Abstract

Imprime PGG binds to neutrophils through complement, Fc, and Dectin-1 receptors, priming these cells for enhanced ROS production and tumor cell cytotoxicity

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Background

- Imprime is a soluble 1,3/1,6-glucan (Figure 1)
- Imprime has shown promise in randomized Phase 2 clinical trials in combination with monoclonal antibodies
- As a PAMP, Imprime is recognized by and binds to cells of the innate immune system enabling the innate immune system to orchestrate a coordinated anti-cancer immune attack
- Herein, we explore the effects of Imprime on neutrophil function

Figure 1. A general structure of yeast-derived Imprime

Imprime PGG binds to neutrophils through complement, Fc, and Dectin-1 receptors, priming these cells for enhanced ROS production and tumor cell cytotoxicity

Figure 2. Imprime treatment enhances neutrophil survival

Whole blood (WB) was treated with vehicle or 25µg/ml of Imprime. WB was incubated 2hrs and neutrophils harvested and tested for viability.

Figure 3. Imprime treatment primes neutrophils to produce ROS in response to monoclonal antibody decorated tumor cells

WB was treated with vehicle or 25µg/ml of Imprime. WB was incubated 2hrs and neutrophils harvested and tested for ROS production.

Figure 4. Imprime ROS priming is mediated by enhanced Fc receptor function but not surface upregulation

Imprime was mixed with 25µg/ml of Rituximab and incubated with or without Imprime for 2hrs. Neutrophils were harvested and tested for ROS production.

Figure 5. Imprime priming of neutrophils leads to increased tumor cell cytotoxicity

Neutrophils were treated with or without Imprime for 2hrs. Neutrophils were then mixed with Raji cells and incubated for 2hrs. Cytotoxicity was assessed with the LDH assay.

Figure 6. Imprime binds with neutrophils via complement receptors and Fc receptors

WB incubated with vehicle or Imprime for 30mins in the presence of (A) anti-CR1 (1B4 at 20µg/ml), (B) CR3 (IB4 at 10µg/ml), (C) CR4 (IB4 at 5µg/ml), or (D) anti-Fc receptor (clone 2G11, 3G8 at 25µg/ml, CD32, A10 at 25µg/ml) blocking antibodies. Imprime binding quantified by using a mouse IgM anti-β-glycan antibody (BDPharm) and an anti-mouse IgM-FITC secondary antibody.

Figure 7. Imprime binds with neutrophils via Dectin-1

(A) WB was incubated with anti-Dectin-1 antibody (clone 62E2, previously shown to block Dectin-1 binding and neutrophil-mediated, antibody-stimulated tumor cell killing) and % cytotoxicity was measured in the presence or absence of Imprime.

Summary

Imprime binds to and elicits functional alterations in neutrophils:
1. Imprime enhances neutrophil survival;
2. Imprime elicits the generation of Reactive Oxygen Species (ROS) from neutrophils specifically in response to antibody-coated tumor cells;
3. Imprime enables neutrophil-mediated, antibody-stimulated tumor cell killing;
4. Imprime binds directly to neutrophils through complement receptors, Fc receptors and Dectin-1.

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