Imprime PGG, a soluble yeast β-glucan, primes innate immune effector cells to recognize and eradicate tumor cells

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Abstract

Imprime PGG (Imprime), a soluble yeast β-glucan, is being developed as a novel cancer immunotherapy in conjunction with anti-tumor antibodies in several cancers. In clinical studies, including randomized Phase 2 clinical trials in the 1st-line treatment of stage IV non-small cell lung cancer with bevacizumab, Imprime treatment has shown promising efficacy in both objective tumor response rates and survival. Mechanistic studies in humans have now demonstrated that Imprime, a fungal pathogen associated molecular pattern (PAMP), forms an immune complex with endogenic anti-β-glucan antibodies (ABG), which is then opsonized by complement. This immune complex binds complement receptor 3 (CR3) and FcγRIII on innate immune cells (macrophages, monocytes and neutrophils). In numerous syngeneic and xenograft mouse tumor models, Imprime treatment in combination with an anti-tumor antibody reduced tumor growth and prolonged survival beyond that observed with antibody alone. We sought to understand better the anti-tumor effect elicited by Imprime binding to these innate immune effector cells by exploring both phenotypic and functional changes driven by Imprime binding to neutrophils, monocytes and macrophages.

Neutrophils were examined in human whole blood treated with Imprime. Neutrophile treatment elicited an activated phenotype as demonstrated by downregulation of CR3 (CD11b) and CR4 (CD33), along with the upregulation of CR1 and CR3, and enhanced ROS production. These surface changes coincided with functional changes as well. Neutrophils from Imprime-treated whole blood showed a profound increase in the generation of reactive oxygen species (ROS) when stimulated with phorbol myristate acetate (PMA). Similarly, Imprime-treated neutrophils exhibited further enhanced ROS production when co-cultured specifically with B cell lymphoma cells (Raji) that had been coated with the anti-C020 antibody rituximab. ROS were not generated by vehicle-treated neutrophils nor when Imprime-treated neutrophils were co-cultured with Raji B cells that had not been coated with antibody. These data support the notion that Imprime specifically "primes" neutrophils enhancing the recognition of, and responsiveness to, antibody-coated tumor cells.

Monocytes showed similar "priming" effects post Imprime treatment, including down- modulation of CR3 (CD11b), upregulation of CR1 and CR3, and enhanced ROS production in response to PMA co-treatment. Differentiation of monocytes to macrophages, post Imprime treatment of human whole blood, enabled enhanced antibody-dependent cellular phagocytosis (ADCP) of multiple B cell lymphoma cell lines decorated with the anti-CD20 antibodies rituximab, daratumumab and alemtuzumab.

Collectively, these data highlight the impact of Imprime on key cell lineages of the innate immune system, priming neutrophils, monocytes and macrophages to better recognize and exert cytotoxic effector functions in response to antibody-coated tumor cells.

Background

- Imprime is a PAMP capable of initiating a robust response against tumor cells by utilizing immune functions normally used against bacterial and fungal pathogens
- Imprime has been administered to >400 humans intravenously with a strong safety profile
- We have previously shown that Imprime can bind to innate immune effector cells, activate and enhance DC antigen presentation, and generate greater numbers of functional T cells
- A screening assay has been developed to assess the ability of neutrophils from individual donors to bind to Imprime. Based on these data, donors are classified as high binders or low binders.
- Here we look closer at how Imprime treatment (in both low and high binders) primes the innate immune system to directly respond to tumors

Figure 1. A general structure of yeast-derived Imprime PGG

Figure 2. Imprime primes Neutrophils via the modulation of CR1, CR3, CD88, and CD62L
Whole Blood (WB) from healthy donors (both low and high binders) were treated with Imprime (10µg/ml) or vehicle control for 30 min. Neutrophils were then analyzed for modulation in surface receptor expression. The impact of Imprime treatment is represented as percent change versus vehicle treatment for both high and low binders. *p<0.05, **p<0.01

Figure 3. Imprime treated Neutrophils produce more reactive oxygen species (ROS) in response to PMA
WB was treated with vehicle (open bar) or Imprime (black bar) for 2hrs. PBMCs obtained and monocytes subsequently isolated. Monocytes were then cultured in X-Vivo10 + 10% human serum for 6 days with 50ng/ml M-CSF and with 10ng/ml of IL-10 for the last 2hrs. Raji cells were labeled with cell trace (CT) violet and then incubated with 1ug/ml rituximab at 4°C for 20 minutes. Macrophages and tumors were mixed 1:1 and incubated at 37°C for 4 hours and Macrophages were labeled with CD11b/ F, phosphofascin was calculated as (CD11b/CT violet) (CD11b/CT violet + CT violet) X100. Data are representative of 3 high and 3 low binders, healthy volunteers. ***p<0.001

Figure 4. Imprime treated Neutrophils produce more ROS in response to antibody coated tumor cells
WB from (A) low binders or (B) high binders were treated with vehicle or Imprime and neutrophils were isolated by negative selection. Cells were then mixed with or without Phorbol Myristate Acetate (PMA, 40ng/ml) in the presence of tumor (50µM). Cells monitored for production of reactive oxygen species (ROS) measured by relative light units (RLU). *p<0.05, **p<0.01

Figure 5. Imprime primes Monocytes via the modulation of CR1, CR3, and CD62L
WB from healthy donors (both low and high binders) were treated with Imprime (10µg/ml) or vehicle control for 30 min. Monocytes were then analyzed for the modulation in surface receptor expression comparing the Imprime treated samples and vehicle.

Figure 6. Imprime enhances ADCP of antibody coated tumor cells
WB was treated with vehicle (open bar) or Imprime (black bar) for 2hrs. PBMCs obtained and monocytes subsequently isolated. Monocytes were then cultured in X-Vivo10 + 10% human serum for 6 days with 50ng/ml M-CSF and with 10ng/ml of IL-10 for the last 2hrs. Raji cells were labeled with cell trace (CT) violet and then incubated with 1ug/ml rituximab at 4°C for 20 minutes. Macrophages and tumors were mixed 1:1 and incubated at 37°C for 4 hours and Macrophages were labeled with CD11b/ F, phosphofascin was calculated as (CD11b/CT violet) (CD11b/CT violet + CT violet) X100. Data are representative of 3 high and 3 low binders, healthy volunteers. ***p<0.001

Summary

1. Imprime can prime innate immune cells by:
   - Modulating CR1, CR3, CD88, and CD62L
   - Enhancing ROS production by neutrophils in response to PMA and antibody coated tumor cells
   - Enhancing ADCP by macrophages of antibody coated tumors

2. Imprime mediated changes in surface expression and function are evident in "high binders" but not in "low binders".

3. Parameters of binding may represent a patient selection biomarker delineating likelihood of responsiveness to Imprime-based therapy.