2016 AACR Annual Meeting Abstract #3280

Imprime PGG synergizes with anti-angiogenic antibodies to repolarize the immune microenvironment, suppressing xenograft tumor growth in vivo

Kathryn Fraser, Nadine Ottoson, Xiahong Qiu, Anissa SH Chan, Adria Jonas, Takashi Kangas, Jeremy Graff, Nandita Bose

Biothera Pharmaceuticals, Inc., Eagan, MN, USA 55121; *kfraser@biothera.com*

Abstract

Anti-angiogenic antibodies (Ab) such as bevacizumab (αVEGF) and ramucirumab (αVEGFR2) suppress tumor growth by disrupting the leaky, tortuous vasculature characteristic of growing tumors. Recent work now indicates that these Ab may also promote a shift from an immunosuppressive tumor microenvironment to one more permissive for immune recognition and tumor eradication. These data suggest that combining anti-angiogenic Abs with immunotherapies, particularly those that may also drive repolarization of the immunosuppressive tumor microenvironment, may enhance therapeutic efficacy.

Imprime PGG (Imprime) is a β glucan PAMP (Pathogen Associated Molecular Pattern) that has demonstrated promising efficacy in phase 2 randomized clinical trials with the bevacizumab (bev)-based therapy. Preclinical mechanistic work has shown that Imprime can promote repolarization of the suppressive M2 macrophages and MDSCs that typically reside within the tumor microenvironment. We now show that, when combined with DC101 (murine anti-VEGFR2 Ab), Imprime significantly enhances the inhibition of H441 human NSCLC xenograft tumor growth in athymic nude mice. Moreover, we also show that the combination of Imprime plus DC101 promotes a more pronounced and significant shift in myeloid function than either agent alone. Specifically, mice treated with Imprime plus DC101 had reduced numbers of immunosuppressive, splenic MDSCs and an increase in the number of CD68+F4/80+ cells expressing the critical co-stimulatory marker CD86, indicating an increase in activated splenic macrophages. Tumor associated macrophages from these mice also showed significantly increased expression of CD86. qRT-PCR analyses of these tumor tissues likewise revealed that the combination specifically elicited a profound shift in the polarization state of the microenvironment, increasing M1 markers (TNFα, iNOS, IL-6) and decreasing M2 markers (CD206, IL-10, TGFβ and CCL22). Similarly, in H1299 NSCLC xenograft-bearing mice, the addition of Imprime to bev also elicited a profound shift in the polarization state of myeloid cells. Macrophages and neutrophils from spleen and tumor tissue of mice treated with the combination showed significant upregulation of CD86. Moreover, when compared to mice treated only with bev, splenic MDSCs from Imprime plus bev treated mice showed increased iNOS expression and reduced Arg-1 expression- a shift typifying the M1 polarization state. These data reveal that the addition of Imprime to anti-angiogenic Ab therapy prompts a substantial shift in the tumor immune microenvironment *in situ* and enhances the efficacy of anti-angiogenic therapy.

Background

- Imprime is a soluble yeast-derived β-1,3/1,6 glucan immunomodulator (Figure 1) being developed for cancer treatment in combination with anti-tumor antibodies.
- In a randomized phase II clinical study, stage IV NSCLC patients treated with Imprime plus the anti-VEGF antibody bevacizumab (bev), carboplatin and paclitaxel showed a median overall survival of 16.1 months versus 11.6 months in patients not receiving Imprime.
- Imprime, a pathogen associated molecular pattern (PAMP), forms an immune complex with endogenous anti-β-glucan antibodies, then binds and primes innate and adaptive immune cells including macrophages, monocytes, neutrophils and DCs. Activation of the above innate cells is central to influencing adaptive immune cell responses. Generating functional and long-lived anti-tumor innate and adaptive immune responses is key to providing durable tumor control.
- **OBJECTIVE: To evaluate the ability of Imprime to complement the effect of anti-angiogenics on the immune microenvironment in *in vivo* xenograft models of NSCLC.**

BIOTHERA

Results

Figure 1: The general structure of yeast-derived Imprime

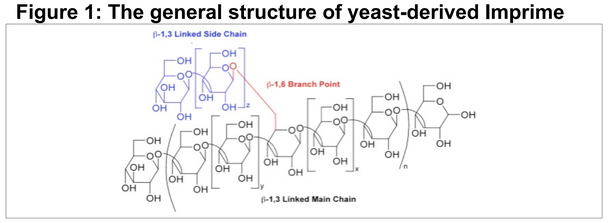
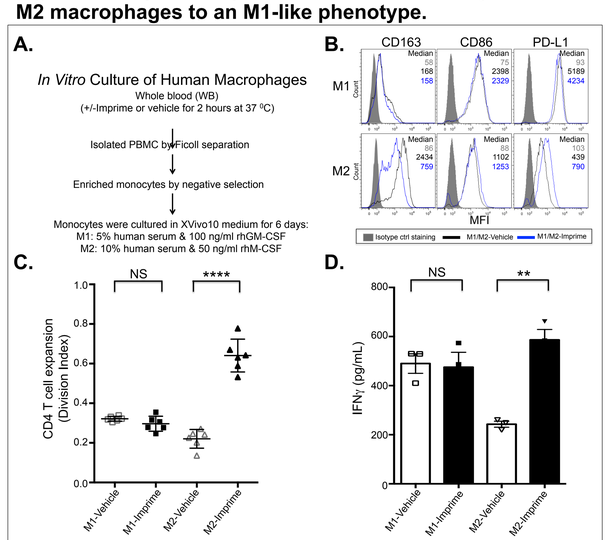
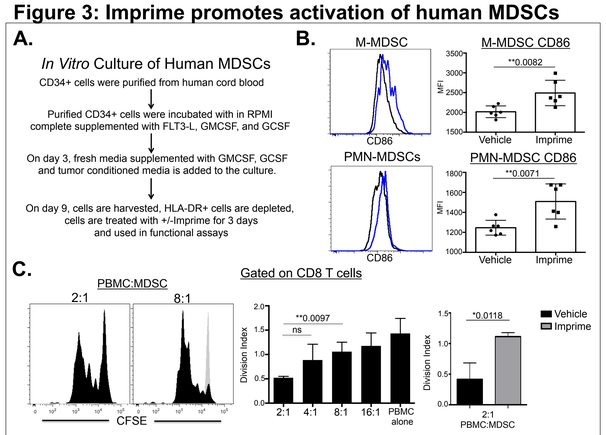


Figure 2: Imprime repolarizes human monocyte-derived M2 macrophages to an M1-like phenotype.



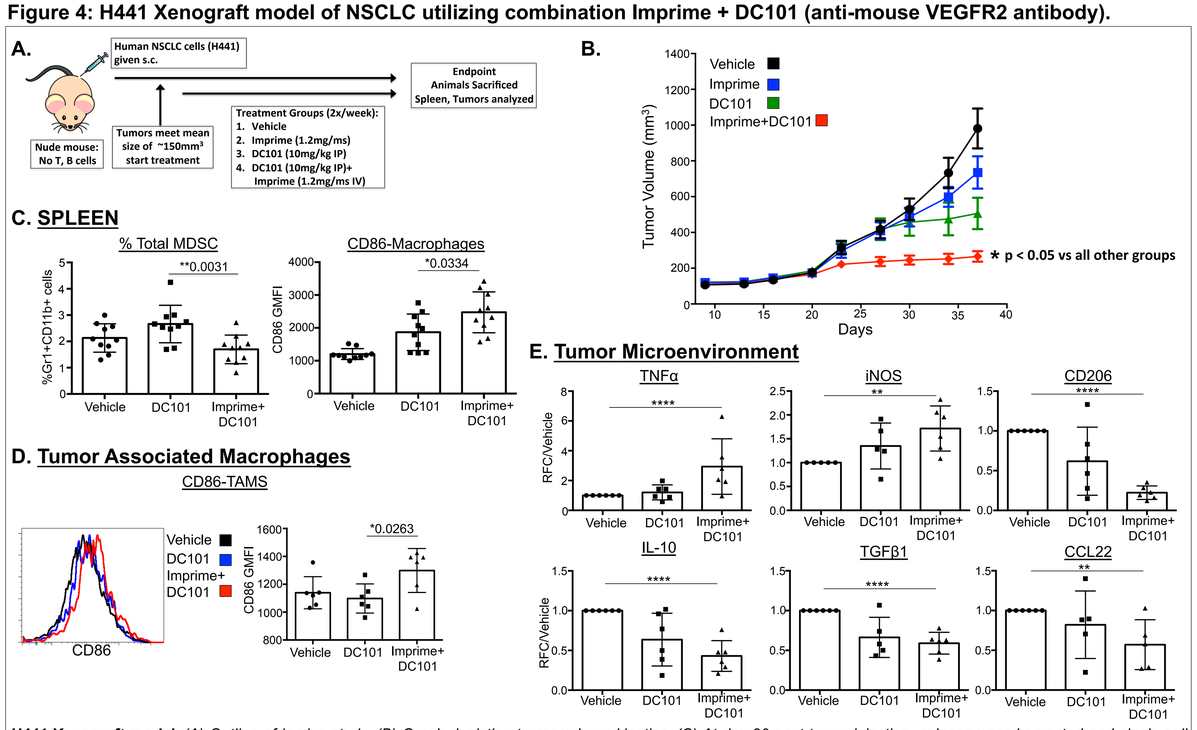
Phenotypic and functional evaluation of Imprime treated M1 and M2 macrophages. *(A) Protocol for generation of M1 or M2 macrophages from CD14+ monocytes purified from human whole blood. (B) Phenotype of Imprime treated M1 or M2 macrophages was obtained by flow cytometry. (C) CD3- & CD28-stimulated, CFSE-labeled CD4 T cells were cultured with M1 or M2 macrophages at ratio at 10:1. T cell proliferation was measured on days 3-5, and (D) modulation of IFN-γ production was analyzed by ELISA.*

Figure 3: Imprime promotes activation of human MDSCs



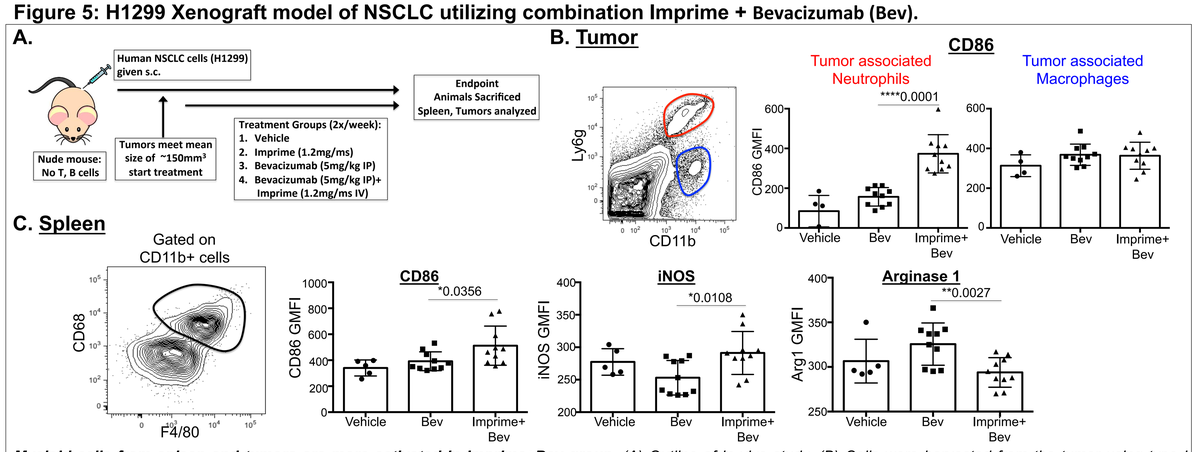
Phenotype and function of human MDSCs. *(A) Protocol for generation of human monocytic (M-MDSC) or* polymorphonuclear *MDSCs (PMN-MDSC). (B) Phenotype of MDSCs after treatment with Imprime. (C) Different ratios of CD3- & CD28-stimulated, CFSE labeled PMBCs were incubated with MDSCs +/-Imprime for 3 days. Cells were harvested and CD8 T cell proliferation was analyzed by flow cytometry.*

Figure 4: H441 Xenograft model of NSCLC utilizing combination Imprime + DC101 (anti-mouse VEGFR2 antibody).



H441 Xenograft model. *(A) Outline of in vivo study. (B) Graph depicting tumor volume kinetics. (C) At day 20 post tumor injection, spleens were harvested and single cell suspensions were analyzed by FACS. GMFI was calculated in Flowjo. (D) Cells were harvested from the tumor using type I collagenase. GMFI of CD86 was calculated in Flowjo after gating on Gr1-CD11b+CD68+ cells. (E) mRNA levels from tumor single cell suspensions were analyzed by qRT-PCR.*

Figure 5: H1299 Xenograft model of NSCLC utilizing combination Imprime + Bevacizumab (Bev).



Myeloid cells from spleen and tumors are more activated in Imprime+Bev group. *(A) Outline of in vivo study. (B) Cells were harvested from the tumor using type I collagenase. GMFI was calculated after gating on Ly6g+CD11b+ (red)or Gr1-CD11b+ (blue) cells. (C) Spleens were harvested and single cell suspensions were analyzed by FACS. GMFI was calculated in Flowjo after gating on CD11b+CD68+F4/80+ cells.*

Summary

- **Imprime interacts with multiple human myeloid subsets *in vitro* including macrophages and MDSCs resulting in a more immunostimulatory phenotype and function.**
- **Imprime treatment *in vivo* can activate myeloid cells within both the tumor and spleen to orchestrate a profound shift in the immune microenvironment which promotes tumor recognition and suppression.**