Poster # A015

Imprime PGG treatment enhances antibody-dependent cellular phagocytosis (ADCP) of tumor cells by monocyte-derived macrophages

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Abstract

Imprime PGG (Imprime), a soluble, yeast β-1,3/1,6 glucan, is currently in clinical development for the treatment of cancer in conjunction with several therapeutic monoclonal antibodies (MAbs). Some of the MAbs that are being evaluated in combination with Imprime include, rituximab in an investigator initiated study for chronic lymphocytic leukemia, bevacizumab in a phase 2 study for non small cell lung cancer, and erbitux in a phase 3 study for metastatic colorectal cancer. Previous studies have demonstrated that Imprime, a pathogen- associated molecular pattern (PAMP), forms an immune complex with endogenous anti-β-glucan antibodies, then binds and primes innate immune cells (including macrophages, monocytes and neutrophils) to kill antibody-targeted cancer cells via a complement-dependent cellular mechanism (CDCC). The objective of this study was to evaluate the effect of Imprime on other innate immune effector mechanisms that are employed by the tumor-targeting MAbs, including antibody dependent cellular phagocytosis (ADCP) - a function largely attributed to macrophages. We therefore sought to investigate whether Imprime PGG treatment might enhance ADCP.
Monocytes enriched from Imprime PGG- or vehicle-treated whole blood were cultured in media containing the appropriate cytokines for differentiation of different macrophage subtypes: GM-CSF for M1 macrophages; M-CSF for M2 macrophages; M-CSF plus IL-4 for M2a macrophages; and M-CSF plus IL-10 for M2c macrophages. ADCP was evaluated by using specific macrophage subtypes with various tumor cell lines, including Raji (Burkitt's lymphoma) and Z138 (Mantle cell lymphoma) in the presence of rituximab, ofatumumab, and obinutuzumab (anti-CD20 MAbs), or SKBR3 (Her2 positive breast cancer) and MDA-MB-231 (Her2 negative breast cancer) in the presence of trastuzumab (anti-Her2). A flow cytometric phagocytosis assay was performed by incubating macrophages and tumor cell lines that are labeled with different flourochromes, and subsequently quantitating the phagocytosed cells that are rendered double positive. The results demonstrated that among all the different sub-types of macrophages, M1 and M2c macrophages have higher phagocytic capacity. In comparison to the vehicle control, Imprime treatment enhanced the ability of M2c macrophages to phagocytose both Raji and Z138 tumor cells in the presence of each of the anti-CD20 antibodies - rituximab, ofatumumab, and obinutuzumab. For the breast cancer cell lines, Imprime treatment enhanced the ability of M1 macrophages to phagocytose trastuzumab-decorated SKBR3 cells. Mechanistic investigation demonstrated that increased surface expression of Fc receptors, especially CD16 and CD32, on Imprime-treated macrophages may drive increased tumor cell phagocytosis. Further, preliminary data have suggested that CD47-SIRPa and FcgRIIb may regulate Imprime-mediated ADCP. Together, these data indicate that Imprime can potentiate the anti-tumor activity of tumor-targeting antibodies by enhancing macrophage-mediated ADCP.

Background/Objective

- Imprime has demonstrated synergistic anti-tumor activity in multiple syngeneic and xenografted tumor models with multiple anti-tumor mAbs.[1-5]
- In the clinic, in combination with antibody therapies, Imprime has shown promising results in early studies in colorectal cancer (CRC), non-small cell lung cancer (NSCLC), and chronic lymphocytic leukemia (CLL) with additional studies ongoing.[6-12]
- β-glucans are conserved microbial structures found in the cell wall of unicellular and multicellular pathogens. These are considered pathogen-associated molecular patterns (PAMPs) and are efficiently recognized by the innate immune system.

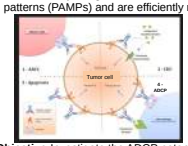

Figure 1. Mechanisms of MAb tumor killing
ADCC – Antibody-dependent cellular cytotoxicity
CDC – Complement-dependent cytotoxicity
Apoptosis
ADCP – Antibody-dependent cellular phagocytosis

Objective-Investigate the ADCP potential of various macrophage subtypes derived from Imprime-bound monocytes

Macrophage subtypes:
- M2c – Immunosuppressive, yet retains ability to phagocytize tumor cells
- M1 – Known to be immunostimulatory

Mabs: Anti-CD20 - Rituximab (Ritux; chimeric IgG1), Ofatumumab (Ofat; humanized IgG1), and Obinutuzumab (Obin; Fc modified humanized IgG1), Anti-Her2/neu – Trastuzumab (Tras; humanized IgG1)

Tumor cell lines:
- Raji (Burkitts lymphoma) & Z138 (Mantle cell lymphoma), SKBR3 (Her2^{hi} breast cancer line) and MDA-MB-231 (Her2^{lo})

Mechanism utilized by macrophages to regulate ADCP
- Expression of FcγR on macrophages
- Role of CD47 – CD172 ("Don't eat me" signal for macrophages)

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Results

Characteristics of *In Vitro* Cultured M1 and M2c macrophages

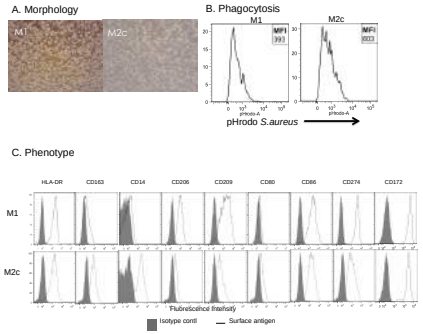


Figure 2. Macrophages can be matured *in vitro* into M1 and M2c like and M2c are more phagocytic. Macrophages were differentiated, as described in Methods, to be M1 and M2c like and were **A.** Observed for morphology **B.** Combined with pHrodo *S. aureus* to investigate phagocytosis.15 minutes at 37°C. M2a cells were also assayed but demonstrated lower phagocytosis than M2c and were not tested further. **C.** Collected and stained with directly conjugated differentiation/ activation MAbs.

Methods

***In Vitro* Culture of Human Macrophages**

Whole blood (WB) (Untreated or treated with Imprime for 2hr @ 37°C)
↓
Isolated peripheral blood mononuclear cells (PBMC) by Ficoll separation
↓
Enriched monocytes by negative selection
↓
Monocytes were cultured in X-Vivo10 medium for 6 days:
M1- X-Vivo10 medium with 5% human serum & 100 ng/ml rhGM-CSF and activated with 100ng/ml LPS and 20ng/ml IFN-gamma for additional 24hr (M1-vehicle/M1-Imprime)
M2c- X-vivo10 with 10% serum & 50ng/ml M-CSF with 10 ng/ml IL-10 for the last 72 hours (M2c-vehicle/M2c-Imprime)
↓
Morphology/Phenotype
Phagocytic capacity
ADCP withtumor cells with therapeutic MAb in absence of serum

ADCP Method

Effector: Macrophages

Target: Raji, Z138, SK-BR-3, MDA-MB-231 cell trace violet labeled and treated with Mab at 4°C for 20 min
- Ritux (1μg/ml)
- Ofat (0.01μg/ml)
- Obin (0.001μg/ml)
- Tras (1μg/ml)

↓
E:T 1:1 ratio at 37°C for 1 or 4 hr
↓
Stain with CD11b; fix and quantify with counting beads
↓

$$\%\text{Phagocytosis} = \frac{(\text{CD11b}^+\ \text{CellTrace Vio}^+\ \text{cells})}{(\text{CD11b}^+\ \text{CellTrace Vio}^+\ \text{cells} + \text{CellTrace Vio}^+\ \text{tumor cells})} \times 100$$

Enhanced phagocytosis of CD20 MAb-bound tumor cells by M2c-Imprime

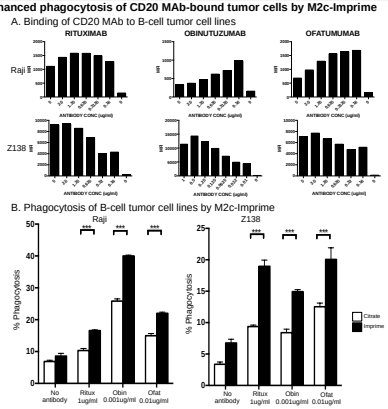


Figure 3. M2c-Imprime macrophages exhibit higher phagocytosis of B cell tumor cell lines. A. Ritux, Obin and Ofat were serially diluted and incubated with Raji or Z138 tumor cells for 20 mins at 4°C and were detected with a directly conjugated α-human IgG Ab. **B.** M2c macrophages were assayed for ADCP. Raji cells and Z138 cells were labeled with cell trace violet and then incubated with 1 μg/ml Ritux, 0.001μg/ml Obin or 0.01μg/ml Ofat at 4°C for 20 minutes. Macrophages and tumors were mixed 1:1 and incubated at 37°C for 4 hrs. ***p<0.001, n=3.

Enhanced phagocytosis of Her2 MAb-bound solid tumor cells by M1-Imprime

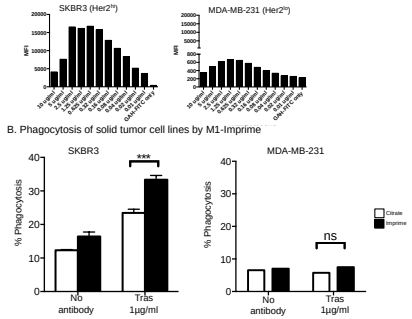


Figure 4. M1-Imprime exhibit higher phagocytosis of Tras-bound breast cancer cells. A . Tras was serially diluted and incubated with SK-BR-3 and MDA-MB-231 tumor cells for 20 mins at 4°C and detected using a directly conjugated α-human IgG antibody. **B.** Macrophages were assayed for ADCP. SKBR3 cells and MDA-MB-231 cells were labeled with cell trace violet and then incubated with 1μg/ml Tras at 4°C for 20 mins. Macrophages and tumors were mixed 1:1 and incubated at 37°C for 1 hour, then measured for phagocytosis as described in Figure 3. ***p<0.001 n=3.

Potential Mechanism for Enhanced Phagocytosis

Increased FcγR expression on M1- and M2c-Imprime

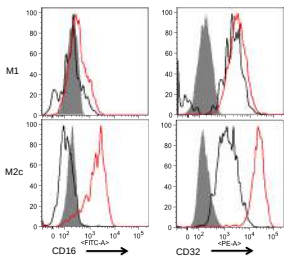
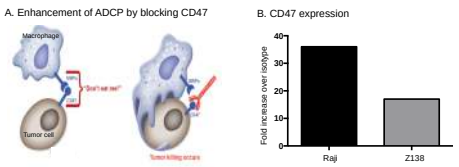


Figure 5. M1and M2c macrophages were prepared as described, incubated with directly conjugated antibodies to FcγR, CD16 and CD32, and expression levels were then determined by flow cytometry.

Potential Mechanism for Enhanced Phagocytosis-blocking CD47



C. Phagocytosis of liquid tumors is increased with CD47 blocking

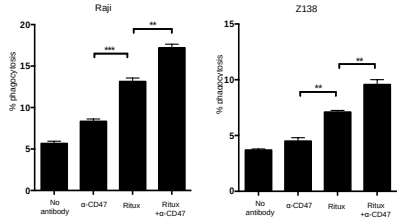


Figure 6. Raji and Z138 cells express CD47 and blocking with an anti-CD47 MAb increases the phagocytosis of Ritux bound cells by M2c. A. CD47 on the surface of tumor cell interacts with CD172 (SIRPα) on the surface of macrophage, signaling "Don't eat me". α-CD47 MAb blocks this signal and tumor cell allows for phagocytosis. **B.** Raji and Z138 tumor cells were stained with α-CD47 antibody or isotype at 4°C for 20 minutes. **C.** Raji and Z138 incubated with and without 20μg/ml α-CD47 blocking mAb at 4°C for 10 minutes prior to the measurement of M2c-mediated phagocytosis as described in Figure 3. **p<0.01 and ***p<0.001.

Summary

- Monocytes can be differentiated to M1 and M2c macrophages *in vitro*.
- In accordance with the literature, M2c macrophages are more phagocytic than M1.
- Imprime treatment enhances the phagocytic activity of M2c macrophages against antibody-decorated B Cell Lymphomas.
- Imprime treatment enhances M1 macrophage mediated phagocytosis of trastuzumab-decorated HER2+ Breast cancer cells.
- FcγR are upregulated on both M1- and M2c-Imprime macrophages compared with the vehicles
- M2c and M1 express CD172 and Raji/Z138 express CD47. Blocking of the CD172/CD47 ("Don't-eat -me") interaction leads to increased phagocytosis of MAb coated tumors.

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