Abstract
A160

CRI-CIMT-EATI-AACR - The Inaugural International Cancer Immunotherapy Conference: Translating Science into Survival
September 16, 2015

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

Steven M Leonardo, Ross B Fulton, Lindsay R Wurst, Adria Bykowski Jonas, Xiaohong Qiu, Anissa SH Chan, Keith B Gorden and Jeremy R Graff . Biothera, Inc. Eagan, MN
sleonardo@biothera.com

Abstract

Imprime PGG (Imprime), a soluble yeast 1,3/1,6 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. In numerous syngeneic and xenogeneic mouse tumor models, Imprime treatment in combination with an anti-tumor antibody reduced tumor growth and prolonged survival beyond that observed with antibody alone. Mechanistic studies have now demonstrated that, with endogenous anti-β-glucan antibodies (ABA), Imprime forms an immune complex. This Imprime-ABA complex then activates the classical complement pathway and is subsequently opsonized by iC3b. This immune complex then binds to and primes innate immune cells, including macrophages, monocytes and neutrophils, triggering a coordinated immune attack against antibody-targeted cancer cells. In this study, we sought to explore more fully the functional consequences of Imprime-ABA immune complex binding to, and priming of, neutrophils.

Neutrophils are the first line of defense against fungal infections. Neutrophils detect fungal infections through recognition of β-glucans on the surface of yeast and fungi. These β-glucans serve as fungal pathogen associated molecular patterns (PAMPs) that are efficiently and effectively recognized by receptors on innate immune cells, initiating a coordinated immune response to infection. Here we demonstrate that Imprime, in individuals with high ABA levels, is recognized as a PAMP, directly interacting with neutrophils via multiple receptors, priming these cells and ultimately leading to enhanced effector responses including reactive oxygen species (ROS) generation and tumor cell killing. Our data now show that Imprime elicits enhanced neutrophil survival as measured by cell viability dye exclusion using flow cytometry. Imprime treatment also enhances the responsiveness of neutrophils to Fc receptor activation. As measured by luminol-based read-outs, ROS production in response to anti-Fc receptor antibody-coated beads is profoundly enhanced by Imprime treatment. Moreover, Imprime treated neutrophils show a remarkable surge in ROS production compared to vehicle–treated neutrophils when exposed to B cell lymphoma cells decorated with anti-CD20 monoclonal antibodies (i.e. rituximab). These Imprime-treated neutrophils also exhibit a robust increase in cytotoxicity against these B cell lymphoma cells coated with rituximab using *in vitro* Calcein AM dye release assays. We next sought to delineate which receptors might be most critical for Imprime binding and priming of neutrophils. As demonstrated by antibody blocking studies, the Imprime-ABA complex can bind to both complement receptors (notably CR3) and Fc receptors. Imprime can also block binding of a dectin-1 agonizing antibody to dectin-1 and can bind directly to HL-60 and HEK cells lines engineered to overexpress dectin-1 by retroviral transduction. Collectively, these data show for the first time that Imprime directly primes neutrophils to recognize and kill antibody-decorated tumor cells and interacts with the Fc, complement and dectin-1 receptors on the surface of neutrophils.

Background

- Imprime is a soluble 1,3/1,6 β-glucan (Figure 1)
- Imprime has shown promising efficacy 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

BIOTHERA
the immune health company

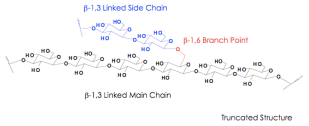

Figure 1. A general structure of yeast-derived Imprime

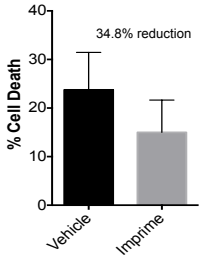

Figure 2. Imprime treatment enhances neutrophil survival
Whole blood (WB) was treated with vehicle or 25μg/ml of Imprime. WB was incubated 2hrs and neutrophils isolated by negative selection (STEMCELL cat#19257). Cells cultured overnight and viability examined the following day using viability dye staining (eBioscience cat# 65-0864)

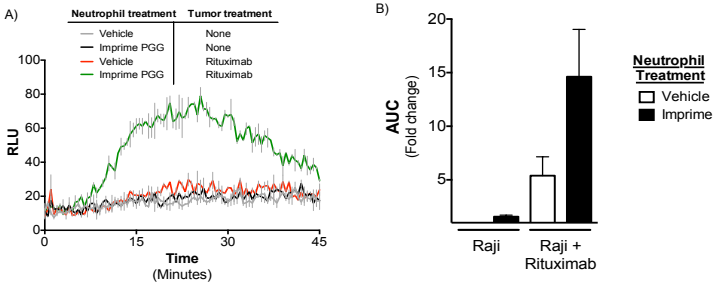

Figure 3. Imprime treatment primes neutrophils to produce ROS in response to monoclonal antibody decorated tumor cells
WB was treated with vehicle or 25μg/ml of Imprime. WB was incubated 2hrs and neutrophils isolated by negative selection (STEMCELL cat#19257). Cells were then mixed with Raji cells with or without Rituximab treatment (1μg/ml) at a 25:1 neutrophil:Raji ratio in the presence of luminol (50μM). A) Cells monitored for production of reactive oxygen species (ROS) measured by relative light units (RLU). B) Graphical representation of n=6 individuals plotting the area under the curve (AUC) fold change.

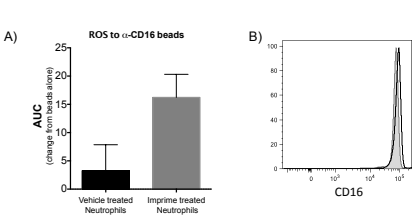

Figure 4. Imprime ROS priming is mediated by enhanced Fc receptor function but not surface upregulation
Neutrophils were treated as described in Figure 3 and incubated with beads coated with the anti-CD16 antibody (3G8) in the presence of luminol buffer, RLUs measured and AUC calculated. A) Graphical depiction in the increase of ROS from uncoated beads. Data indicate Imprime priming of CD16 function. B) Flow cytometry showing the level of CD16 expression on vehicle (gray) or Imprime (black) treated neutrophils

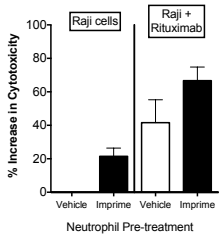

Figure 5. Imprime priming of neutrophils leads to increased tumor cell cytotoxicity
Neutrophils were treated as described in Figure 3 and incubated with Raji cells that had been labeled with Calcein AM dye with or with out Rituximab at a 50:1 effector to target ratio. Cells incubated for 3hrs and then the co-culture was stained with a live/dead dye and analyzed by FACS to determine cytotoxicity. Raji cells were identified as Calcein+ and % cytotoxicity was calculated by live/dead dye staining. Data show the % increase in cytotoxicity over vehicle treated neutrophils co-cultured with Raji without RItuximab.

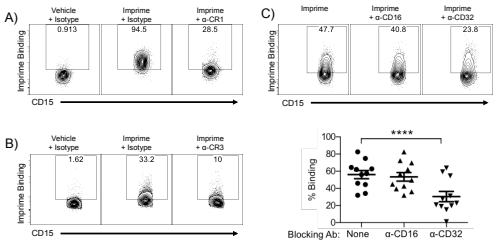

Figure 6. Imprime binds with neutrophils via complement receptors and Fc receptors
WB incubated with vehicle or Imprime for 30mins in the presence of (A) anti-CR1 (1B4 at 20μg/ml), (B) anti-CR3 (LM2/1, VIM12, IB4 at 10, 10, 5μg/ml), or (C) anti-Fcγ receptor (CD16, 3G8 at 20μg/ml, CD32, AT10 at 20μg/ml) blocking antibodies. Imprime binding quantified by using a mouse IgM anti-β-glucan antibody (BFDIV) and an anti-mouse IgM-FITC secondary antibody.

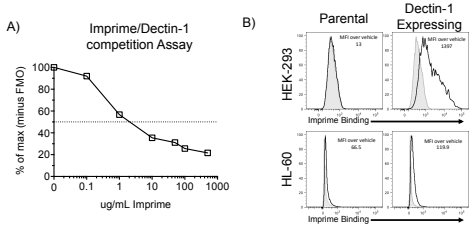

Figure 7. Imprime binds with neutrophils via Dectin-1
(A) WB was incubated with anti-Dectin-1 antibody (clone GE2, previously shown to block β-glucan binding) alone or with increasing amounts of Imprime. Median fluorescence intensity (MFI) was determined for Dectin-1 staining and graphed as the %max relative to the sample receiving Dectin-1 antibody alone. (B) HEK-293 and HL-60 cells lines were transduced to express human Dectin-1a and subsequently analyzed for their ability to bind Imprime (binding done as described in Figure 6)

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.