SITC 2017
P74

Increasing the levels of anti-beta glucan antibodies by administration of intravenous immunoglobulin (IVIG) induces immunopharmacodynamic (IPD) responses of a novel immunotherapeutic Imprime PGG

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Abstract

Background and Objective: There is a critical need for rational combination immunotherapies that have mechanism-driven predictive biomarkers. Imprime PGG (Imprime), is a novel, intravenously (i.v.) administered innate immunomodulator currently in clinical development as a combination therapy with checkpoint inhibitors in biomarker-selected patients. Imprime is a soluble β-glucan PAMP that requires immune complex formation with serum anti-beta glucan antibodies (ABA) for its functionality. *Ex vivo* human studies, a healthy volunteer phase I trial and retrospective analyses of clinical studies have demonstrated that IPD changes and clinical responses mediated by Imprime correlated with serum ABA levels. *Ex vivo* studies have also shown that innate immune functionality in subjects with lower ABA values can be restored by supplementation with purified ABA or ABA-containing IVIG. Herein we present a case study of a cancer patient with low ABA levels demonstrating enhanced Imprime-induced PD responses post IVIG administration.

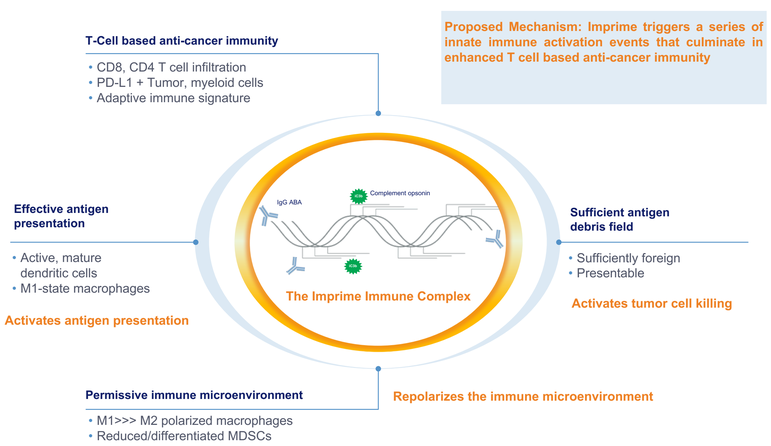
Methods: A 54-year old female with metastatic colorectal adeno-carcinoma was offered immunotherapy with bevacizumab, cetuximab and Imprime as part of a compassionate use study after she could not tolerate first line therapy with FOLFOX and bevacizumab. The patient was dosed in 4 week cycles for 12 cycles. Imprime and cetuximab were administered i.v. weekly. Bevacizumab was administered every 4 weeks from cycle 2 through 8. Evaluation of serum ABA levels from cycles 1-6 confirmed low values in this patient. To boost ABA levels, IVIG was added to dosing beginning at cycle 6. ABA, complement, and cytokine levels in serum (ELISA and Luminex), Imprime binding and immune cell phenotyping (flow cytometry) were measured prior to and within 30 minutes after Imprime administration.

Results: Compared to the weeks prior to adding IVIG to the dosing regimen, IVIG infusion resulted in increased serum ABA levels at the End of Infusion (EOI), which then dropped to the baseline levels in the subsequent weeks. Concomitant with the ABA increase, serum C5a levels peaked at the EOI. Furthermore, serum chemokines such as IL-8 also increased at EOI with the most pronounced change observed during cycles 7 and 8. Increased ABA levels also correlated with significant Imprime binding on neutrophils and monocytes. Importantly, minimal PD changes were observed with Imprime dosing alone in the cycles prior to IVIG administration. Disease remained stable for 10 months.

Background

- Imprime PGG, a yeast-derived pharmaceutical-grade soluble 1,3/1,6 β-glucan is being developed for the treatment of cancer in conjunction with tumor targeting and immunomodulatory antibodies (Abs).
  - Imprime has shown promising results in multiple Phase 2 clinical trials in non-small cell lung cancer (NSCLC) and chronic lymphocytic leukemia (CLL) with additional studies ongoing.
- β-glucans are conserved microbial structures found in the cell wall of unicellular and multicellular pathogens. They are considered pathogen-associated molecular patterns (PAMPs) recognized by the pattern recognition receptors including Dectin-1 and Complement Receptor 3 (CR3). Imprime forms an immune complex with endogenous serum immunoglobulin IgG or IgM anti-beta-glucan antibodies (ABA) before being recognized by CR3 and FcgRIIA on innate immune cells.

Figure 1. Imprime (PAMP) binds to innate immune cells and triggers and coordinated Anti-Cancer Immune response.



All experiments funded by Biothera Pharmaceuticals, Inc.
No external funding was received to support the work.

Results

Healthy Human Volunteer Phase 1: Imprime-Induced IPD Effects Are Restricted to ABA+ Subjects

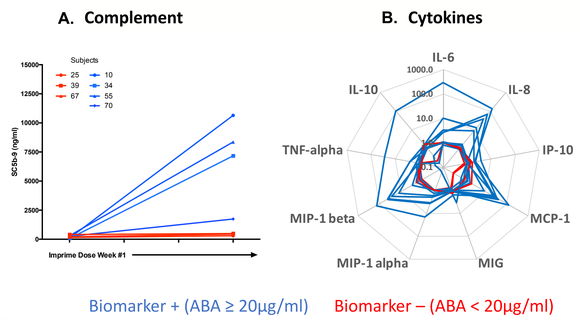


Figure 2. Healthy Human Phase I data:
Whole blood or serum was drawn from healthy volunteers at various time points before and after a single dose of Imprime infusion. ABA were measured in serum by Biothera-developed ELISA. **A.** Complement activity was measured by ELISA using the SC5b-9 Plus kits (Quidel). **B.** Cytokines and chemokines were measured in serum using Luminex XMAP technology. Fold over pre-dose values are plotted. Data from subjects who were treated with Imprime (4 mg/kg) and no pre-medications.(Trials data analysis ongoing.)

In the Clinic: High ABA Levels Correspond with Better OS in Imprime Treated Patients: Primus 3rd line CRC Trial

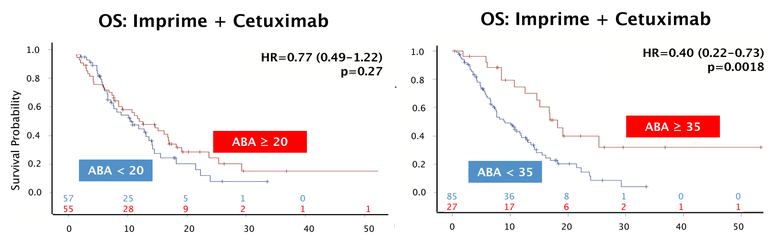


Figure 3. Overall response data from Primus: Retrospective analyses: Primus trial, 3rd line CRC cetuximab ± Imprime. Pre-treatment ABA levels were measured by ELISA. Sample "cutpoints" for ABA are noted at 20 and 35 ug/ml.

Ex vivo: IVIG addition rescues Imprime binding and function in low ABA subjects

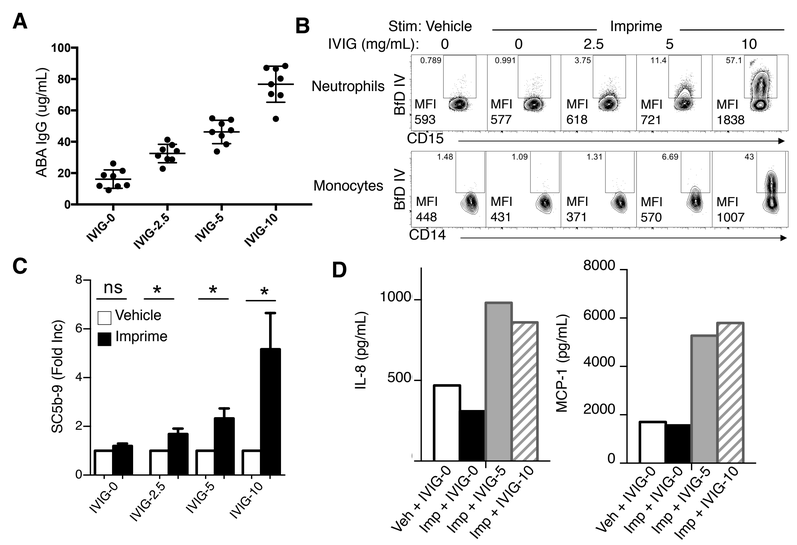


Figure 4. **A**. Plasma isolated from WB samples spiked with PBS (IVIG-0) or IVIG at final concentrations of 2.5 mg/mL (IVIG-2.5), 5 mg/mL (IVIG-5) and 10 mg/mL (IVIG-10) was analyzed for IgG ABA antibodies by ELISA. Data represent mean ± SEM of 8 subjects. WB from LB samples were treated with vehicle control or 10 µg/mL Imprime in the presence of the indicated IVIG concentrations and then assessed for, **B** Imprime binding to neutrophils and monocytes by flow cytometry, **C** induction of SC5b-9 by ELISA after 30 mins and **D** chemokine production by Luminex after 24 hrs. Results are representative of 3 independent experiments performed with different donors.

Treatment of a Low ABA Colorectal Cancer Patient with Imprime plus IVIG

Patient info:
- 54-year old female with colorectal adeno-carcinoma with lymph node metastases.
- 3 cycles of first line chemotherapy with FOLFOX and bevacizumab was not well tolerated, and therefore FOLFOX chemotherapy was stopped and bevacizumab was continued in different intervals for several months. Eventually, tumor started to grow rapidly in metastases in retroperitoneal lymph nodes causing persistent abdominal pain.
- Biothera supported an investigator-initiated study which provided treatment with cetuximab in combination with Imprime every week (1 cycle=4 weeks).
- She had improvement in pain and went off her pain medications. She had fatigue, acneiform rash, diarrhea, hypomagnesemia from her cetuximab therapy.
- Radiographically she appeared to have stable disease.
- After 6 cycles of Imprime, due to her low ABA status, she was co-administered IVIG to boost ABA IgG levels.
- She remained on treatment through cycle 12.

Treatment Schema

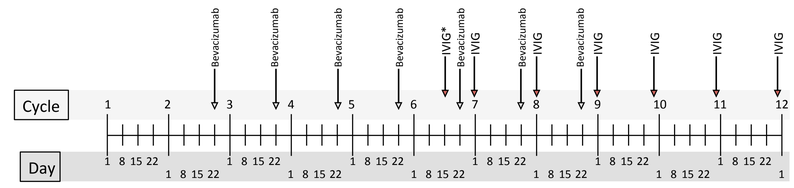


Figure 5. Imprime and Cetuximab administered on days 1, 8, 15 and 22 of each cycle.
***IVIG = 500 mg/kg dose of IVIG on day 15 of cycle 6. The remaining doses of IVIG were at 1000 mg/kg.**

Imprime plus IVIG rescues Imprime-driven IPD in a CRC Patient

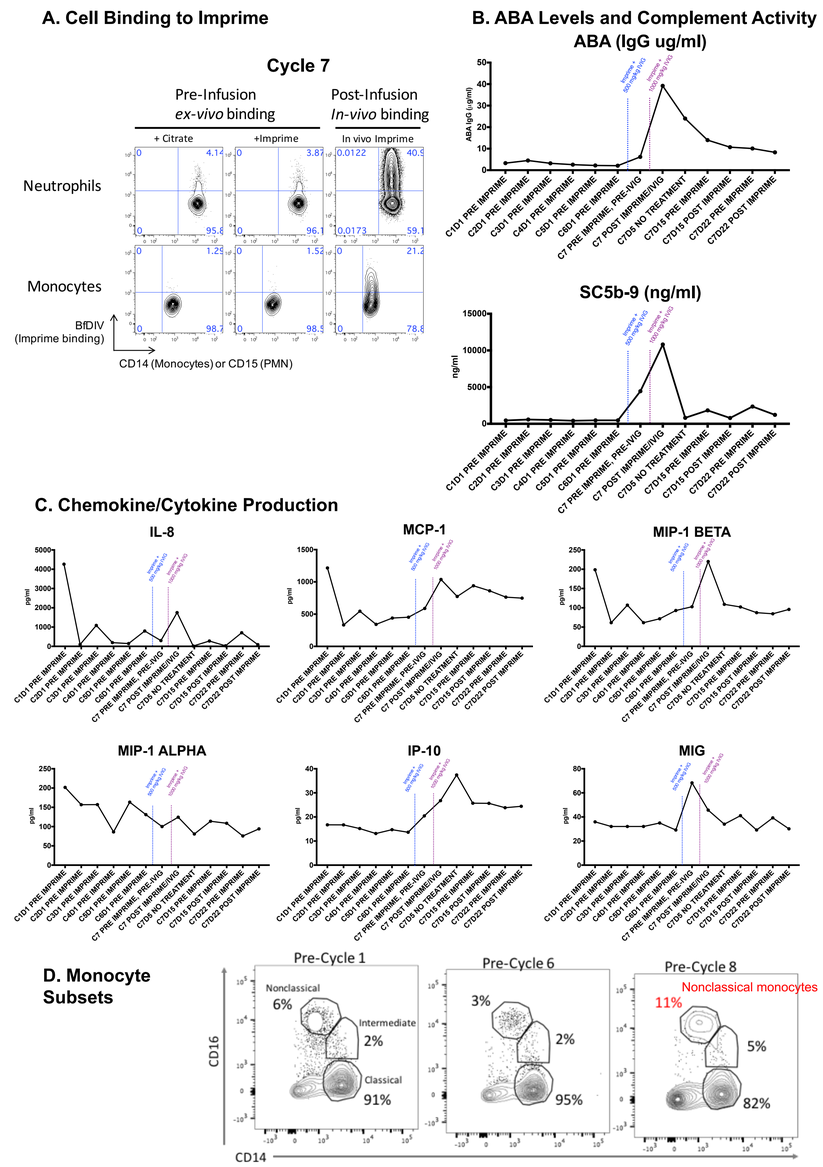


Figure 6. Analysis of *in vivo* soluble β-glucan treatment response after administration of Imprime PGG.
A. Assessment of *in vivo* Imprime binding to PMNs and monocytes. Pre- and post- Imprime PGG dose whole blood patient samples were stained with CD14, CD15 and anti-β glucan antibody BfDIV and analyzed by flow cytometry. *In vivo* binding was determined by comparison of the level of β-glucan binding in the post-Imprime PGG dose to the pre-dose sample on the same day of treatment. **B**. Patient serum samples were analyzed for ABA IgG antibody levels by ELISA, with values reported as ug/mL. Activation of the complement cascade was determined by assaying SC5b-9 levels in pre- and post dose serum samples. **C**. Chemokine/Cytokine production was measured in serum or plasma from blood collected at indicated time points using Luminex XMAP technology. **D**. PBMC isolated from indicated time points were stained with CD14 and CD16 to determine Monocyte subset percentages.

Clinical Response: Disease remained stable for 10 months

Conclusions

These human data provide the first evidence of rescue of Imprime-driven IPD responses in a cancer patient by supplementation of ABA, a crucial pre-requisite for the therapeutic activity of Imprime:
- Increased ABA upon IVIG administration
- Increased complement activity
- Enhanced cytokine/chemokine production
- Higher frequency of circulating nonclassical monocytes:
  - Enhanced ADCC potential
  - $CD86^{hi}HLA\text{-}DR^{hi}$

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