Imprime PGG, a yeast β-glucan PAMP elicits a coordinated immune response in combination with anti-PD-1 antibody

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Background

- Imprime PGG (BTH1677), a yeast-derived soluble β-1,3-β-1,6 glucan, is currently in clinical development as an i.v. administered immunotherapy in combination with tumor targeting and anti-angiogenic antibodies as well as checkpoint inhibitors.
- Randomized Phase 2 clinical trials of Imprime PGG in the 1st-line treatment of stage IV non-small cell lung cancer have shown promising efficacy in terms of both objective tumor response and survival.[1] Similarly, in high-risk CLL patients, the addition of Imprime PGG to rituximab and alemtuzumab achieved a higher complete response rate as compared to an earlier study with only rituximab and alemtuzumab.[2-3]
- Imprime PGG has also demonstrated synergistic anti-tumor activity in multiple preclinical syngeneic and xenograft mouse tumor models with multiple anti-tumor mAbs.[4-8] These studies have revealed the critical role of complement and complement receptor 3 (CR3) and Gr-1 positive myeloid immune cells in the anti-tumor activity of Imprime PGG.[4,8]
- As a PAMP, Imprime PGG first forms an immune complex with endogenous anti-β glucan antibodies. This complex activates complement via the classical pathway and then engage both the CR3 and FcgRIIA receptors. Imprime PGG is readily recognized by innate immune cells, triggering immune responses including repolarization of M2 macrophages and dendritic cell (DC) maturation.

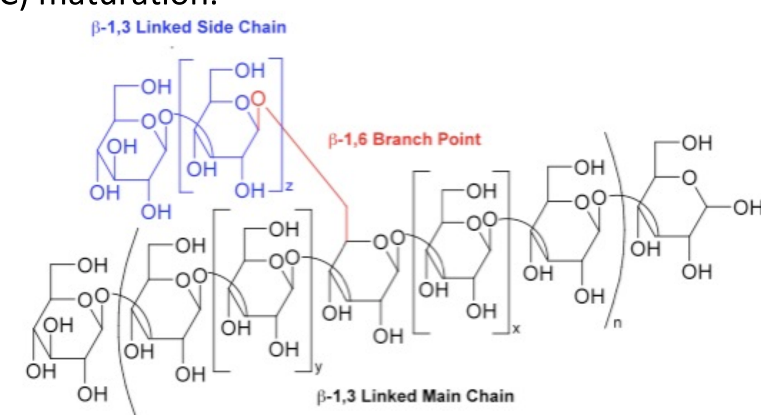

Figure 1. A general structure of Imprime PGG

Imprime PGG drives M2 macrophage repolarization to an M1-like phenotype

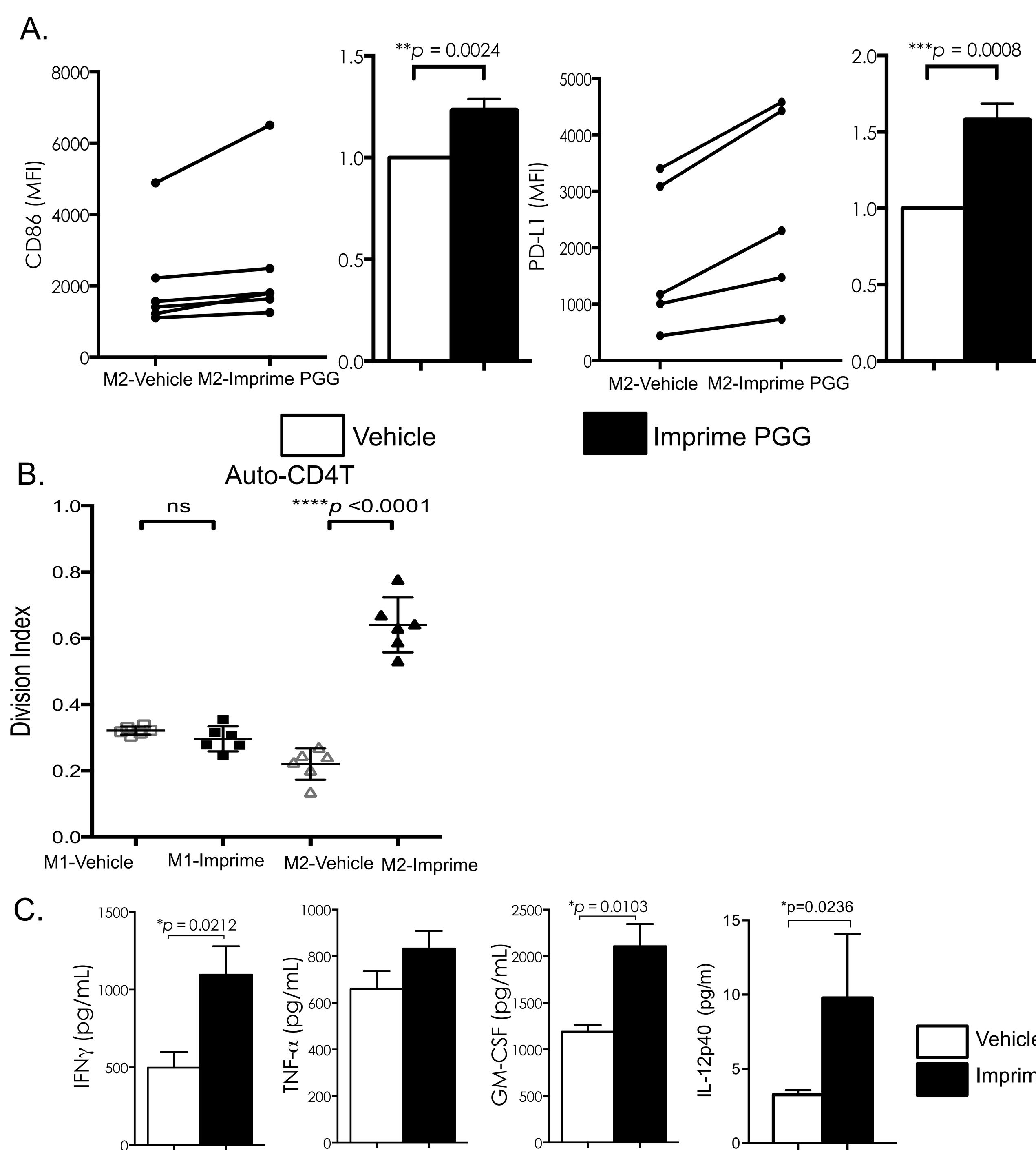

Figure 2. Imprime PGG-treated M2 are more M1-like with increased PD-L1 and CD86 expression on the surface of the cells and enhanced functional characteristics. Briefly, whole blood was treated with vehicle or Imprime PGG for 2hrs at 37°C. Monocytes were enriched by negative selection and cultured in X-Vivo 10 with 5% or 10% serum and rhGM-CSF (100ng/ml) or M-CSF (50ng/ml) for M1 and M2 respectively. **A.** M1 and M2 were evaluated for phenotype by flow cytometry and an increase in CD86 and PD-L1 was noted on M2 (n=5). **B.** ability to modulate CD3- and CD28-costimulated CD4 T cell proliferation by CFSE dilution assay (six replicates in each condition), and **C.** Culture supernatant of M2 co-cultured with CD3- and CD28-stimulated autologous CD4T cells, for 3-5 days, were analyzed for the production of IFN-γ, TNF-α, GM-CSF and IL-12p40 by Luminex. Shown here are representative results from 2 different experiments from 3 donors.

Results

Imprime PGG-treated MoDC consistently exhibits increased expression of CD86 and PD-L1 and enhanced T cell function

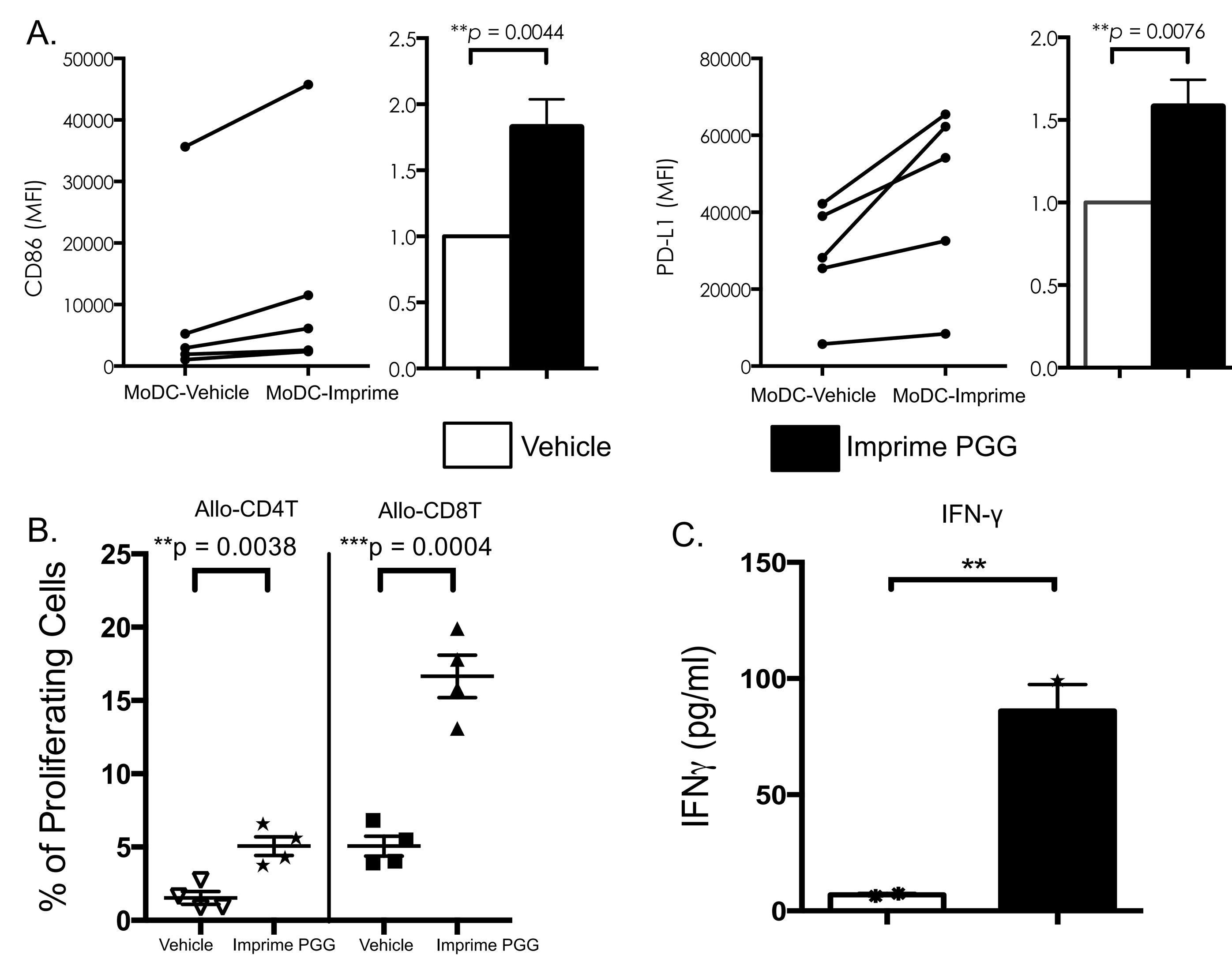

Figure 3. Imprime PGG-treated MoDC cells increases PD-L1 and CD86 expression on the surface of the cells and enhance functional characteristics Monocytes was prepared as in Figure 1 and monocytes were differentiated into MoDC, which were cultured in X-Vivo 15 with 10% human serum, rhGM-CSF (100 ng/ml) and IL-4 (100 ng/ml) for 7 days, and maturation was further induced with LPS (50 ng/ml) and TNF-α (25 ng/ml) for additional 48 hrs. **A.** MoDC were subsequently evaluated for phenotype by flow cytometry, and an increase in CD86 and PD-L1 was noted on MoDC. n=5 for each cell type. **B.** immunogenicity by allo-MLR with allogeneic CD4 and CD8T cells (four replicates in each condition) for 5 days. **C.** IFN-γ production in the MoDC allo-MLR. Shown here are representative data from more than three experiments.

Combination of Imprime PGG and Anti-PD-1 Ab Nivolumab enhances T cell proliferation when co-cultured with M2 macrophages or MoDCs

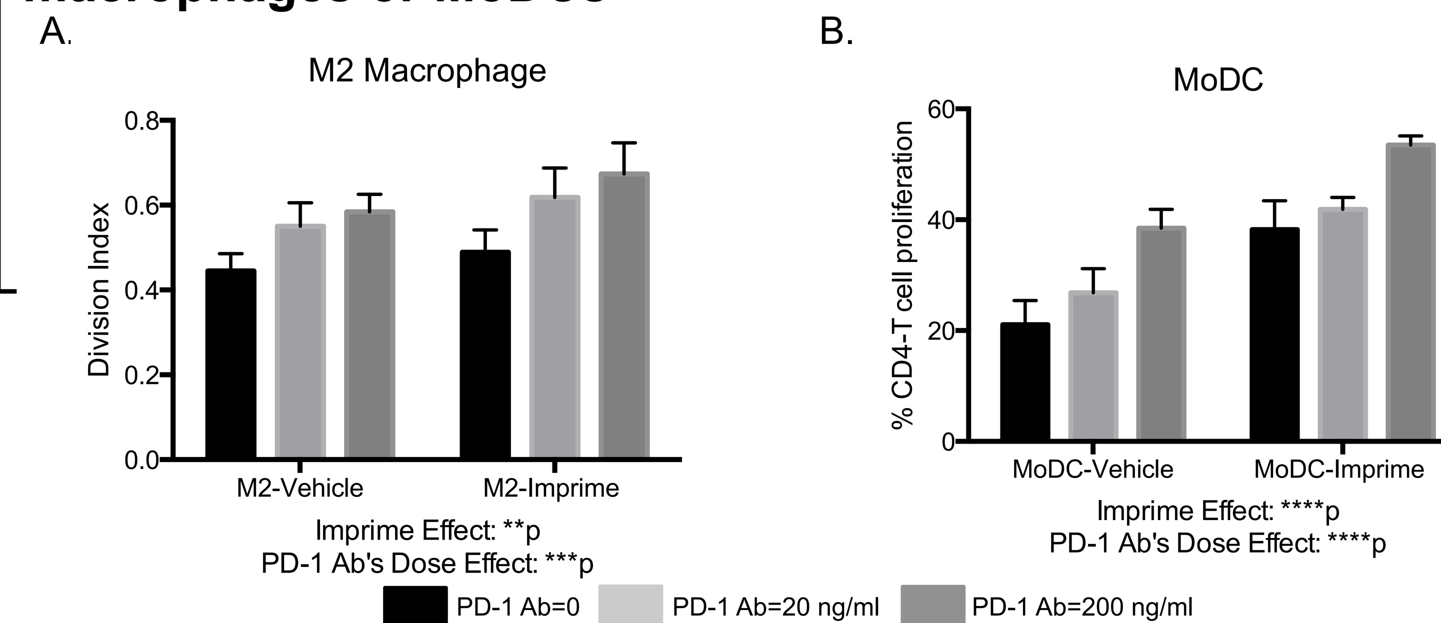

Figure 4. Synergistic effect of Imprime PGG and anti-PD-1 Mab on T-cell proliferation. Monocytes treated with Imprime PGG or vehicle then differentiated and polarized into M2 macrophages and MoDC as described in Figure 2 and Figure 3. 10^5 purified CD4+ T cells were co-cultured with 10^4 autologous M2 or allogeneic MoDC, in the presence of a titration of nivolumab for 6 days. **A.** ability to modulate CD3- and CD28-costimulated CD4 T cell proliferation by CFSE dilution assay, and **B.** immunogenicity by allo-MLR with. (four replicates in each condition) Shown here are representative results from 2 different experiments from 2 donors.

Increased cytokine production from anti-PD1 Ab treated T cells co-cultured with Imprime PGG-treated M2 macrophages

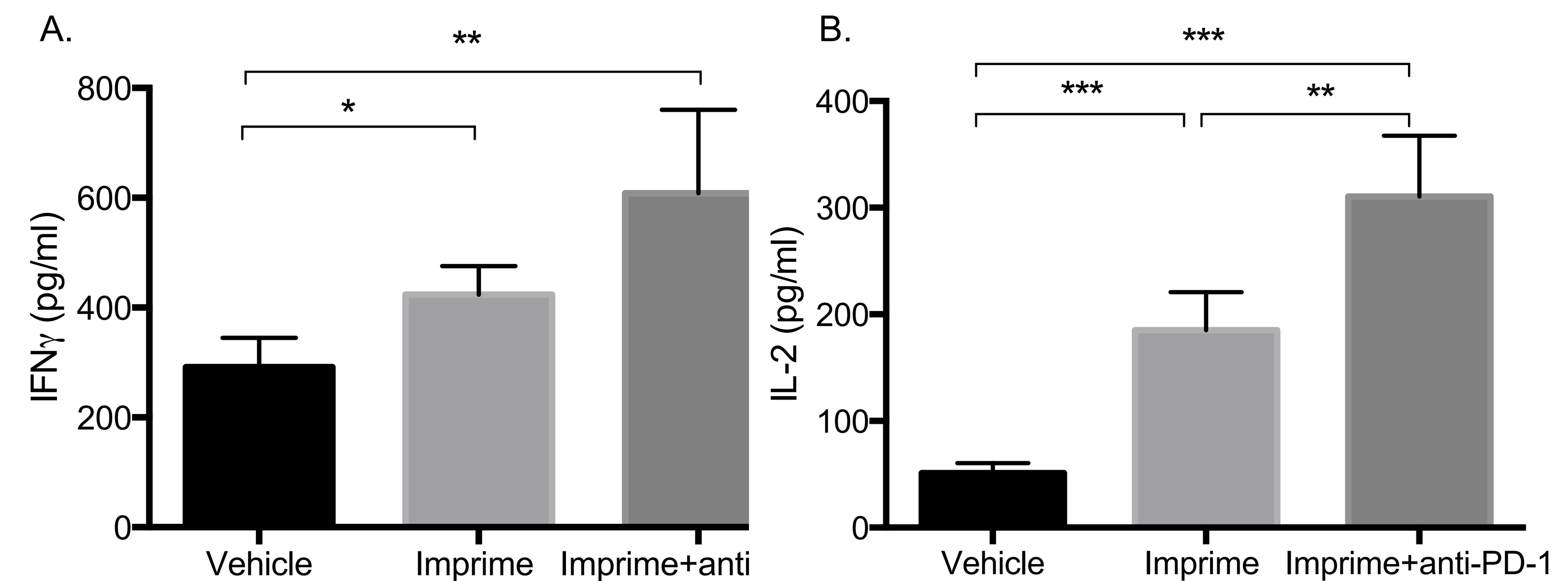

Figure 5. Synergistic effect of Imprime PGG and anti-PD-1 Mab on T-cell function. Monocytes treated with Imprime PGG or vehicle then differentiated and polarized into M2 macrophages as described in Figure 2. CFSE dilution assay in the presence of a titration of nivolumab as described in Figure 4. IFN γ (A) and IL-2 (B) were analyzed from the supernatants collected after 6 days with Milliplex Map Kit. (four replicates in each condition) Shown here are representative results from 2 different experiments from 3 donors.

Imprime PGG treatment increases efficacy of checkpoint inhibitor, anti-PD-1 Ab in a syngeneic mouse model

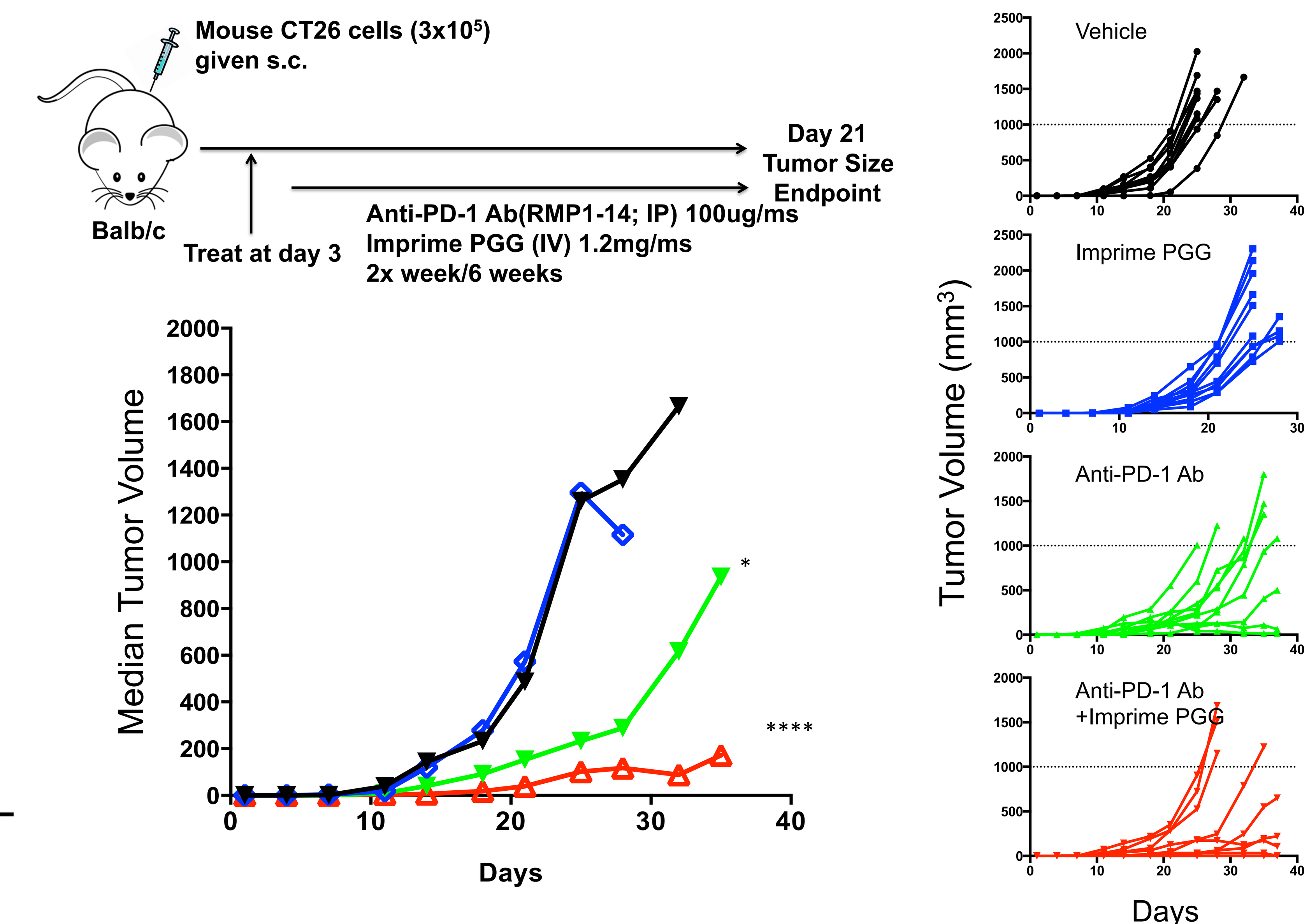

Figure 6. Imprime PGG in combination with an anti-PD-1 antibody reduces CT-26 tumor volume in a syngeneic mouse model. CT-26 colorectal tumor cells were injected subcutaneously into the flanks of 40 BALB/C mice. Three days later, mice were randomized and treated with vehicle, Imprime PGG, anti-PD-1 Ab or combination anti-PD-1 Ab and Imprime PGG. Remarkably, the median tumor volume in the group receiving Imprime PGG plus the anti-PD-1 antibody was significantly reduced compared to anti-PD-1 Ab alone. Overall, 60% of the mice in the anti-PD-1 Ab group had tumors that reached the pre-determined endpoint whereas combination yielded only 30% of mice reaching that same level. N=10 mice per group.

Summary

- M2 macrophages differentiated *in vitro* from monocytes of Imprime PGG-treated WB displayed a M1-like phenotype, with elevated levels of PD-L1 and CD86 expression and ability to increase proliferation of CD4 T cells. The co-culture supernatant yielded a greater increase in Th1 cytokine production.
- Imprime PGG treated MoDC exhibited an enhancement in PD-L1 and CD86 expression and increased the proliferation of CD4 and CD8 T cells. The co-culture yielded an increase in IFN-γ production
- Ex- vivo treatment of T cells with anti-PD-1 antibody, nivolumab showed increased proliferation in response to CD3/CD28 stimulation. Co-culture of these T cells with Imprime PGG-treated M2s or DCs drove further T cell expansion and increased production of several cytokines including IFN-γ and IL-2.
- Imprime PGG in combination with checkpoint inhibitor anti-PD-1 Ab increased efficacy against CD26 syngeneic mouse of colon carcinoma compared to anti-PD-1 Ab alone.

References

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