**Abstract**

Anti-angiogenic antibodies (Ab) such as bevacizumab (aVEGF) and ramucirumab (aVEGFR2) suppress tumor growth by disrupting the leaky, tortuous vasculature characteristic of growing tumors. Recent work now indicates that these Abs not only 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 (Imprime) is a β-glucan PAMP (Pathogen Associated Molecular Pattern) that has demonstrated promising efficacy in phase 2 randomized clinical trials with the bevacizumab (aVEGF)-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 (TNF-α, iNOS, 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.

**Results**

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

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

**Figure 3: Imprime promotes activation of human MDSCs**

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

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

**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.