Background and Objective: The success of cancer immunotherapy is often limited by multiple mechanisms of tumor-induced immunosuppression; M2-like TAMs being one of the critical mediators of immunosuppression. Imprime PGG (Imprime), an intravenously administered soluble yeast β-1,3/1,6 glucan is being clinically developed in combination with tumor-targeting antibodies, anti-angiogenics, and checkpoint inhibitors. Imprime has shown promising results in two randomized phase 2 studies in non-small cell lung cancer (NSCLC). Imprime acts mechanistically as a PAMP enlisting innate immune functions including cytotoxic effector mechanisms, reversal of immunosuppression and cross-talk with the adaptive immune system. With respect to immunosuppression, Imprime has been shown to repolarize M2 macrophages to an anti-tumor M1-like orientation in human ex vivo studies. The objective of this study was to expand on this finding in an in vivo setting.

Methods: Imprime’s M1-polarization effect was evaluated in tumor-free mice, and xenogenic and syngeneic tumor models. Bone marrow-derived macrophages (BMDM) prepared from Imprime- or vehicle-treated tumor-free mice were evaluated by qRT-PCR. Imprime was tested in combination with an anti-angiogenic agent, DC101 (a VEGFR2 Mab) in H441 NSCLC xenograft model in athymic nude mice, and in combination with anti-TNFα tumor-targeting antibody TA-99 in B16 experimental lung metastasis model. Immunohistochemistry of FFP- tumor tissue or lung tissue were evaluated.

Results: qRT-PCR analyses of Imprime-treated BMDM from tumor-free mice revealed an increase in M1 markers (iNOS, PD-L1, IL-12, TNF-α, CCL3, CCL10, and CCL11) and a coincident decrease in M2 markers (CD206, YM-1, Fiz1, and CCL22). Imprime’s M1-polarization effect was also observed in H441 NSCLC tumor model where Imprime treatment alone upregulated M1-like genes in TAMs as well as significantly suppressed tumor growth when combined with DC101, an agent that also modulates tumor microenvironment. Imprime-mediated M1-polarization was also observed in the B16 experimental lung metastasis model where the combination of Imprime with TA-99 significantly reduced both the number and size of B16 lung metastases. Immunohistochemistry analysis showed an increase in the number of tumor-infiltrating CD11b+ cells with an M1 phenotype evidenced by increased iNOS expression.

Conclusion: Collectively, these data indicate that Imprime, by reorienting the M2 macrophages to an M1-like polarization state can re-establish the suppressive tumor microenvironment to be more sensitive to other immunotherapeutic modalities.

## Abstract

**Background**

- Imprime PGG, a yeast-derived pharmaceutical-grade soluble 1,3/1,6 β-glucan is being developed for the treatment of cancer in conjunction with tumor-targeting and immunomodulatory antibodies (Abs).
- Imprime has shown promising results in multiple Phase 2 clinical trials in non-small cell lung cancer (NSCLC) and chronic lymphocytic leukemia (CLL) with additional studies ongoing.
- β-glucans are conserved microbial structures found in the cell wall of unicellular and multicellular pathogens. They are considered pathogen-associated molecular patterns (PAMPs) recognized by the pattern recognition receptors including Dectin-1 and Complement Receptor 3 (CR3). Imprime forms an immune complex with endogenous IgG or IgM anti-beta-glucan antibodies (ABA) before being recognized by CR3 and FcgRIIIa on innate immune cells.

**Figure 1. Imprime (PAMP) binds to innate immune cells and triggers coordinated Anti-Cancer Immune response.**

**Results**

### Polarization to M1: In vivo effect on macrophages in tumor-free mice

#### A) Splenic macrophages

- **Bone marrow-derived macrophages (BMDM)** exhibit M1 phenotype after Imprime treatment. (A) Splenic macrophages from Imprime- or vehicle-treated mice (received single dose of 1.2 mg for 16 hrs) were evaluated for M1/M2 markers by flow cytometry. (B) Bone marrow-derived macrophages (BMM) was prepared by culturing bone marrow cells harvested from Imprime-treated mice in RPMI medium supplemented with 10% fetal cell serum and 20 ng/ml rmM-CSF for 7 days (received single dose of 1.2 mg for 16 hrs). M1/M2 markers were then measured by qRT-PCR.

#### B) Bone marrow-derived macrophages

- **Mouse BMM-Vehicle**
- **Mouse BMM-Imprime**

### Polarization to M1: In vivo effect on TAM in xenograft tumor models

#### A) H441: NSCLC Tumor Model

- **Imprime**
- **DC-101**
- **DC-101 + Imprime**

#### B) H1299: NSCLC Tumor Model

- **Vehicle**
- **Imprime**
- **DC-101**
- **DC-101 + Imprime + Control Liposomes**
- **DC-101 + Imprime + Cytokine Liposomes**

### Polarization to M1: In vivo effect on TAM in syngeneic tumor models

#### MC38 – Colon Adenocarcinoma Tumor Model

- **Imprime**
- **Vehicle**

#### B16 – Melanoma Tumor Model

- **Imprime**
- **Vehicle**

**Figure 2.** Splenic macrophages and bone marrow derived macrophages (BMDM) exhibit M1 phenotype after Imprime treatment. (A) Splenic macrophages from Imprime- or vehicle-treated mice (received single dose of 1.2 mg for 16 hrs) were evaluated for M1/M2 markers by flow cytometry. (B) Bone marrow-derived macrophages (BMM) was prepared by culturing bone marrow cells harvested from Imprime-treated mice in RPMI medium supplemented with 10% fetal cell serum and 20 ng/ml rmM-CSF for 7 days (received single dose of 1.2 mg for 16 hrs). M1/M2 markers were then measured by qRT-PCR.

**Figure 3.** Imprime treatment induces M1 polarization of TAM in non-small cell lung cancer (NSCLC) xenograft models and enhances efficacy of anti-angiogenic agent, DC101 (murine ramucirumab). H441 and H1299 NSCLC xenograft tumour (reached ~100 mm³ bearing nude mice were administered DC101 (10 mg/kg twice weekly IP for up to six weeks) and/or Imprime (1.2 mg/mouse IV twice weekly for up to six weeks). (A) In the H441 model, changes in the mRNA expression of M1/M2 markers of Imprime vs. vehicle-treatment tumor cell suspension is shown on the left and the tumor growth in the different treatment groups is shown in the right panel. (B) In the H1299 model, changes in the mRNA expression of M1/M2 markers of Imprime vs. vehicle-treated tumor cell suspension is shown on the left and the tumor growth in the different treatment groups is shown in the middle panel. The right panel shows the abrogation of the enhanced efficacy imparted by Imprime after depleting macrophages by administering adenosine liposomes. Cytokine or control liposomes (100 μl/mouse) were administered on days 1, 3, 6, 10, 13, 17, 20, 23, 24 and 26. (***p<0.001, **p<0.01, *p<0.05, ***p<0.0001)

**Figure 4.** Imprime treatment promotes M1 polarization of TAM in MC38 colon adenocarcinoma and B16 melanoma syngeneic models and enhances efficacy of checkpoint inhibitors and tumor-targeting antibody (A). In the MC38 model, changes in the mRNA expression of M1/M2 markers of Imprime vs vehicle-treated tumor cell suspension is shown on the top (**p<0.05, ***p<0.001) and ***p<0.0001. The phenotypic changes in the M1/M2 markers on splenic monocytes (MDC-130) was assessed by flow cytometry is shown in the bottom. (B) Top panel shows % tumor free CS/BJ6 mice in the MC38 syngeneic model with the single and combination treatment of Imprime and anti-CD8 antibody. Briefly, three days after subcutaneous injection of tumor, the mice were administered Imprime (1.2 mg/mouse IV twice weekly) and/or anti-PD-L1 (100 μg/mouse twice weekly IP for up to five weeks). Bottom panel shows tumor growth in the different treatment groups in another MC38 syngeneic in vivo study. Once tumors reached ~100 mm³, mice were administered anti-PD1 (200 μg/mouse twice weekly IP for up to six weeks) and/or Imprime (1.2 mg/mouse IV twice weekly for up to six weeks). (C) MC38 tumor-bearing CS/BJ6 mice were treated with Imprime as above or anti-PD1 for 1 week. Tumors and spleens were harvested and analyzed by flow cytometry. Imprime TAM (CD11b+Ly6G) and sM-MSCD (-CD11b+Gr-1+Ly6C) increase expression of activation markers. (D) The top panel is a representative immunohistochemical staining of lung tissue in B16 lung metastasis tumor model showing down-modulation of M2 marker, Arg-1 in the Imprime + tumor-targeting antibody TA99 combination group vs TA99 alone. The bottom panel shows the count of lung metastases in each of the treatment groups.

**Figure 5.** Imprime treatment results in polarization of human monocyte-derived macrophages. M1-like macrophages were prepared by culturing imPrime-bound monocytes enriched from human whole blood in the presence of M2-polarizing cytokine RM-445 (50 ng/ml) in XVivo 10 medium for 6 days. Macrophages were subsequently evaluated for M1/M2 phenotype. (A) CD14/CD11b T cell proliferation by CSF1-dilution assay. (B) Production of cytokines by multiple imPrime/vehicle (C) M2 imPrime/vehicle were prepared as described and TCM was added on imPrime 1:4, 1:8, 1:16, and 1:32 at 24 hours. (D) M2 imPrime/vehicle and TCM were evaluated for phenotype (top panel) T cell proliferation and cytokine production (bottom panel).

**Conclusion**

- Imprime’s ability to polarize macrophages to M1 orientation is evident in several in vivo tumor models.
- Imprime polarizes the human monocyte-derived macrophages to be M1-like and have reduced ability to suppress T cell proliferation and functions.
- Imprime, by reorienting the M2 macrophages to an M1-like polarization state can re-establish the suppressive tumor microenvironment to be more sensitive to other immunotherapeutic modalities, including tumor-targeting antibody, anti-angiogenics, and checkpoint inhibitors.

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