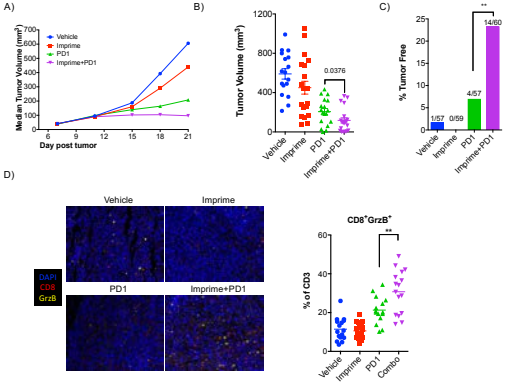
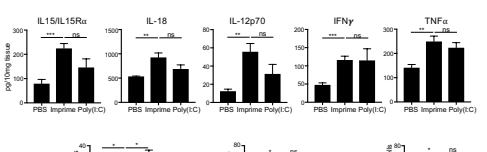


Fig 1. Imprime PGG synergy with anti-PD1 results in a significant decrease in tumor burden in a mouse model of colon carcinoma



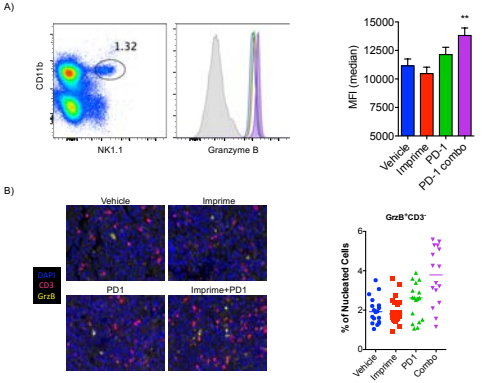
C57BL/6 mice were injected s.c. with 5e5 MC38 tumor cells. When the tumors reached an average of ~50mm3, mice were randomized and treatments started. Mice were treated 2x weekly with vehicle (PBS), Imprime (1.2mg), PD1 (100ug), or Imprime+PD1 . A) Representative experiment illustrating delayed tumor growth with combination treatment over two weeks and (B) a statistically reduced tumor volume at D14 post treatment. C) Combined tumor studies (n=3) demonstrated enhance proportion of tumor free mice with combination treatment. D) Representative Multiplex immunohistochemistry (mIHC) staining of tumor tissue from D14 post treatment. Combination treatment statistically increases the proportion of CD3 T cells that are activated CTLs (CD8+GranzymeB+).

Fig 4. Imprime PGG treatment results in increased cytokines that drive enhanced NK activation and functional phenotype



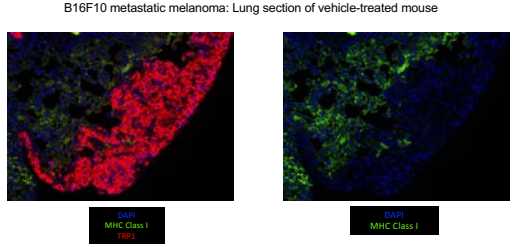
C57BL/6 mice were treated as in Fig 3. Recipient mice lymphnode and splenic cells were harvested after O.N. incubation. (A) Lysates were generated from LNs, and samples were analyzed on a Luminex cytokine plex. *n* = 5 mice and data are from a single experiment. (B) Splenic cell lysates were stained with indicated antibodies and analyzed via Flow Cytometry. Percent expression of activation markers on NK1.1+, CD3-, CD45+ live cells.

Fig 2. Combination of Imprime PGG with anti-PD1 leads to increase in NK cell activation



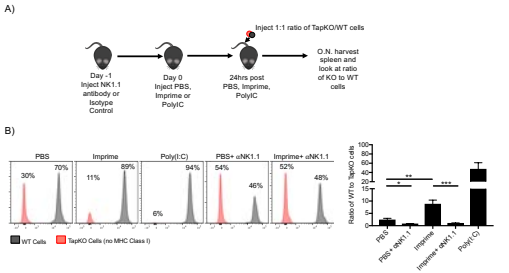
A) Representative flow cytometry showing peripheral blood from MC38 bearing mice 7d post start of treatment. Combination treatment shows enhanced expression (MFI) of granzymeB in NK cells. B) Upon examining the tumor tissue 14d post treatment via mIHC we observed a CD3$^-$ GranzymeB$^+$ population that we believe to be NK cells (these cells were negative for F4/80, B220 but positive for CD45). This effector population is enhanced with combination treatment.

Fig 5. B16F10 melanoma cells have decreased MHC Class I expression



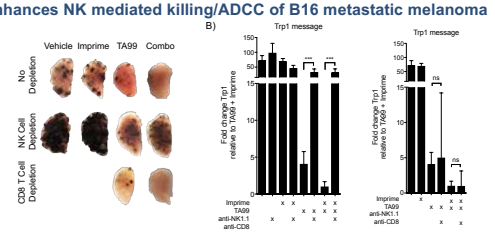
C57BL/6 mice were injected i.v. with 1e5 B16F10 cells tumor cells. At day 10 post tumor inoculation, mice were treated with PBS for 72 hrs and lungs were harvested for mIHC. Images show lung tissue 13 days post tumor inoculation stained for MHC Class I and Trp1 (Left) and the same field with the Trp-1 channel turned off (Right). Tumor cells staining for the antigen Trp1 were negative for MHC Class I, indicating that that B16F10 may serve as a target of NK cells.

Fig 3. Imprime PGG treatment results in enhanced killing of NK cell targets



(A) C57BL/6 mice were injected i.p. with NK1.1 antibody (PK136) or isotype control. The next day, mice were treated with PBS, Imprime (1.2 mg) or PolyIC (20ug). 24hrs post treatment, mice were injected with a 1:1 ratio of labelled WT (CFSE) to TapKO (CTV) splenic cells. Recipient mice splenic cells were harvested after O.N. incubation. (B) Representative experiment depicting the significant decrease in frequency of TapKO cells in comparison to WT cells in Imprime treated mice. All decreases in TAPKO frequency were dependent on NK1.1 expressing cells.

Fig 6. Combination of Imprime PGG with a tumor targeting antibody enhances NK mediated killing/ADCC of B16 metastatic melanoma



B16 lung melanoma model. 1e5 B16F10 cells were administered i.v. and treatments were administered as follows. Imprime (1.2mg) D1, 3, 7, 10, 14; TA99 (50ug) D1,D3,D5,D7,D10;anti-NK1.1 and anti-CD8 200ug D1, 100ugx2/wk. Mice were euthanized on D21 and lungs removed. (A) Picture of mouse lungs day 21 post tumor inoculation. (B) Relative expression of Trp1 message in the lungs on day 21 post tumor inoculation. Combination treatment showed enhance tumor efficacy vs TA99 alone. Upon NK depletion (left), but not CD8 T cell depletion (right graph), the enhancement was lost indicating that the tumor efficacy mediated via combination treatment was NK cell dependent.

Conclusions

- Imprime synergizes with anti-PD-1 antibody therapy in the murine MC38 tumor model. Combination therapy results in increased clearance of established tumors as well as increased GrB$^+$ CD8 TIL, and CD3- GrB$^+$ NK cells.
- Imprime treatment results in a significant increase in NK activation and functional markers as well as increased killing of MHC Class I negative cells.
- Combination of Imprime and TA99 enhances NK mediated tumor killing/ADCC in metastatic melanoma.

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