Imprime (RMP1-14), an engineered yeast β-glucan, is currently in clinical development with tumor-targeting, antiproliferative and checkpoint inhibitor antibodies. The multi-stimulatory mechanism of action for this different therapeutic combination is that Imprime, a pathogen-associated molecular pattern (PAMP), primes innate immune effector functions to drive a coordinated anti-tumor immune response, mediated by a peritumoral macrophage network with known novel immunomodulatory properties. Studies have shown that Imprime, a yeast-derived pharmaceutical-grade soluble 1,3/1,6 β-glucan is being developed alone, indicating that these cells could play a role in T-cell activation. We therefore assessed the principal influence the TME (tumor microenvironment) eliciting a tumor microenvironment (TME) that activates T cell-dependent anti-tumor immunity.

Studies have shown that mature neutrophils and immature myeloid-derived suppressor cells (PMN-SDC) are increased in the tumor microenvironment. Increased levels of these cells show that neutrophils have a role in Imprime-mediated remodeling of the tumor microenvironment in Imprime-treated cancer patients (multiple Ph2 trials) are underway.

**Proposed Mechanism:** Imprime triggers a series of innate immune activation events that culminate in enhanced T-cell anti-tumor immunity.

**Background**
- Imprime (RMP1-14) is an engineered yeast β-glucan, being developed for the treatment of cancer in conjunction with tumor targeting and immunomodulatory antibodies.
- Imprime has shown promising results in multiple Phase 2 clinical trials in various solid tumors (NSCLC, HNSCC, CRC, and others), and as a single agent in Phase 1.
- In the current study, neutrophils were phenotypically activated in Imprime-treated cancer patients, as observed by increased CD11b and Gr-1 expression.
- Studies have shown that Imprime, when combined with anti-PD-1, results in increased CD86 expression on Ly6Ghi cells in the spleen and tumor compared to vehicle treatment. The increase of these cells in the spleen coincided with increased levels of chemokine production and mobilization into the bloodstream, indicating a role in Imprime-mediated remodeling of the tumor microenvironment.

**Results**
- **Figure 2:** Imprime mobilizes Ly6Ghi neutrophils into the blood and secondary lymphoid organs. (A) Systemic administration of Imprime induces chemokine production and mobilizes Ly6Ghi cells in tumor-free C57BL/6 mice in vivo. (B) Bevacizumab (5 mg/kg IP) + Imprime (1.2 mg/mg IV) in combination with anti-PD-1 was administered to H1299 xenograft mouse model. No T, B cells were observed in any treatment group. Cytokine by multiplex ELISA showed significantly less suppression to CD3/CD28-mediated proliferation of CD3+ splenocytes. Collectively, these data show that neutrophils have a role in Imprime-mediated remodeling of the tumor microenvironment, driving a stronger immune response in vivo.

- **Figure 4:** Imprime stimulates lactoferrin and suppresses pro-inflammatory cytokines. (C) PD-1 blockade reduces tumor microenvironment (TME) inflammation. (D) Imprime (1.2 mg/mg IV) and Bevacizumab was administered to H1299 xenograft mouse model. No T, B cells were observed in any treatment group. Cytokine by multiplex ELISA showed significantly less suppression to CD3/CD28-mediated proliferation of CD3+ splenocytes. Collectively, these data show that neutrophils have a role in Imprime-mediated remodeling of the tumor microenvironment, driving a stronger immune response in vivo.

**Summary**
- Systemic treatment of Imprime results in the phenotypic activation and enhancement of functions of Ly6Ghi cells.
- Ly6Ghi cells could potentially have a role in Imprime-mediated remodeling of the immunosuppressive TME, driving a stronger immune response in vivo.
- Systemic treatment of Imprime results in expansion of neutrophils as measured in the peripheral blood of healthy volunteers. Evaluation of peripheral blood neutrophils and those infiltrating the tumor microenvironment in Imprime-treated cancer patients (multiple Ph2 trials) are underway.

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