Abstract #423

Imprime PGG, an innate immunomodulator for cancer immunotherapy has the potential to modulate macrophages in tumor and spleen to an anti-tumor M1-like phenotype

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Abstract

Imprime PGG (Imprime) in combination with an anti-VEGF monoclonal antibody, bevacizumab has shown promising clinical efficacy in randomized phase 2 clinical trials in non- small cell lung cancer (NSCLC) patients. Imprime, a soluble, yeast β-glucan acts as a pathogen associated molecular pattern (PAMP). As such, Imprime is efficiently and effectively recognized by the cells of innate immune system -macrophages, monocytes and neutrophils- and triggers a coordinated anti-cancer immune attack in concert with other cancer therapies.

In vitro mechanistic studies using whole blood from human volunteers have demonstrated that Imprime may alter the polarization and functionality of monocyte-derived M2 macrophages, reducing surface expression of CD163, upregulating PD-L1, driving CD4 and CD8 T cell expansion and enhancing Th1 polarization. In this study, we sought to evaluate whether Imprime treatment may also similarly affect the polarization state of the macrophages *in vivo*, particularly at a tumor site. Athymic nude mice were injected with the H1299 NSCLC cells and randomized into treatment groups (n = 10 per group) once tumors reached a group mean of ~100mm³. Mice were treated with vehicle, bevacizumab (5 mg/kg twice weekly IP), Imprime (1.2mg/ mouse twice weekly IV) or bevacizumab + Imprime. Tumor growth inhibition was calculated at the end of study (% TGI). Tumor and spleen tissue was harvested at the end of study and analyzed by flow cytometry and RT-PCR. CD11b+/CD68+/F4/80+ cells (i.e. murine macrophages) harvested from the spleen of mice treated with Imprime + bevacizumab showed a significant increase in protein expression of inducible nitric oxide synthase (iNOS) in concert with a significant decrease in Arginase-1 (Arg-1) when compared to the same cells harvested from mice treated only with bevacizumab. Furthermore, *ex vivo* treatment with LPS significantly enhanced the production of TNFα in the CD11B+/CD68+/F4/80+ cells from mice treated with Imprime + bevacizumab. Remarkably, CD11b+ cells harvested from the tumor tissue of these mice showed a similar shift toward an M1-like phenotype, demonstrating significant increases in iNOS and PD-L1 expression, and significantly reduced expression of Arg-1, at both RNA and protein levels. Furthermore, ELISA data from these tumors showed a significantly reduced expression of the potent immunosuppressor, TGFβ, when compared to tumors from mice treated only with bevacizumab, particularly in the most-responsive tumors (> 50% TGI). Collectively, these data show for the first time that Imprime treatment alters the immune microenvironment of a tumor *in situ*, driving a shift to an M1-like polarization state.

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 2 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, B cells 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 alter the immunosuppressive nature of macrophages both *in vitro* as well as in an *in vivo* xenograft model of NSCLC.**

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Results

Figure 1: The general structure of yeast-derived Imprime PGG

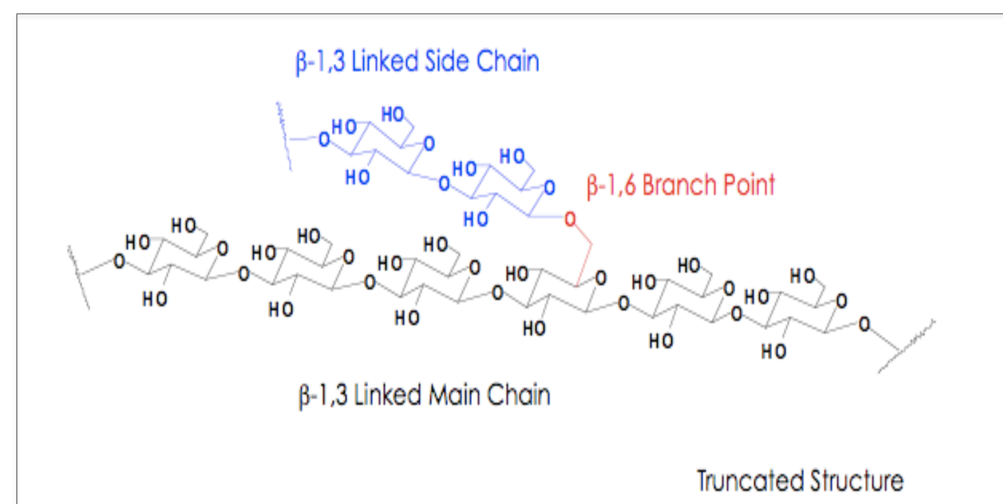
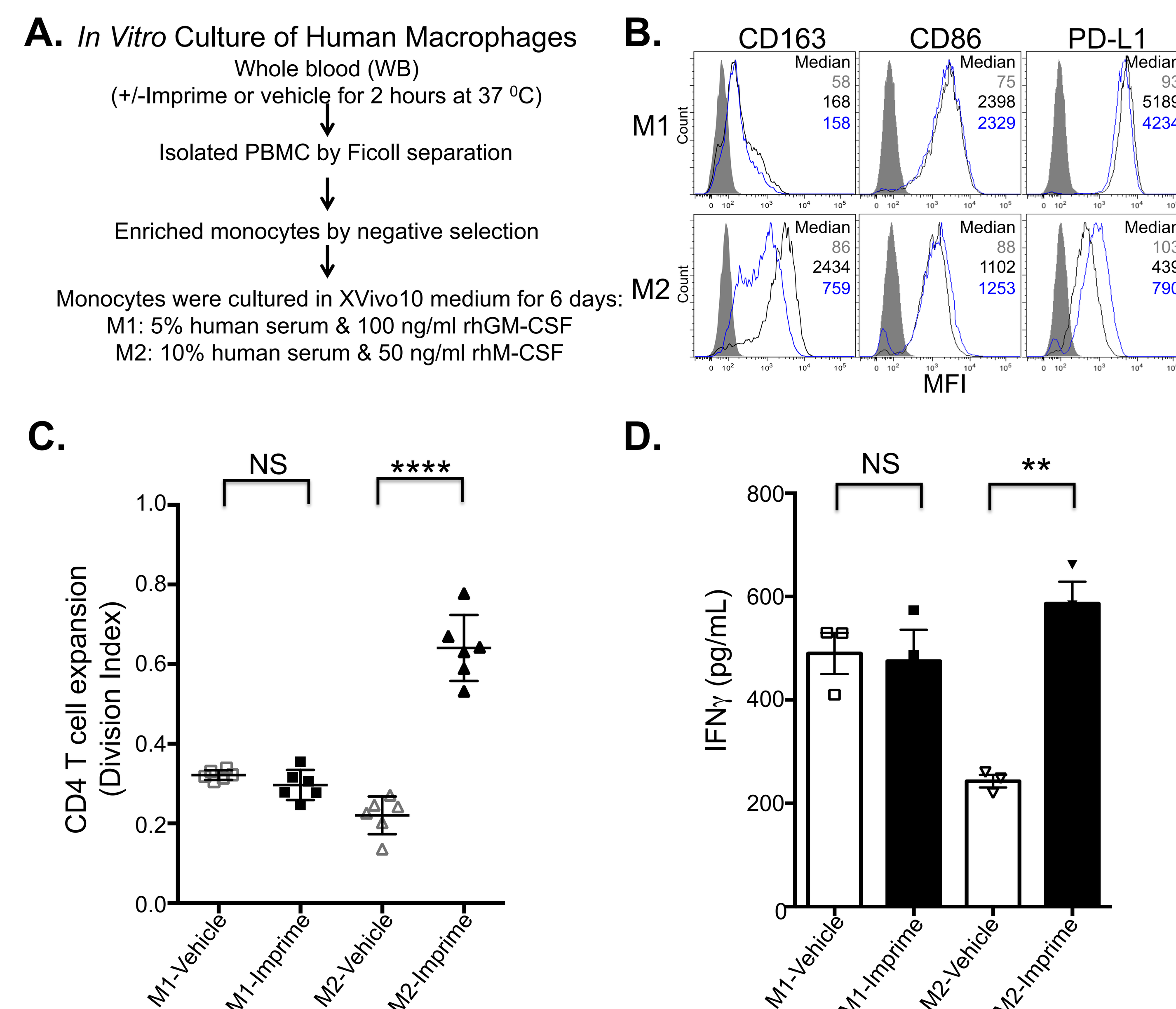
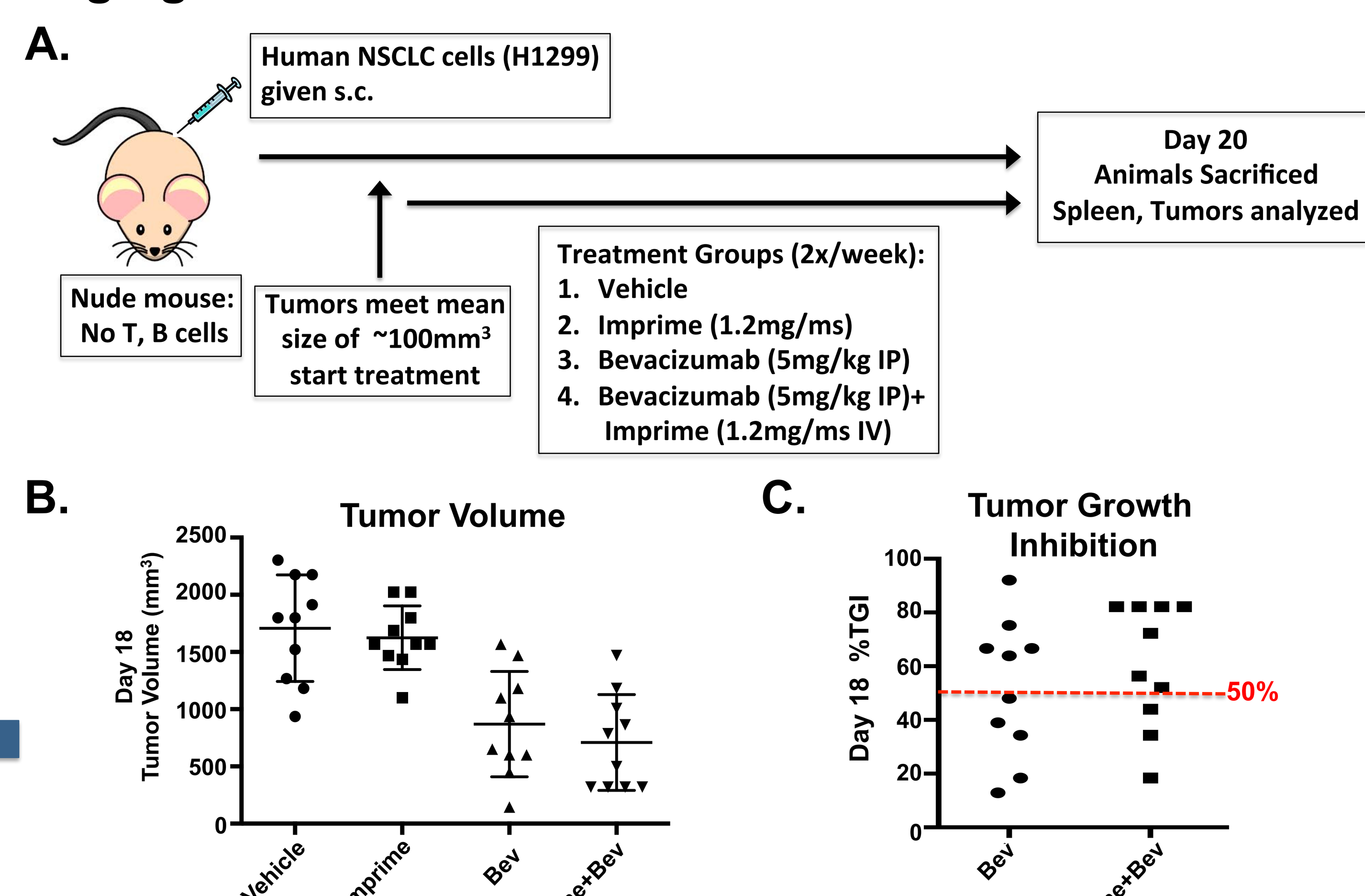


Figure 2: Imprime treated human monocyte-derived M2 macrophages display M1-like characteristics.



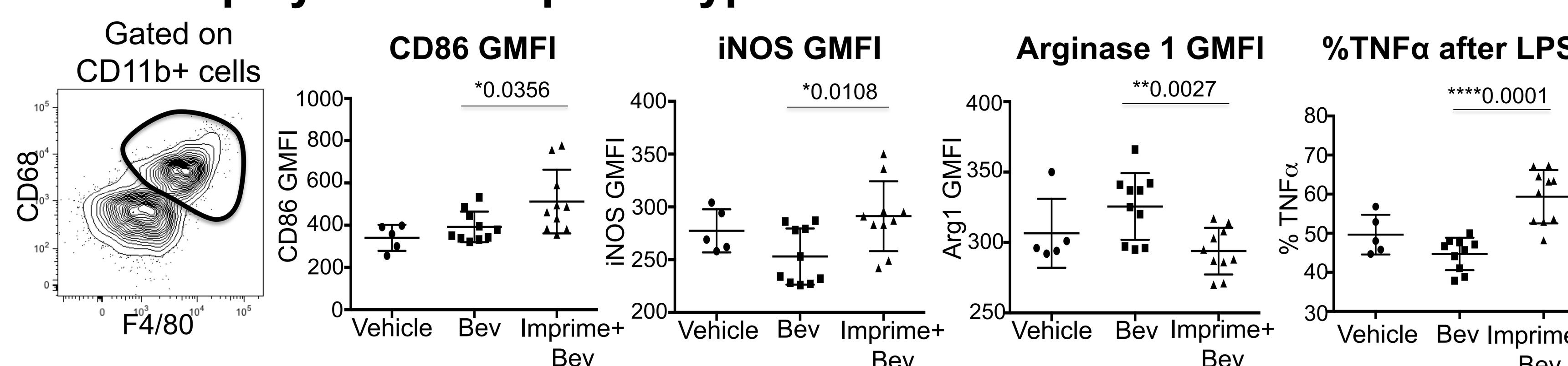
Phenotypic and functional evaluation of Imprime treated M1 and M2 macrophages. (A) Protocol for generation of M1 or M2 macrophages from CD14+ monocytes purified from human whole blood. (B) Phenotype of +/- Imprime treated M1 or M2 macrophages was obtained by flow cytometry. (C) CD3 & CD28-stimulated, CFSE-labeled CD4 T cells were cultured with 50% M1 or M2 macrophages. T cell proliferation was measured on day 11, and (D) modulation of IFN-γ production was analyzed by ELISA.

Figure 3: Anti-tumor efficacy of Imprime with anti-angiogenics *in vivo*.



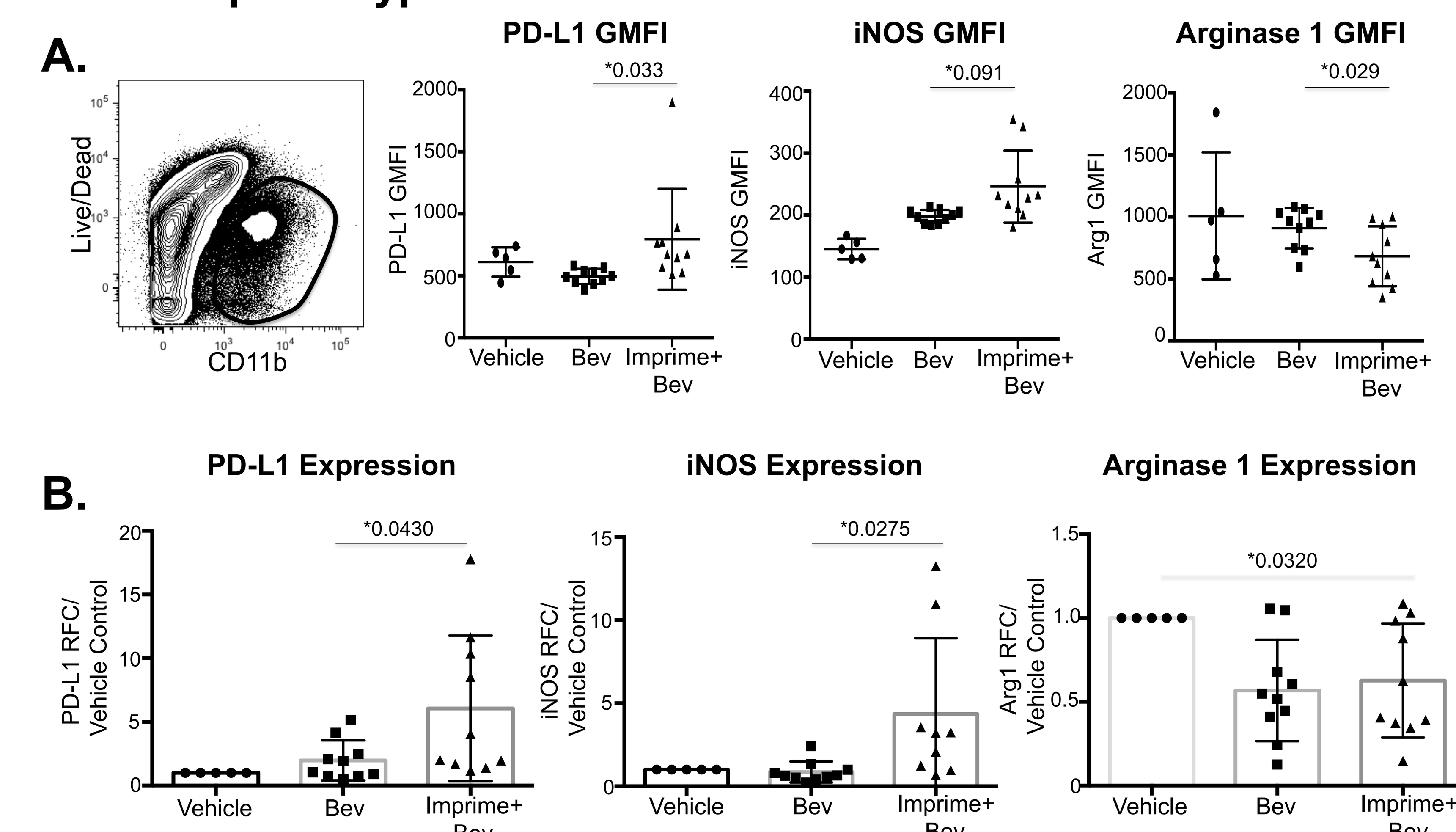
H1299 Xenograft model. (A) Outline of *in vivo* study. (B) Graph depicting tumor volume of individual mice at day 18 post challenge. (C) %TGI was calculated by %TGI= (1-individual treated mouse/median control mice)*100.

Figure 4: Splenic macrophages isolated from Imprime+Bev treated mice display a M1-like phenotype.



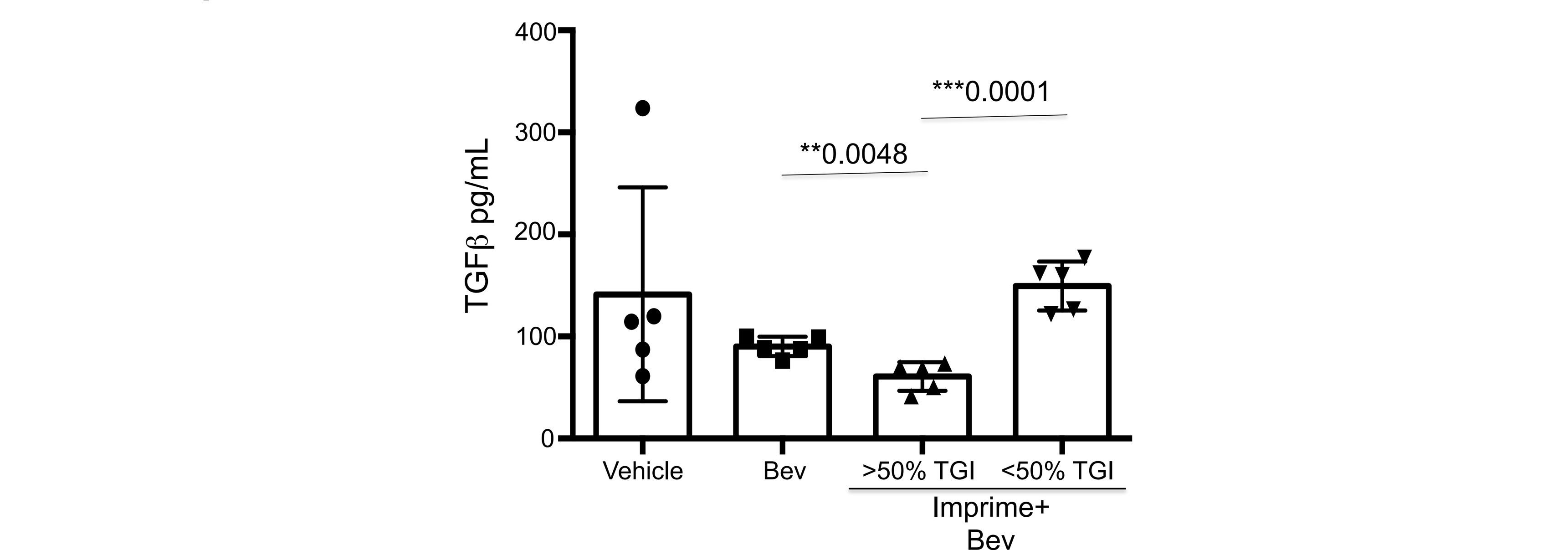
Splenic macrophages are M1. At day 20 post tumor injection, spleens were harvested and single cell suspensions were stained with mouse antibodies and analyzed by FACS. GMFI or frequency was calculated in Flowjo after gating on CD11b+CD68+F4/80+ cells. For cytokine stimulation, splenic cells were stimulated overnight with 100ng/mL LPS.

Figure 5: Imprime+Bev tumor associated myeloid cells show a more activated phenotype and an increase in PD-L1.



Myeloid cells from tumors are more activated. Cells were harvested from the tumor using type I collagenase. (A) GMFI was calculated after gating on CD11b+ cells. (B) mRNA levels of PD-L1, iNOS and Arg1 from single cell suspensions were analyzed by qRT-PCR.

Figure 6: Imprime+Bev treated animals had reduced concentration of TGFβ within the tumor.



Tumors from Imprime+Bev treated animals made less TGFβ. Cells were harvested from the tumor using type I collagenase and incubated overnight in XVivo10 media. Supernatants were then analyzed for TGFβ concentration by ELISA. %TGI was calculated by %TGI= (1-individual treated mouse/median control mice)*100.

Summary

1. Imprime PGG interacts with human macrophages *in vitro* resulting in a more immunostimulatory phenotype and function.
2. Imprime PGG treatment *in vivo* activates myeloid cells within both the tumor and spleen to orchestrate a profound shift in the immune microenvironment which promotes tumor recognition and suppression.