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 (CIPI). Recently, Imprime, a yeast β-glucan (PGG, also known as β-glucan 1,3/1,6), demonstrated, in a recent phase 1 study, the ability of this novel TLR-2 agonist to sensitize tumors to respond to CIPI in several ways, including activation of innate and adaptive immunity, leading to CD8 T cell expansion and IFN-γ production. In multiple studies, Imprime has been shown to be safe, well-tolerated, and improves the direct tumor killing function of innate and adaptive immune cells by activating macrophages, natural killer cells, and tumor specific CD8 T cells and increasing immunosuppressive processes to the effector function of T cells at the tumor site. However, many PAMPs exist, and research has shown that certain PAMPs, such as cytokine osteomalacia (CPI) agonists, are not well-tolerated. This research further demonstrated that immunomodulatory processes against CPIs are associated with both single and combination therapy. Despite the clear benefits of Imprime, little is known about the cytokine profile and effect of Imprime on the tumor microenvironment. Here, we analyzed serum cytokine and chemokine profiles following Imprime treatment.

Results and Summary
Imprime has been safely administered to mice and human volunteers. Promising efficacy was observed in a variety of cancer models, including mice and human volunteers infected with human epidermal growth factor receptor 2 (HER2) positive breast cancer xenografts, and Nevalin®-positive melanoma xenografts. Preclinical studies showed that Imprime has potential as a cancer therapy and as a vaccine adjuvant. In a study with HER2-positive breast cancer xenografts, Imprime demonstrated a trend towards enhanced therapeutic efficacy when combined with anti-PD-L1 antibody. In addition, preclinical studies have shown that Imprime has synergistic effects with anti-PD-L1 antibody, suggesting that Imprime may be a valuable therapeutic agent in the treatment of cancer. Preclinical studies have also shown that Imprime has the potential to sensitize tumors to respond to CIPI in several ways, including activation of innate and adaptive immunity, leading to CD8 T cell expansion and IFN-γ production. Imprime has been shown to be safe, well-tolerated, and improves the direct tumor killing function of innate and adaptive immune cells by activating macrophages, natural killer cells, and tumor specific CD8 T cells.

Objective
1. Study the properties of Imprime that allow it to elicit a tumor-specific immune response to PAMPs that must be given intravenously.
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 Imprime infusion.

In Vivo Human Whole Blood Cytokines

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>In Vivo Mouse mRNAs Level</th>
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<tbody>
<tr>
<td>IFN-γ</td>
<td>CD-80 (B7-1)</td>
</tr>
<tr>
<td>IFN-α</td>
<td>CD-86</td>
</tr>
<tr>
<td>IL-1β</td>
<td>MHC-II</td>
</tr>
<tr>
<td>IL-6</td>
<td>CD-103</td>
</tr>
<tr>
<td>IL-12</td>
<td>CD-87</td>
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<tr>
<td>IL-18</td>
<td>CD-88</td>
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</tbody>
</table>

Figure 1. Imprime induces a unique anti-infectious and anti-inflammatory cytokine profile in mice in vivo. (A) Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 2. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 3. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 4. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 5. Imprime-induced transcription of chemokines, cytokines, and activation markers. Normal MC38 mice (wild type or Dectin-1 KO) were injected i.v. with Imprime (D) or vehicle (Ctrl) for the duration of the experiment, and then were sacrificed for RNA isolation. A total of 10 mice were assayed in each group. Data are presented as fold change compared to the untreated group. ****p < 0.0001.

Figure 6. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 7. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 8. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 9. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 10. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 11. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.

Figure 12. Unique in vivo whole blood cytokine profile of Imprime compared to other PAMPS. Imprime was administered intravenously (i.v.) to WT mice with or without PAMPs. Imprime administration resulted in a significant increase in IFN-γ and IL-1β production. (A) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (B) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (C) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6. (D) Imprime administration was associated with a decrease in the expression of IL-10 and IL-6.