ENDOGENOUS ANTI-ß-GLUCAN ANTIBODIES, A POTENTIAL PREDICTIVE BIOMARKER FOR THE EFFICACY OF SOLUBLE YEASt ß-1,3/1,6 GLUCAN (IMPRIME PGG®) IMMUNOTHERAPY IN CANCER PATIENTS

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BACKGROUND

- Imprime PGG is a yeast-derived, soluble ß-glucan being developed for the treatment of cancer in conjunction with anti-tumor monoclonal antibodies (MAbs).
  - In syngeneic and xenogenic tumor models, Imprime PGG has demonstrated synergistic anti-tumor activity with multiple anti-tumor MAbs.[1, 2, 3, 4]
  - In the clinic, in combination with antibody therapies, Imprime PGG 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, including a Phase 3 study in CRC.[5, 6, 7, 8, 9]
- Imprime PGG mediates anti-tumor activity by directing innate immune cells to recognize and kill antibody-targeted tumor cells through a novel mechanism involving complement receptor 3 (CR3) (studies summarized in Table 1).
  - Anti-tumor activity *in vivo* is dependent on the presence of neutrophils, complement receptor 3 (CR3), and complement.[1, 2, 3]
  - Binding of Imprime PGG to human innate immune cells (neutrophils and monocytes) is also CR3 and complement dependent.[10]
    - The presence of natural anti-ß-glucan antibodies (ABA), either IgG or IgM, is an absolute pre-requisite for initiation of the classical pathway of complement activation and subsequent opsonization and binding of Imprime PGG to innate immune cells.[11]
    - ABA levels measured differ among individuals.[11]

Table 1. STUDIES ELUCIDATING THE MECHANISM OF ACTION OF IMPRIME PGG

Anti-Tumor Activity In Combination with MAbs (*In Vivo* Mouse Studies)	Imprime PGG Binding to Innate Immune Cells (*In Vitro* Human Studies)
Role of innate immune cells: • RMAS-MUC1 (T-cell lymphoma overexpressing mucin 1) tumor/anti-MUC1 Mab - anti-tumor activity demonstrated in wild-type mice - Loss of anti-tumor activity in neutrophil-deficient mice[1]	**Binding to innate immune cells :** • Binding to neutrophils and monocytes in whole blood - but only in a subset of individuals[10]
Role of CR3 receptor: • RMAS-MUC1tumor/anti-MUC1 MAb - Loss of anti-tumor activity in CR3-deficient mice[2]	**Role of complement receptors:** • Inhibition of binding after blocking of CR3 by receptor-specific antibodies[10]
Role of complement: • RMAS-MUC1tumor/anti-MUC1 MAb - Loss of anti-tumor activity in C3-deficient mice[3]	**Role of complement in binding:** • ABA above a threshold level are required for initiation of the classical pathway of complement activation followed by opsonization and binding of Imprime PGG[11]

OBJECTIVES

- Evaluate if there is a specific ABA level in healthy subjects above which Imprime PGG binds and induces functional activities in innate immune cells
  - Evaluate healthy subjects' ability to bind Imprime PGG and induce function at multiple threshold levels
- Evaluate if ABA level has the potential to be used as a biomarker to predict patients whose innate immune cells are most likely to respond to Imprime PGG therapy when used in the clinic
  - Evaluate whether there is improved clinical outcome in patients with elevated ABA levels as compared to those with ABA levels below the threshold identified in the healthy volunteers

METHODS

- Healthy male and female volunteer subjects were recruited and informed consent was obtained from each subject prior to blood collection
- Binding and functional studies were performed as described in the respective figure legends

RESULTS

CORRELATION BETWEEN IgG ABA LEVEL AND IMPRIME PGG BINDING TO NEUTROPHILS IN WHOLE BLOOD OF HEALTHY SUBJECTS

There was a correlation between a subject's IgG ABA level and the ability of their neutrophils to bind Imprime PGG. The majority of subjects with higher ABA levels appeared to have greater than 5% of their neutrophils binding Imprime PGG (defined as high binders). A similar correlation between a subject's IgG ABA level and the ability of their monocytes to bind Imprime PGG was also observed (data not shown).

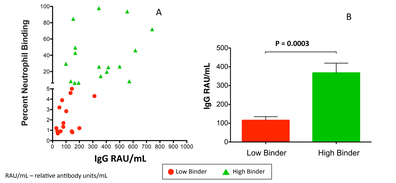


Figure 1. Neutrophil binding to Imprime PGG was assayed in whole blood samples from healthy subjects after incubation with 10 µg/mL of Imprime PGG or vehicle for 30 minutes at 37°C. Surface bound Imprime PGG was determined by staining with a glucan-specific antibody (BTD IV) and assayed by flow cytometry. Serum was also collected from these healthy volunteers and IgG ABA levels were measured by ELISA with relative levels measured against a pooled human serum used as reference standard being the reportable value (relative antibody units/mL; RAU/mL). (A) Plot of percentage of subject's neutrophils binding Imprime PGG vs their respective IgG ABA level. Each symbol represents an individual subject. (B) Comparison of ABA level of low binders (N=18; ≤ 5% of neutrophil binding) with that of high binders (N=26; > 5% neutrophil binding). Data expressed as mean ± SE.

EVALUATION OF IGG ABA THRESHOLD LEVELS AND IMPRIME PGG BINDING TO NEUTROPHILS IN WHOLE BLOOD OF HEALTHY SUBJECTS

Given the strong correlation between IgG ABA levels and binding to the neutrophil population, several arbitrary threshold levels were evaluated in an attempt to discriminate between high and low binders.

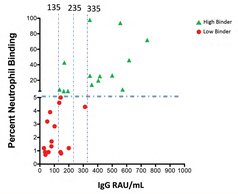


Figure 2. Visual inspection of the IgG ABA vs binding data presented in Figure 1 suggested that an arbitrary threshold level drawn at 335 IgG RAU/mL should exclude all the subjects whose neutrophils were not able to bind Imprime PGG, while enriching for those whose cells could bind. However, to expand on this for functional correlations, effects at IgG ABA thresholds of 235 and 135 RAU/mL were also evaluated.

Table 2. IgG ABA THRESHOLD LEVELS vs IMPRIME PGG

IgG ABA THRESHOLD LEVELS vs IMPRIME PGG MEDIATED FUNCTIONS

Functions	135 RAU/mL	235 RAU/mL	335 RAU/mL
Binding	p=0.0155	p=0.0039	p=0.0013
C5a production	p=0.0203	p= 0.0003	p= 0.0003
CR3 upregulation	NS	p = 0.0112	p = 0.0118
CD88 downmodulation	NS	p = 0.0324	p = 0.0306
CD62L shedding	NS	p= 0.0018	p= 0.0022
IL-8 production	NS	p= 0.0002	p= 0.0094

NS = Not significant

Table 2 summarizes results based on p values for differences in binding and functional responses between high and low binders in the healthy subject population at the three ABA thresholds illustrated in Figure 2 (unpaired two sided t-test). A threshold level between 135 and 235 RAU/mL appeared to differentiate between the high and the low binders and their ability to bind and respond to Imprime PGG treatment ex vivo. Data plotted for each subject for each function at the 235 RAU/mL threshold is shown in Figure 3.

EVALUATION OF NEUTROPHIL IMPRIME PGG BINDING AND FUNCTIONS AT THE 235 IgG RAU/mL THRESHOLD

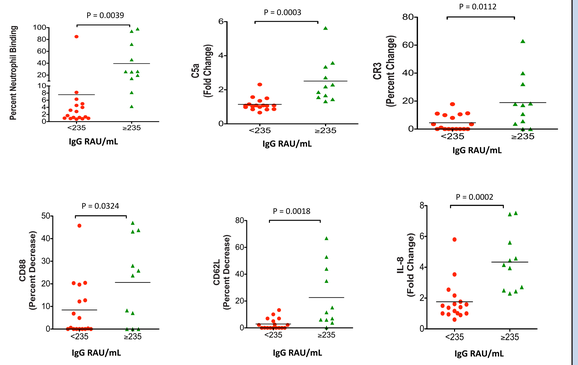


Figure 3. IgG ABA levels and Imprime PGG binding to neutrophils were determined in whole blood samples from healthy subjects as described in Figure1. From blood samples from the same subjects, surface expression of CR3, CD88, and CD62L on CD15+ neutrophils was analyzed by FACS after a 30 minute incubation with Imprime PGG (10 µg/mL) and detection by marker-specific antibodies. Surface receptor expression is presented as percentage change in mean fluorescence change over citrate control. For C5a and IL-8 evaluations, subjects' whole blood was incubated 30 minutes (for C5a) or 24 hours (IL-8) with Imprime PGG (10 µg/mL) and C5a and IL-8 in the plasma measured by commercially available ELISA kits. Unpaired two-sided t-test was used for statistical evaluations.

EVALUATION OF IgM ABA THRESHOLD LEVELS AND IMPRIME PGG BINDING TO NEUTROPHILS IN WHOLE BLOOD OF HEALTHY SUBJECTS

There was a correlation between a subject's IgM ABA level and the ability of their neutrophils to The correlation between a subject's IgM ABA level and the ability of their neutrophils to bind Imprime PGG was not as strong as that for IgG ABA

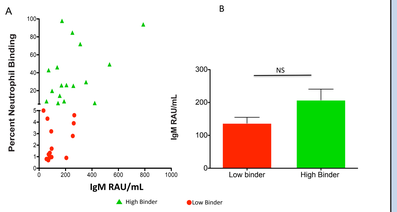
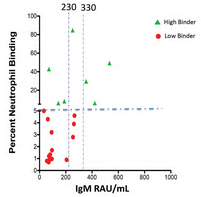


Figure 4. Serum from healthy subjects was also evaluated for IgM ABA levels by ELISA with relative levels measured against a pooled human serum used as reference standard. (A) IgM ABA RAU/mL values and the corresponding neutrophil Imprime PGG binding values (% binding) are presented for the same subjects shown in Figure 1. (B) Comparison of ABA level of low binders (N=18; ≤ 5% of neutrophil binding) with that of high binders (N=26; > 5% neutrophil binding). Data expressed as mean ± SE.



Two arbitrary threshold levels were evaluated in an attempt to discriminate between high and low binders. Although functional evaluations at IgM thresholds were performed, due to the small number of subjects (7 at IgM RAU/mL > 230 and 3 at IgM RAU/mL > 330), statistical significance between groups were not seen (data not shown)

Figure 4. After exclusion of subjects with high IgG ABA (>235), visual inspection of the IgM ABA vs binding data suggested that a cut point of 330 would exclude all low binders, while enriching for high binders. To expand on this for functional correlations, effects at an IgM ABA threshold of 230 RAU/mL were also evaluated.

EVALUATION OF ABA LEVELS AS A BIOMARKER FOR CLINICAL RESPONSE TO IMPRIME PGG THERAPY

Biothera recently completed a randomized Phase 2 study evaluating the effect of Imprime PGG in combination with cetuximab, carboplatin and paclitaxel vs cetuximab, carboplatin and paclitaxel alone in 1st line treatment of advanced Non-Small Cell Lung Cancer (NSCLC) patients.[9]

- The primary endpoint for the study was objective response rate, which was significantly improved in the Imprime PGG treated patients vs control patients (48% vs 23%, P <0.05).
- IgG and IgM ABA levels were measured in serum samples originally drawn for pharmacokinetic analysis and subjects were categorized as biomarker positive or biomarker negative based on having ABA levels above or below the independently established threshold cut points of 235 for IgG or 330 for IgM respectively.
- In a post-hoc subgroup analysis, clinical outcomes from this study were analyzed by biomarker status (Table 3).

Table 3. CLINICAL OUTCOMES ACCORDING TO BIOMARKER STATUS IN NON-SMALL CELL LUNG CANCER PATIENTS TREATED WITH IMPRIME PGG[a]

	Control (N=26)	All Imprime PGG (N=46)	Biomarker Positive Imprime PGG (N=15)	Biomarker Negative Imprime PGG (N=31)
Objective Response Rate	(6/26) 23%	(22/46) 48% vs. Control P=0.0478	(10/15) 67% vs. Control P=0.0088	(12/31) 39% vs. Control P=0.2592
Median Overall Survival	11.3 mo	12.4 mo vs. Control P=0.8830 HR =1.04	16.7 mo vs. Control P=0.3650 HR = 0.70	9.4 mo vs. Control P=0.4332 HR =1.28

[a] Patient response and survival data obtained in Biothera study LCA0821 (Investigator Primary Efficacy Population).
HR = Hazard ratio

SUMMARY

- Evaluation of ABA thresholds revealed higher Imprime PGG binding to neutrophils in healthy subjects with IgG ABA above 235 RAU/mL and with IgM ABA above 330 RAU/mL.
  - All of the innate immune functions tested were significantly modulated above the IgG ABA cut off.
  - Additional studies to further evaluate the IgM cut off to Imprime PGG's functional activities are ongoing.
- These cut off values were used to retrospectively identify biomarker positive (above the ABA cut offs) and biomarker negative (below the ABA cut offs) patients in a recently completed NSCLC trial evaluating Imprime PGG therapy
  - Ad hoc sub-analysis by biomarker status showed that, compared to control patients, biomarker positive patients had a significantly increased objective response rate and a marked survival benefit not seen in the biomarker negative patients
- Ongoing work continues to evaluate the use of ABA levels as a potential predictive biomarker to identify patients who are most likely to respond to Imprime PGG therapy

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