Abstract

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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Results

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

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

A. In Vitro Culture of Human Macrophages Whole blood (WB) (+Imprime or vehicle for 2 hours at 37°C) isolated PBMC by Ficol separation

B. CD163 CD68 PD-L1

C. CD1a cell expansion %

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 & CD68-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.

A. Human NSCLC cells (H1299) given s.c.

B. Nude mouse No 1, 8 cells Tumors mean size: ~100 mm² start treatment

C. Treatment Groups (x/week): 1. Vehicle 2. Imprime (1.2mg/kg) 3. Bevacizumab (5mg/kg IP) 4. Bevacizumab (5mg/kg IP)+Imprime (1.2mg/kg+IV)

Day 20 Animals Sacrificed Spleen, Tumors analyzed

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

Gated on CD11b+ cells

CD68 GMFI INOS GMFI Arginase 1 GMFI %TNFα after LPS

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

A. PD-L1 Expression

B. iNOS Expression

C. Arginase 1 Expression

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

TGFβ pmol

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. %TGIF was calculated by %TGIF= (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.