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Imprime PGG, a Yeast β-Glucan PAMP Induces a Unique Cytokine Profile and Enhances Immune Checkpoint Inhibitor Therapy

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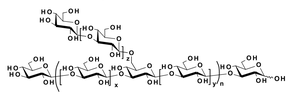
Abstract

Significant preclinical and clinical research has focused on the prospect of using PAMPs to spark a coordinated anti-cancer immune response in combination with checkpoint inhibitors (CPI). PAMPs are unique combination partners as they can sensitize tumors to respond to CPI in several ways, including activation of antigen presenting cells to prime tumor-specific CD8 T cells and thwarting immunosuppression to boost the effector function of T cells at the tumor site. However, many PAMPs elicit intolerable, and sometimes fatal, cytokine storms when administered systemically (TLR and STING agonists). Imprime PGG (Imprime), a soluble yeast β-1,3/1,6 glucan, is a PAMP that has been successfully administered intravenously, is well-tolerated, and shows promising efficacy in a series of clinical trials. Imprime enhances the direct tumor killing function of innate effector cells, promotes re-polarization of the immunosuppressive tumor microenvironment, and drives the activation of antigen presenting cells, enabling CD8 T cell expansion and IFN-γ production. In multiple preclinical cancer models, Imprime has shown profound anti-tumor efficacy in concert with tumor-targeting and anti-angiogenic antibodies. In this study, we sought to explore the ability of Imprime to synergize with CPI and also evaluate what makes Imprime a unique PAMP, especially in the context of its cytokine profile.
In the murine CT26 colorectal tumor model, Imprime and an anti-PD-1 antibody given in combination repressed tumor growth more than either single agent. In the MC38 tumor model, 33% of mice receiving an anti-PD-L1 antibody were tumor free whereas >80% of those dosed with Imprime and the anti-PD-L1 antibody were tumor free. Moreover, the tumor-free mice remained tumor free upon re-challenge with MC-38 tumor cells, suggesting that Imprime based therapy enhanced immunologic memory. For comparison of cytokine profiles, a collection of 18 different PAMPs representing ligands for a variety of Pattern Recognition Receptors (PRRs- TLR-2, -3, -4, -5, -7/8, -9, NOD-1, -2, RIG-1, STING, β-glucan receptors) were tested for their ability to produce cytokines in whole blood from healthy human subjects. Unlike the other classes of PRR agonists, Imprime consistently induced chemokines involved in leukocyte trafficking (IL-8 and MCP-1), but not the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α that contribute to toxicity. Furthermore, transcriptional profiling after *in vivo* dosing of Imprime in mice also showed increased mRNA levels of select chemokines and a strong type I IFN signature in lymph nodes. Importantly, Imprime's unique chemokine profile was also confirmed in a Phase 1 healthy volunteer translational trial. Collectively, these data show that Imprime is a novel PAMP that has the potential to enhance the efficacy of CPI without the systemic toxicity typical of other PAMPs.

Background

- Imprime is a pathogen associated molecular pattern (PAMP) molecule under development as an immunotherapy for cancer treatment.



- Imprime is isolated from the cell wall of a proprietary strain of *Saccharomyces cerevisiae* as a pharmaceutical grade, water soluble beta 1,3/1,6 linked glucose polymer. The structure is an aggregate of triple helices with a Mw of ~150 kDa.
- Imprime has been safely administered **by i.v. injection into over 400 human subjects**.
- Promising efficacy was observed in multiple clinical trials with tumor targeting antibodies including phase 2 clinical trials in lung cancer (NSCLC) and larger, randomized clinical trial in colorectal cancer. Retrospective analyses of these trials have shown an association between anti beta glucan antibody level and clinical outcome.
- Imprime also shows synergy with anti-angiogenic and checkpoint inhibitor mAbs in murine *in vivo* tumor models (Figure 1).

Anti-angiogenics – immunomodulatory effects of anti-VEGFR2 | **Checkpoint inhibitors – Anti-PD-1/PD-L1 antibodies**

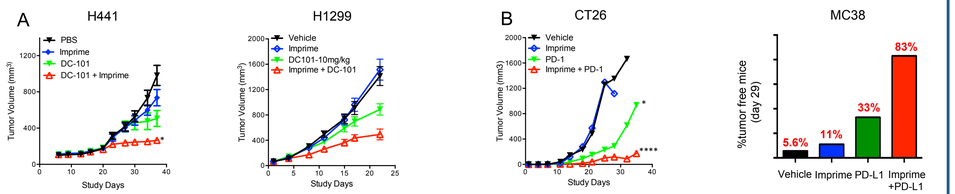


Figure 1. Imprime enhances *in vivo* anti-tumor efficacy of anti-angiogenics and checkpoint inhibitors. **(A)** Imprime in combination with DC101 (murine surrogate for ramucirumab) in H441 and H1229 NSCLC xenograft models, respectively. Once tumors reached ~100mm³, mice were administered DC101 (10 mg/kg twice weekly IP for up to six weeks) and/or Imprime (1.2 mg/mouse i.v. twice weekly for up to six weeks) . **(B)** Imprime in combination with anti-PD-1 antibody was tested in CT26 colon cancer-bearing BALB/c mice or with anti-PD-L1 antibody in MC38 colon cancer –bearing C57Bl/6 mice. Three days after subcutaneous injection of tumor, the mice were administered Imprime and/or anti-PD-1/ PD-L1 (100 µg/mouse twice weekly IP for up to five weeks).

Objective

1. Study the properties of Imprime that allow i.v. administration, versus other PAMPS that must be given intra-tumorally, by comparing the cytokine profile of human blood treated *in vitro* with Imprime versus other PAMPS.

2. Measure the *in vivo* activity of Imprime in non-tumor bearing mice by analyzing mRNA levels in lymph nodes 16 hours post-treatment.

3. Measure the cytokine and chemokine profile in healthy human volunteers following i.v. Imprime infusion.

In vitro Human Whole Blood Cytokines

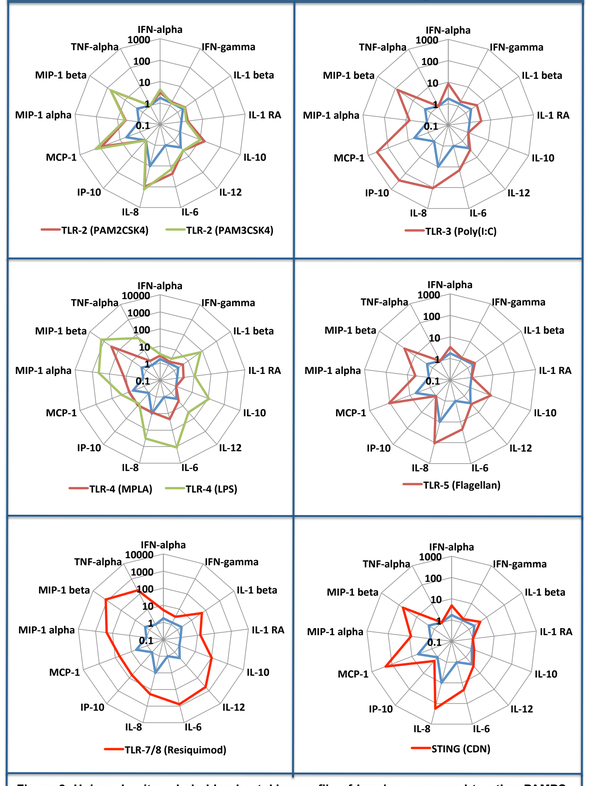


Figure 2. Unique *In vitro* whole blood cytokine profile of Imprime compared to other PAMPS. Luminex (14-plex kit or 25-plex kit) was used to measure cytokine production in the plasma of anti-coagulated WB (0.5 mL) treated with test articles for 24 hrs. One representative donor of four is displayed. Imprime is represented in blue in each of the radar plots and was tested at 10 µg/mL. The TLR-2 agonists PAM2CSK4 and PAM3CSK4 (tlrl-pms and tlrl-pm2s, Invivogen), the TLR-3 agonist Poly(I:C) (tlrl-piclv, Invivogen), the TLR-4 agonists MPLA (L6895, Sigma) and LPS (L4516, Sigma), the TLR-7/8 agonist Resiquimod (4536, Tocris), and the STING agonist (tlrl-cga2srs, Invivogen) were all tested at 10 µg/mL. The TLR-5 agonist Flagellin (tlrl-epstfla, Invivogen) was tested at 1 µg/mL. The ranges on each of the radar plots represent a fold change over PBS.

In Vivo Mouse mRNA Levels

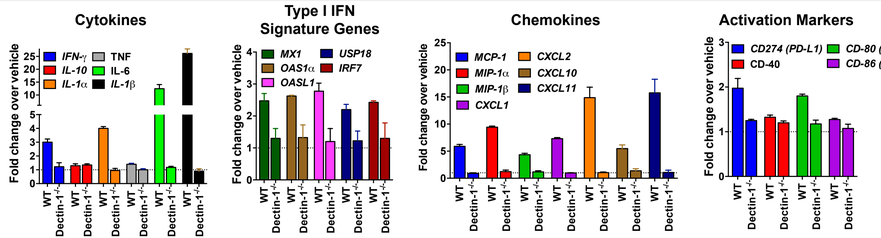


Figure 3. Imprime induces transcription of chemokines, cytokines, and activation markers. Naïve C57BL/6 mice (wild type or Dectin-1 KO) were injected i.v. with 1.2 mg Imprime or vehicle and skin-draining LNs were harvested 16 hours post injection. Gene transcription levels were determined using QuantiGene (Affymetrix) and data were normalized to vehicle-treated mice.

In Vivo Human Cytokine Levels

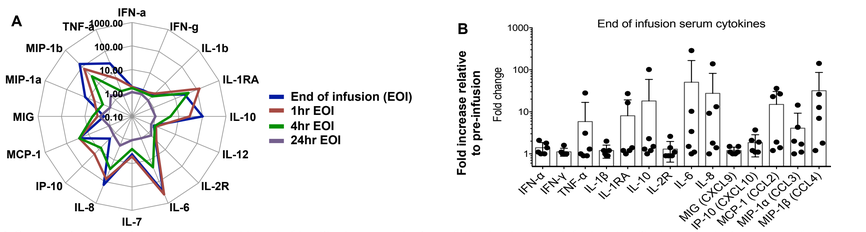


Figure 4. Serum cytokines detected in peripheral blood of healthy human volunteers following Imprime infusion. Healthy human volunteers were infused i.v. with 4mg/kg Imprime. At the end of infusion, serum was isolated from peripheral blood and cytokines/chemokines were detected using the Luminex platform. Analyte levels are shown as fold-change over pre-infusion levels. **(A)** Radar plot showing serum cytokines from 1 individual. **(B)** Cumulative data from 6 individuals.

Results and Summary

- Imprime showed synergy with both anti-VEGFR2 and checkpoint inhibitor mAbs when i.v. administered in tumor bearing mice. In H441 and H1299 NSCLC tumor models Imprime plus DC101 demonstrated better anti-tumor efficacy than vehicle or either agent alone. Likewise, Imprime plus a PD-1 mAb outperformed vehicle or either agent alone in a CT26 colon tumor model. In the MC38 colon tumor model, the Imprime plus PD-L1 mAb group had 83% mice without tumor at day 29 versus 33%, 11%, and 5.6% for the PD-L1 alone, Imprime alone, or vehicle groups, respectively.
- Imprime has been safely administered to over 400 cancer patients and healthy human volunteers by i.v. administration. When isolated human whole blood was treated with Imprime, only IL-8 and MCP-1 were detected, supporting the saftey profile, whereas most of the other PAMPS produce pro-inflammatory cytokines such as TNFα , IL-6, IL-1β , and IL-10.
- *In vivo* activation of mouse innate immune cells was observed when Imprime was i.v. administered and mRNA levels were measured in harvested lymph nodes. Specifically, cytokines and chemokines responsible for immune cell mobilization, antigen presentation, and type I IFN production were produced. Imprime's unique *in vivo* chemokine profile was also confirmed in a Phase 1 healthy human volunteer translational trial providing rationale for dosing in future human cancer trials.
- Together, these data demonstrate that Imprime acts as a unique i.v.-administered PAMP that primes the immune system and inspires a coordinated adaptive immune response. These qualities make Imprime an attractive candidate to synergize with cancer immunotherapies.

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