Imprime PGG, a soluble yeast β-glucan, is a systemically administered PAMP that activates DCs and supports T cell priming, showing synergy with cancer immunotherapies

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Background
Pathogen-associated molecular patterns (PAMPs) provide crucial non-self "danger" signals that potentially activate the immune system and may be useful therapeutically to trigger anti-cancer immunity. TLR and STING agonists act as PAMPS but can cause systemic toxicity and are limited to direct subcutaneous or intratumoral injection. Imprime PGG (Imprime) is a soluble β-1,3/1,6 glucan PAMP isolated from yeast. Imprime has been safely administered intravenously (i.v.) to >400 human subjects (>325 cancer patients). Imprime has shown promising clinical efficacy in multiple phase 2 studies when combined with tumor targeting or anti-angiogenic antibodies. Imprime also enhances the anti-tumor efficacy of immune checkpoint inhibitors in pre-clinical mouse tumor models and is currently being explored clinically in combination with anti-PD1 therapy. We now provide data in vivo mouse studies that Imprime activates dendritic cells (DCs) to induce T cell priming. Similarly, in human healthy volunteers, i.v. administration of Imprime also drives innate immune cell activation.

Methods
To examine in vivo effects of Imprime, C57BL/6 mice were injected i.v. with 1.2 mg of Imprime. 16 hr post-treatment, lymph nodes were harvested and mRNA levels were determined (Quantigene Plex platform, Affymetrix). To study Imprime’s effect on CD8 T cell priming, 1x10⁶ OT-I CD8 T cells were transferred into congenic hosts and one day later immunized with H-2Kb/OVA257-264 peptide +/- Imprime. Separately, healthy human volunteers were infused with 4mg/kg Imprime and serum cytokines/chemokines were examined using the Luminex platform.

Results
Following i.v. administration in non-tumor bearing mice, Imprime rapidly bound resident and migratory DC subsets, caused DC maturation, and increased DC recruitment into lymph node (LN) subpopulations. Transcriptional profiling in LNs showed increased mRNA levels of chemokines important in immune cell trafficking, pro-inflammatory cytokines, and a strong type I interferon signature. Many of these chemokines were also increased in the blood of healthy volunteers, as was detection of Imprime binding to DCs. In congenic mouse recipients that were immunized with peptide +/- Imprime after transfer of OVA-specific OT-I CD8 T cells, those primed in the presence of Imprime demonstrated greater overall expansion and acquisition of effector functions than peptide alone. Imprime’s transcriptional profile and ability to enhance T cell priming was dependent on the C-type lectin receptor Dectin-1.

Conclusions
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.

Figure 1. Imprime binds to and matures mouse dendritic cells. C57BL/6 mice were injected i.v. with 1.2mg DAFAF-conjugated Imprime and spleens and peripheral LNs were harvested 30min post injection. (A and B) DC binding of Imprime in the spleen (A) and lymph nodes (B). (C) DC expression of CD86 and CD40 following Imprime injection. Gray = vehicle, colored = Imprime.

Figure 2. Imprime induces transcription of chemokines, cytokines, and type I IFN. Naïve C57BL/6 mice were injected i.v. with 2mg Imprime and skin-traing LNs were harvested 16 hours post injection. Gene transcription levels were determined using QuantiGene (Affymetrix) and data were normalized to vehicle-treated mice. Filled bars represent wild-type mice and white bars represent Dectin-1 KO mice.

Figure 3. Imprime enhances expansion and effector differentiation of antigen-specific CD8 T cells. (A) Vaccination model. Naïve WT or Dectin-1-/- mice received OVA CD8 T cells. The following day they were injected with 100µg OVA257-264 peptide i.v. and either PBS, Imprime, or LPS (2µg). The magnitude of the OT-I response was assessed on day 5 in the blood (B) and day 7 in the spleen (C). (D) OT-1 CD44 and Tbet expression on day 7 in the spleen. (E) On day 7, splenocytes were stimulated with OVA peptide for 5hrs with brefeldin A. Cells were then stained for intracellular IFN-γ, TNF-α, and IL-2.

Figure 4. Imprime synergizes with anti-PD-1/L1 in a murine colorectal tumor model. C57BL/6 mice were injected 5x10⁶ MC38 cell s.c. Three days after inoculation, mice were treated 2x weekly with Imprime and 100µg oPD-L1. Data represents the % of mice on day 29 that were tumor free. MC38 re-challenge of tumor-free mice showed that they had developed memory (data not shown).

Figure 5. Imprime binds in vivo to human peripheral DCs. Healthy human volunteers were infused i.v. with 4mg/kg Imprime. Blood was drawn at various time pre- and post-infusion. (A) Representative plots of extracellular Imprime binding to DCs that they had developed memory that were tumor free. MC38 re-challenge of tumor-free mice showed that they had developed memory (data not shown).

Figure 6. Serum cytokines detected in peripheral blood of healthy human volunteers following Imprime infusion. Healthy human volunteers were infused intravenously 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.