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Imprime PGG, a β-glucan PAMP (pathogen-associated molecular pattern) activates the direct killing functions of innate immune cells in concert with tumor targeting antibodies

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Abstract

Imprime PGG (Imprime) is a soluble yeast 1,3/1,6 β-glucan PAMP (pathogen-associated molecular pattern). As a PAMP, Imprime triggers innate immune function, activating the direct killing functions of innate immune cells, facilitating MDSC differentiation and macrophage repolarization as well as enabling dendritic cell maturation and antigen presentation, driving T cell expansion and activation. In the clinic, Imprime is administered intravenously and is well-tolerated. In multiple clinical trials (> 400 subjects), including randomized phase 2 studies in NSCLC, Imprime has consistently shown promising increases in both objective tumor response and patient survival. To date, the clinical experience with Imprime has centered on combinations with tumor targeting monoclonal antibodies (Mabs). For instance, Imprime combined with rituximab and alemtuzumab in CLL patients yielded a 65% complete response rate (vs 37% historical CR rate for alemtuzumab plus rituximab).

We sought to better characterize the effect of Imprime in concert with tumor-targeting mAbs. We show that Imprime enhances the effector functions of multiple innate immune cell lineages. We first evaluated the generation of Reactive Oxygen Species (ROS) in neutrophils isolated from human healthy volunteer whole blood. These neutrophils, but not those from vehicle treated whole blood, specifically recognized B cell lymphomas (Raji) only after opsonization with anti-CD20 Mabs (rituximab, ofatumumab, obinatuzumab), generating a substantial ROS burst that coincided with enhanced tumor cell cytotoxicity. Similarly, increased antibody dependent cellular phagocytosis (ADCP) mediated by monocyte-derived macrophages was evident against antibody-opsonized lymphomas (Z138 B cell lymphomas with obinutuzumab) and solid tumor cells (SKBr3 breast cancer cells with trastuzumab) from Imprime-treated whole blood. Likewise, antibody dependent cellular cytotoxicity (ADCC) was evident only after Imprime treatment against antibody-opsonized cancer cells (SKBR3 with trastuzumab). *In vivo*, we now show that Imprime administered intravenously significantly enhances the anti-tumor efficacy of trastuzumab in a patient derived xenograft model of breast cancer, reducing mean tumor volume to ~ 50% of that achieved by trastuzumab alone. In the B16 lung experimental metastasis model, the addition of Imprime to the anti-TRP1 tumor targeting antibody TA-99 significantly reduces both the number and size of B16 lung metastases. Together, these data show that Imprime stimulates the innate immune system, augmenting the anti-tumor efficacy of a diverse array of tumor targeting antibodies in multiple tumor types.

Background

- As a PAMP, Imprime is recognized by and binds to cells of the innate immune system enabling the innate immune system to orchestrate a coordinated anti-cancer immune attack
- Imprime has been administered to >400 humans **intravenously** and is well tolerated
- Clinically Imprime has been used in randomized phase 2 studies in NSCLC, consistently showing promising **increases in both objective tumor response and patient survival**
- We have previously shown that Imprime can bind to and activate cells of the innate immune system via association with CR3, FcRs, and Dectin-1
- Here we investigate multiple immune effectors in vitro and in vivo for the ability of Imprime to synergize with monoclonal antibody therapy

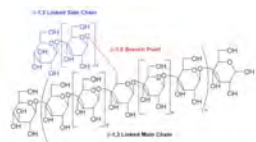
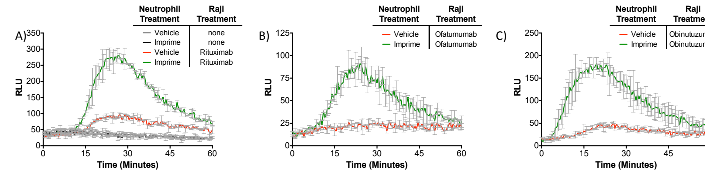


Figure 1. A general structure of yeast-derived Imprime PGG



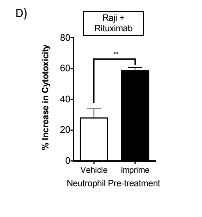


Figure 2. Imprime enhances Neutrophil ROS production against tumor cell lines bound by monoclonal antibodies

Whole Blood (WB) was treated with vehicle or Imprime (25μg/mL) at 37°C for 2 hours. Neutrophils were isolated via negative selection directly from WB (Stemcell technologies) and cells were re-suspended at between 2-3e6 cells per well in a 96 well plate. Neutrophils were stimulated in the presence of luminol (50μM) with Raji B cell lymphoma cells that were pretreated with or without (A) rituximab (1μg/mL), (B) ofatumumab (1ug/mL), (C) obinutuzumab (0.01ug/mL). Luminescence (RLU) was read at 30 second intervals corresponding to reactive oxygen species (ROS) production. (D) These Imprime treated neutrophils also showed enhanced cytotoxicity when co-cultured with Raji cells that had been labeled with Calcein AM dye with Rituximab at a 50:1 effector to target ratio. Cells were incubated for 3hrs and then the co-culture was stained with a live/dead dye and analyzed by FACS to determine cytotoxicity. Raji cells were identified as Calcein$^+$ and % cytotoxicity was calculated by live/dead dye staining. Data show the % increase in cytotoxicity over vehicle treated neutrophils co-cultured with Raji alone. Data representative of 2 independent donors

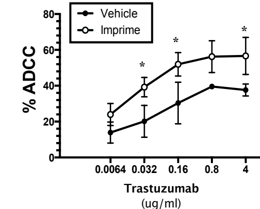


Figure 4. Imprime treatment enhances ADCC against antibody coated tumor targets

PBMCs were treated with Imprime (25 mg/ml) or vehicle in ExVivo15 media with 30% autologous serum for 3 days. PBMCs were washed once with PBS and cultured with SKBR-3 and trastuzumab for 4 hr. T: E ratio = 20 : 1. Cytotoxicity was detected by flow cytometry with Live/Dead staining. Samples were tested in triplicate. Statistical analysis was done by two-way ANOVA (compared to vehicle control). Data are presented as mean +/- SEM.

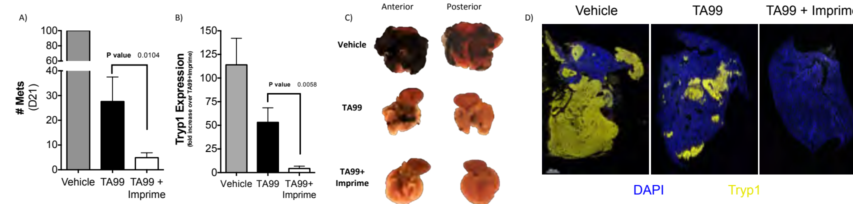


Figure 6. Imprime PGG synergizes with tumor-targeting monoclonal antibody TA99 to reduce B16 lung metastases

C57BL/6 mice were given 1e5 B16F10 melanoma cells i.v. The anti-tryp1 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. Mice were euthanized 21 days after tumor challenge and lungs were harvested. The number of metastases per lung was recorded (A) demonstrating that Imprime+TA99 results in **5.6 fold fewer** metastases than TA99 alone. Similar results were obtained in two other independent experiments. (B) The left lobe from each set of lungs was homogenized and analyzed via qRT-PCR for tumor burden (tryp1) and normalized to 18S. Data show a **26 fold reduction** in tumor burden in the TA99+Imprime group compared to the TA99 only group. Importantly 5 of the 10 mice in the TA99+Imprime group did not amplify tryp1 message, though values for tryp1 were set to a minimum CT of 40. Representative pictures were taken of (C) both the anterior and posterior sides of the lungs and (D) IHC of FFPE lung tissue (inferior lobe) stained with anti-tryp1 and DAPI, demonstrating almost total clearance of lung metastases in the TA99 + Imprime group.

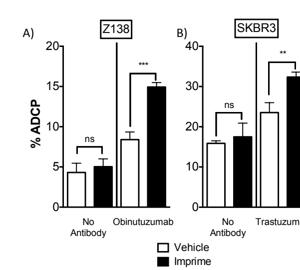


Figure 3. Imprime enhances ADCP by monocyte-derived macrophages of tumor cell lines bound by monoclonal antibodies

WB was treated with vehicle (open bar) or Imprime (black bar) for 2hrs, PBMCs obtained and monocytes subsequently isolated. Monocytes were then cultured in XVivo10 + 10% human serum for 6 days with 50ng/ml M-CSF and with 10ng/ml of IL-10 for the last 72hrs to generate M2c macrophages or cultured in XVivo10 medium with 5% human serum & 100 ng/ml rhGM-CSF for 6 days and activated with 100ng/ml LPS and 20ng/ml IFN-gamma for additional 24hr to generate M1 macrophages. Target tumor cells, Z138 and SKBR3, were stained with cell trace violet (CT) (A) Z138 cells were incubated with 0.001 μg/ml obinutuzumab at 4°C for 20 minutes and then mixed 1:1 with M2c macrophages and incubated for 4hrs at 37. (B) SKBR3 (human breast cancer cells) were incubated with 1-5 μg/ml trastuzumab at 4°C for 20 minutes and then mixed 1:1 with M1 macrophages and incubated at 37 for 1hr. After incubation, macrophages were labeled with CD11b. % phagocytosis was calculated as ((CD11b$^+$/CT violet)/ (CD11b$^+$/CT violet + CT violet)) X100. Data are representative of 3 healthy volunteers.

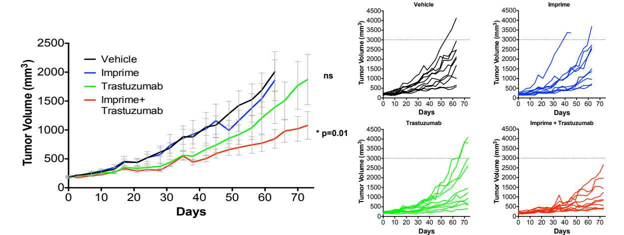


Figure 5. Imprime enhances the anti-tumor efficacy of trastuzumab in a patient derived xenograft model of breast cancer

In collaboration with Champions Oncology, PDX breast cancer tumors were established in mice and allowed to growth to an average group size of ~150mm^3. Mice were then treated with vehicle (black lines), trastuzumab (20mg/kg i.p., green lines), Imprime (1.2mg/kg i.v., blue lines), or Imprime + tratuzumab (red lines). Mice were treated twice per week for a total of 4 weeks with post dosing observation continuing to Day 73. The data show an increase in % tumor growth inhibition (TGI) from a **33%TGI with trastuzumab alone to 64%TGI with Imprime + trastuzumab**. p value = two-way ANOVA comparing tumor volume against vehicle D0-63. n=10 mice per group

Summary

1. Imprime can act to prime multiple cell types of the innate immune system to better respond to and kill monoclonal antibody coated tumor cells
2. These *in vitro* effects of Imprime on innate immune cells translate to efficacy in our *in vivo* mouse studies
3. Imprime's ability to prime immune cells allows for combination with multiple different tumor targeting antibodies in multiple different tumor types, setting the stage for multiple different clinical combinations

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