Immune Pharmacodynamic Responses of the Novel Cancer Immunotherapeutic Imprime PGG in Healthy Volunteers


Imprime PGG (Imprime) is an i.v. administered, yeast β-1,3/1,6 glucan in clinical development with checkpoint inhibitors. Imprime-mediated innate immune activation requires immune complex formation with naturally occurring IgG anti-β glucan Abs (ABA). We administered Imprime to healthy human volunteers to assess the necessity of ABA for Imprime-mediated immunopharmacodynamic (IPD) changes. Imprime (4 mg/kg) was administered i.v. in single and multiple infusions. Subsets of subjects were premedicated with antihistamine and corticosteroid. Peripheral blood was measured before, during and after Imprime administration for IPD changes (e.g., ABA, circulating immune complexes, complement activation, complete blood counts, cytokine/chemokine, and gene expression changes). IPD changes were analyzed based on pretreatment serum ABA levels: low-ABA (<20 μg/ml), mid-ABA (20–50 μg/ml), and high-ABA (≥50 μg/ml). At the end of infusion, free serum ABA levels decreased, circulating immune complex levels increased, and complement activation was observed. At ∼1–4 h after end of infusion, increased expression of cytokines/chemokines, a 1.5-4-fold increase in neutrophil and monocyte counts and a broad activation of innate immune genes were observed. Low-ABA subjects typically showed minimal IPD changes except when ABA levels rose above 20 μg/ml after repeated Imprime dosing. Mild-to-moderate infusion-related reactions occurred in subjects with ABA ≥20 μg/ml. Premedications alleviated some of the infusion-related reactions, but also inhibited cytokine responses. In conclusion, ABA levels, being critical for Imprime-mediated immune activation may provide a plausible, mechanism-based biomarker to identify patients most likely to respond to Imprime-based anticancer immunotherapy. The Journal of Immunology, 2019, 202: 000–000.

Imprime is a yeast-derived soluble β-1,3/1,6 glucan (BTH1677) that is currently in clinical development for cancer as an i.v. administered immunotherapy in combination with immune checkpoint inhibitor Abs for the treatment of cancer. In previous preclinical and randomized phase II clinical studies, Imprime has demonstrated the potential to enhance the efficacy of tumor targeting and antiangiogenic Abs. In several xenograft models, Imprime enhanced the antitumor efficacy of the anti-EGFR tumor-targeting Ab cetuximab as well as the anti-VEGFAb bevacizumab (1–3). In a randomized phase II trial, compared with the cohort of advanced nonsquamous non–small cell lung cancer (NSCLC) patients treated with bevacizumab, carboplatin, and paclitaxel, first-line treatment with Imprime plus bevacizumab, carboplatin, and paclitaxel yielded an increased overall response rate and improved median overall survival (4). In high-risk chronic lymphocytic leukemia patients (including those with del 17p, del 11q risk factors), the addition of Imprime to rituximab and alemtuzumab yielded a complete response rate of 65%. In a similar trial run at the same institution during the same time period, patients receiving rituximab and alemtuzumab achieved a 36% complete response (5, 6).

Mechanistic studies in preclinical tumor models have demonstrated the critical role of complement protein C3, complement receptor 3 (CR3), and Grl1-positive myeloid immune cells in the antitumor activity of Imprime (7, 8). More recently, in vivo mouse studies have also highlighted the need for Dectin-1 receptor in Imprime-mediated innate immune functionality (9). Ex vivo human whole blood studies also showed that Imprime

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Abbreviations used in this article: ABA, anti-β-glucan Ab; AE, adverse event; CBC, complete blood count; CH50, total complement activity test; CIC, circulating immune complex; CR2, complement receptor 2; CTCAE, Common Terminology Criteria for Adverse Events; EOL, end of infusion; IPD, immunopharmacodynamic; NSCLC, non–small cell lung cancer; PAMP, pathogen-associated molecular pattern; SC5b-9, serum complement membrane attack complex.

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triggers a myriad of innate immune responses, including complement activation, neutrophil and monocyte binding and phenotypic activation, cytokine/chemokine production, and enhanced myeloid cell-mediated tumor cell killing, in concert with antitumor Abs (10, 11). Interestingly, Imprime binding and functional activation of these innate effector cells was critically dependent on Imprime first forming an immune complex with endogenous IgG anti-β-glucan Ab (ABA). The Imprime–ABA immune complex activates the classical complement pathway, becomes opsonized by complement protein fragments, and can then activate innate effector functionality through CR3 and FcγRIIa (10, 11). Donor blood from healthy subjects with insufficient ABA levels neither bound nor functionally responded to Imprime unless rescued by the addition of purified ABA or commercial IVIG, which is rich in ABA (11). Furthermore, retrospective analyses of historic Imprime-based cancer clinical studies have revealed that patients with higher pretreatment ABA IgG levels derived more clinical response benefit from Imprime-based therapy than patients with low ABA levels (12).

In the present phase I healthy volunteer study, we sought in vivo evidence for the relationship between ABA levels and Imprime-mediated innate immune activation. We monitored several innate immune activation effects in 30 subjects with different pretreatment serum ABA levels dosed with 4 mg/kg, the standard dose of Imprime in clinical trials. Consistent with our ex vivo findings, Imprime dosing resulted in several innate immune activating pharmacodynamic changes that were measurable at both serum/ plasma and the cellular levels. These changes included an acute drop in the free ABA levels concomitant with increased levels of circulating immune complex (CIC), complement activation, production of cytokines and chemokines, expansion of key anticancer effector cells, neutrophils and monocytes, and changes in the expression of a broad set of early innate immune response genes. Notably, these pharmacodynamic changes were minimal in the low-ABA group (<20 μg/ml). Furthermore, the adverse event (AE) profile of Imprime essentially mirrored the immunopharmacodynamic (IPD) profile with significantly lower AE in the low-ABA group. Finally, a premedication regimen that included corticosteroids abrogated the cytokine response to Imprime.

Collectively, these data confirm the critical role of endogenous ABA levels in Imprime-mediated innate immune activation in the clinical setting and also support the use of ABA levels for patient selection in ongoing cancer clinical trials.

Materials and Methods

Trial design and drug administration

The clinical protocol and informed consent forms were reviewed and approved by Salus institutional review board (Austin, TX). The study was conducted in three parts as shown in the schema (Fig. 1). Cohort 1 (12 subjects) received a single dose of Imprime 4 mg/kg i.v. over 2 or 3 h (depending on volume of administration) 1 wk following an infusion of a similarly calculated volume of 0.9% normal saline. Cohort 2 (12 subjects) received an infusion of 0.9% normal saline followed by three weekly i.v. infusions of 4 mg/kg Imprime. Six of the 12 subjects in each cohort 1 and 2 were assigned to receive premedications with low-dose corticosteroids (8 mg of dexamethasone) and a low-dose H1 antagonist (50 mg diphenhydramine i.v.) 1 h before dosing with Imprime. The remaining six subjects in each cohort 1 and 2 did not receive premedication. The nonpremedicated subjects who could not complete a prior dose infusion (e.g., because of AE) could be redosed on schedule with premedication on the subsequent dose. Cohort 3 (six subjects) received i.v. infusions of 4 mg/kg on the first, second, and fifth weeks without premedication. Subjects were observed for AE by clinic staff, including a physician during infusions and for 8 h following infusions. AE were classified and graded by the clinical physician according to the National Institutes of Health/National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (13). The demographics and ABA values for all the subjects who did not receive and those who received premedications are listed in Table I.

All samples were collected in accordance with Salus independent review board–approved protocol and the New England independent review board–approved protocol. All patients provided written consent prior to blood collection.

All data generated or analyzed in this study that are relevant to the results presented in this article are included in this article and its supplementary information files. Other data that were not relevant to the results presented here are available from the corresponding author upon reasonable request.

Laboratory evaluations

Subjects were healthy volunteers as assessed by medical and social history, physical examination, and screening laboratory testing including hepatitis B and C, HIV, serum chemistry, coagulation tests, complete blood count (CBC) with differential, serum pregnancy (β-HCG) test, urinalysis, and electrocardiogram. Throughout the study, subjects were monitored by physical examination, vital signs, serum chemistry, coagulation, and AEs.

IPD evaluations

Human serum was prepared using Vacutainer SST tubes (Becton Dickinson, Franklin Lakes, NJ) according to the manufacturer’s instructions, and the cleared serum samples were used immediately or stored at −80°C. Serum samples were tested for ABA and CIC formation at the following timepoints: predose, approximate end of infusion (EOI), 24, and 168 h post-EOI. Complement activation was measured at predose, ~EOI, and 24 h post-EOI in plasma specimens. Serum chemokine and cytokine production were measured at predose, ~EOI,~1–4 h post-EOI, and 24 h post-EOI. CBC with differential were assessed predose, 15 min to 1 h after start of infusion, ~EOI, and ~4, 24, and 168 h post-EOI. Gene expression was quantified from whole blood samples collected predose, ~1, and 24 h post-EOI.

ELISA for ABA measurement

To measure the concentrations of serum IgG and IgM ABA, solid-phase direct ELISAs were developed. Diluted serum was incubated on a microtiter plate precoated with Imprime. The plates were then incubated with either anti-human γ-chain or anti-human μ chain Abs conjugated to peroxi- disce (Jackson ImmunoResearch, West Grove, PA). A chromogenic substrate (SeraCare, Milford, MA) was subsequently added, and the resulting color change was measured spectrophotometrically at absorbance of 450 nm. A standard curve based off of purified human ABA
was run on each assay to determine microgram per milliliter concentrations of samples.

**CIC measurement**

CIC levels were measured by using MicroVue Complement CIC-Raji Cell Replacement Kit (Quidel Corporation, San Diego, CA) with SpectraMax-M5 plate reader (Molecular Devices, San Jose, CA).

**Complement measurement**

The classical complement activity in plasma was measured by using the MicroVue Complement CH50 Eq EIA Kit (Quidel Corporation), and the complement levels of serum complement membrane attack complex (SC5b-9) were measured by using MicroVue SC5b-9 Plus EIA Kit (Quidel Corporation).

**Cytokine and chemokine measurement**

All or a subset of the cytokines and chemokines, including eotaxin, GM-CSF, IFN-γ, IFN-α1, IL-1β, IL-1RA, IL-10, IL-12, IL-13, IL-15, IL-17, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10 (CXCL10), MCP-1 (CCL2), MIG (CXCL9), MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5), and TNF-α, were tested using multiplex or singleplex Luminex kits (EMD Millipore, Billerica, MA and Thermo-Fisher Scientific, Pittsburg, PA) at various time points before and after Imprime infusion. Results were obtained on Luminex xMAP 200 (ThermoFisher Scientific) using xPonent software and analyzed on Milliplex Analyst software. For ex vivo experiments performed to test the effect of dexamethasone on Imprime-induced cytokine production, whole blood from individuals with high ABA (60–210 mg/ml) was treated ex vivo with vehicle alone or Imprime at 25 mg/ml in the presence of dexamethasone (0.2 and 2 µM) over 24 h. Plasma was collected by centrifugation and assayed by Luminex.

**Complete blood counts**

Automated CBC with differential counts were performed by a Clinical Laboratory Improvement Amendments-certified contract laboratory at the time points mentioned above (MedTox Laboratories, St. Paul, MN).

**Transcriptional regulation**

Human blood samples from various time points before and after Imprime infusion were collected using PAXgene blood RNA tube (BD Biosciences, Franklin Lakes, NJ) according to the manufacturer’s instructions and stored at –80°C. RNA was isolated with RNeasy Mini Kit (Qiagen, Hilden, Germany). A custom-made 80-plex QuantiGene assay kit (ThermoFisher Scientific) using a bead-based multiplex assay was used to measure the expression of 80 genes of interest, including 72 target genes and 8 reference genes with Luminex xMAP 200.
ABA and (change at The change in absolute concentrations 168 h post-EOI using an ELISA method. of the ABA groups. Each line or point represents an individual subject in each of the ABA groups.

Results
To isolate the IPD effects of Imprime alone from those of the premedications, the pharmacodynamic responses of Imprime were assessed in all the subjects across the three cohorts who received a full 4 mg/kg dose Imprime without any premedications (Fig. 1). These subjects were divided into three groups based on their pre-Imprime IgG ABA values (Table I): low-ABA (≤20 μg/ml), mid-ABA (20–50 μg/ml), and high-ABA (≥50 μg/ml). The IPD responses to a single dose and repeated doses measured in this study were changes in the ABA levels, formation of CIC, complement activation, cytokine production, CBC with differentials, and whole blood gene expression levels. As controls, some of these IPD changes were also measured in a subset of subjects in response to saline administration.

Imprime administration results in decreased levels of free ABA with a concurrent increase in IgG-containing CIC in the serum
We hypothesized that Imprime administration should result in Imprime–ABA immune complex formation and, as a result, should cause a measurable drop in the serum levels of free IgG ABA as well as an increase in the levels of CIC. Subsequent results showed the free ABA levels were indeed reduced at the EOI in each of the three groups of subjects with the median drop of 22% (range 0.6- to 0.9-fold) in the low-ABA group, 33% median drop (range 0.5- to 0.9-fold) in the mid-ABA group, and 50% drop (range 0.4- to 0.6-fold) in the high-ABA group. The levels remained reduced at 24 h before returning to baseline between 24 and 168 h postdose in most subjects. Interestingly, several subjects in each of the three groups showed increases in ABA levels ranging from ∼1.5- to 6.1-fold increase over baseline by 168 h (Fig. 2A). Accompanying the drop in the free ABA levels, we saw an increase in the CIC levels with median 1.2-fold (range 0.9–1.8) in the low-ABA group, median 2.0-fold (range 1.3–3.7) in the mid-ABA group, and median 3.9-fold (range 2.3–4.5) in the high-ABA group (Fig. 2B). The peak levels were achieved between EOI and 4 h after EOI before returning to baseline by 24 h. In the mid-ABA group, the cytokine/chemokine production was more sporadic, with two of seven subjects producing some of the aforementioned cytokines/chemokines. Imprime did not stimulate any significant production of the measured cytokines/chemokines in the

FIGURE 2. Imprime administration resulted in drop of free IgG ABA concentrations and increase in CIC levels. Serological changes in IgG ABA and CIC levels with a single dose of Imprime were tested at predose, ~EOI, ~24, and 168 h post-EOI using an ELISA method. The change in absolute concentrations and folds over time and maximum fold change at ~EOI are shown for (A) IgG ABA and (B) CIC in low-, mid, and high-ABA groups. Each line or point represents an individual subject in each of the ABA groups.
Complement activation in response to a single dose of Imprime was measured as changes in plasma levels of SC5b-9 and CH50 activity at predose and ~EOI using commercial enzyme immunoassay kits. The changes in (A) SC5b-9 levels and (B) CH50 activity are shown as absolute concentrations and maximum fold change. Each line or point represents an individual subject in each of the ABA groups.

Imprime induces cytokine production. Serum cytokine levels produced by one dose of Imprime at ~EOI were measured by Luminex multiplex assay. The maximum fold change over predose values are plotted for each of the subjects in (A) low-ABA, (B) mid-ABA, and (C) high-ABA groups. Each line represents an individual subject in each of the ABA groups.

Imprime induces expansion of neutrophils and monocytes in the blood

We next tested whether Imprime administration led to any changes in the absolute numbers of neutrophils, monocytes, and lymphocytes in the blood by performing the CBC with differentials. In the high-ABA group, Imprime mediated a rapid increase in the absolute numbers of neutrophils and monocytes at ~EOI through 4 h post-EOI before returning to near baseline by 24 h. A median 3.1-fold increase in neutrophils (range 2.4–3.4) and 1.6-fold increase in the monocyte count (range 1.3–2.1) were observed (Fig. 5). When we looked at cell count changes at earlier time points (i.e., during Imprime infusion) in a subset of these subjects, we found that Imprime first induced rapid neutropenia and monocytopenia as early as 15 min into the infusion before the expansion at later time points (Supplemental Fig. 1). In contrast, lymphopenia without any further expansion was observed in these subjects, with a median of 65% drop in the lymphocyte count (range 0.3- to 0.4-fold). The leukocyte counts in most of these subjects returned to near baseline in ~24 to 48 h. In the mid-ABA group, the increases in neutrophil and monocyte counts were modest, with median increases of 1.6-fold (range 1.3–2.9) and 1.5-fold (range 1.3–2.3), respectively. Additionally, a 24% median decrease was seen in the lymphocyte counts. There were no changes in the leukocyte counts in the low-ABA group. These alterations in leukocyte counts were not observed with saline administration alone (data not shown). These results again highlighted the ability of Imprime to induce changes in the myeloid cell count in those with ABA ≥20 μg/ml.

Imprime induces a transcriptional signature of innate immune activation in blood

To determine a broad molecular signature of Imprime, we first evaluated the expression of selected gene transcripts in whole blood. This investigation was done as part of cohort 3 only and, as such, had one subject in the low-ABA group, two from the mid-ABA group, and two from the high-ABA group. The genes for which the mRNA transcription was differentially changed (i.e., up or down) from baseline by at least 2-fold are presented as a log2-transformed gene expression heat map (Fig. 6). In the high-ABA group, both subjects substantially upregulated (~2- to 97-fold) several innate immune activation markers: CD83, ICAM1, CD274 (PD-L1), ITGAM (CD11b), FCGR1B, FCGR2A, FCGR3A, FCGR3B, and C5AR1; chemokine/chemokine receptor family of genes: CXCL1, CXCL2, CXCL8, CCL2, CCL3, CCL4, CXCR1, CXCR4, and CCR1; and cytokine genes: IL1B, IL1RN, IL10, and TNFA. CCL5 and CCR5 were the only measured chemokine/chemokine receptor pair to show downregulation. Among the type I IFN-related genes tested, a 2-fold increase in the IFN-stimulated gene MX2 and a decrease in the OAS2 gene were seen in both subjects. Interestingly, among the B cell markers evaluated, SDCBP (CD138), which is primarily expressed on Ab secreting plasma cells, was highly upregulated in both the high-ABA donors. The mid-ABA subjects showed a modest (2- to 4-fold) increase in a subset of the genes upregulated in the high-ABA subjects. In both the high- and mid-ABA groups, maximum upregulation of the genes occurred at about ~1 h post-EOI and then returned to baseline by ~24 h. Consistent with the earlier IPD findings, Imprime did not significantly induce any gene expression changes in the low-ABA subject.

IPD effects of repeated Imprime doses

We next evaluated whether repeated dosing of Imprime altered any of the IPD effects. Ten subjects received multiple doses of Imprime. As shown previously in Fig. 2, some subjects in each of the three ABA groups showed increased IgG ABA levels after one dose of Imprime. Examination of changes in the IgM ABA levels found
that subjects with increased IgG levels did not show concomitant increase in the IgM levels (Fig. 7A). This increase in IgG ABA was generally associated with an enhanced immunological response to Imprime and was most striking in the low-ABA group. Some of these subjects were not responsive to the first dose of Imprime but showed robust responsiveness by the third dose of Imprime, coinciding with increased ABA levels. Representative data presented for one low-ABA subject show that as the ABA levels increased from 15.0 µg/ml (predose 1) to 26.2 µg/ml (predose 3), IPD responses were observed after the third dose of Imprime. A 2.7-fold increase in CIC formation, a 4.9-fold increase in SC5b-9 production, >2-fold increase in cytokine/chemokines IL-6, IL-8, MCP-1, MIP-1α, MIP-1β, MIG, IP-10, and TNF-α, 70% drop followed by 3.2-fold increase in the neutrophil counts (data not shown for monocytes), and >2-fold change in the expression of several innate immune genes were observed (Fig. 7B). Interestingly, the gene expression of the plasmablast/plasma cell marker, CD138, also increased ~3-fold by the third dose of Imprime. Representative data for one subject in each of the mid- and high-ABA groups show that the mid-ABA subject also had enhanced IPD responses as ABA levels increased 2.6-fold by the third dose of Imprime, whereas the high-ABA subject with only a modest increase in ABA levels by the third dose had no significant changes in the responses (Supplemental Fig. 2). The enhanced IPD responses with the increased ABA levels demonstrate that these Abs are not neutralizing Abs but, rather, are critical for Imprime-mediated innate immune responses.

Effects of premedications on Imprime’s IPD

Medications such as corticosteroids, given concomitantly or prophylactically, have been shown to suppress the activity of innate immune cells (14). In our ex vivo experiments, dexamethasone completely inhibited Imprime-mediated cytokine production (Supplemental Fig. 3). Thus, we evaluated the effect of dexamethasone and diphenhydramine, a standard prophylactic premedication regimen administered occasionally with Imprime and often with other biologics and chemotherapy, on Imprime-mediated IPD responses. Eleven subjects who received full doses of Imprime along with premedications were evaluated (Table I).

The premedications profoundly inhibited Imprime-mediated cytokine production. The comparison of mean concentrations of cytokines produced in the high-ABA subjects who were not premedicated (n = 5) versus who were premedicated (n = 7) shows that consistent with the ex vivo data, the premedications considerably inhibited Imprime-mediated cytokine production (Fig. 8A). Furthermore, even with an increase in the ABA levels after repeated dosing of Imprime, the cytokine levels remained blunted in these subjects (Fig. 8B). The other pharmacodynamic responses, including Imprime-mediated drop in free ABA levels, increase in CIC formation, and increased complement activation, were preserved in the mid- and the high-ABA groups (Supplemental Fig. 4). However, consistent with the known leukocytosis effect of corticosteroids, increased expansion of neutrophils, monocytes, and lymphocytes were seen in all subjects independent of ABA levels (15). These results indicate that corticosteroid premedication counteracts some of the key innate immune modulating effects of Imprime.

Imprime induces infusion-related reactions

As the pharmacodynamic effects of Imprime correlate with the ABA levels, we assessed whether there is a relationship between Imprime-induced AEs and ABA levels. We first evaluated the AEs in subjects who did not receive any premedications. In Table II, the IgG ABA values prior to each Imprime dose along with a designation of whether there was an infusion reaction and its highest CTCAE grade of any symptom component are listed. All symptoms were mild to moderate in severity (CTCAE grade 1–2), with the exception of one grade 3 headache. The symptoms typically resolved within approximately one-half hour following EOI. Because the ABA values increased in some subjects with repeated dosing of Imprime, we evaluated the AEs based on each of the predose ABA level. The maximum grade of any symptom component is listed. All symptoms associated with any dose of Imprime was plotted against the predose ABA level. The maximum grade of any component of symptom associated with any infusion reaction and its highest CTCAE grade of any symptom component are listed. All symptoms were mild to moderate in severity (CTCAE grade 1–2), with the exception of one grade 3 headache. The symptoms typically resolved within approximately one-half hour following EOI. Because the ABA values increased in some subjects with repeated dosing of Imprime, we evaluated the AEs based on each of the predose ABA level. The maximum grade of any component of symptom associated with any dose of Imprime was plotted against each of the predose ABA categories (Fig. 9). Most doses were not associated with any infusion reaction. There were two grade 1 events in the category of doses associated with <20 µg/ml predose ABA levels. There were four grade 1 events and two grade 2 events in the doses associated with 20–50 µg/ml predose ABA.
Finally, in the category of doses with $\geq 50$ μg/ml predose ABA levels, there were five grade 1 events, nine grade 2 events, and one grade 3 event. Thus, higher grade AEs appeared to be associated with higher predose ABA levels (≥20 μg/ml), albeit high ABA levels did not always result in infusion reactions.

We next looked at the severity of the AEs in subjects who received premedication (Table III). One high-ABA subject experienced a grade 3 AE, but the symptoms rapidly resolved upon discontinuation of the infusion. No grade 2 AEs were observed in these subjects. Even though some subjects increased their ABA levels by dose 2 or 3, only two subjects developed grade 1 AEs with subsequent doses of Imprime. Thus, corticosteroids may generally mitigate the severity of Imprime-induced infusion reactions but also substantially blunt cytokine responses to Imprime and may therefore defeat Imprime-mediated innate immune responses.

**Discussion**

In our previous studies using ex vivo human whole blood, we have shown that Imprime is pharmacologically active as an immune complex formed between Imprime and naturally occurring ABA (10, 11). The concentration of ABA in serum is a critical determinant of the formation of this complex, subsequent opsonization, binding to innate immune cells, and innate immune functional activation. The role of ABA in mediating the pharmacodynamic effects, as well as the clinical response to Imprime in the clinical trials has not been well established. In this study, we have explored the relationship between the pretreatment ABA levels and the in vivo IPD effects of Imprime.

![FIGURE 7](http://www.jimmunol.org/Downloadedfrom)

**FIGURE 7.** Imprime-induced IPD changes with repeated dosing. Serological changes in ABA levels and IPD changes with repeated dosing of Imprime were measured using the methods previously described. (A) Longitudinal changes in IgG and IgM ABA levels at predose, ∼EOI, and ∼24 h post-EOI for each of the three doses are presented for the three ABA groups. (B) A representative example of a low-ABA subject showing (a) increasing ABA levels with repeated dosing of Imprime and thereby enhanced IPD responses as measured by increased (b) CIC levels, (c) SC5b-9 levels, (d) cytokine production, (e) fold change in neutrophil counts, and (f) gene expression changes.
The systemic effects of Imprime were assessed as serum and cellular immune responses in subjects with varying ABA levels. The formation of in vivo Imprime–IgG ABA immune complex was evident in the drop in the free IgG ABA levels and the simultaneous increase in the levels of IgG-containing CIC observed with the administration of every dose of Imprime (Fig. 2). One of the effects directly downstream of immune complex formation that is proportional to the existing Ab levels is the activation of the classical complement pathway. True to this, Imprime administration resulted in complement fixation, yielding a greater increase in the SC5b-9 and reciprocal drop in CH50 levels in the mid- and high-ABA group (Fig. 3). Importantly, even though higher levels of SC5b-9 were produced in these subjects, clinical symptoms sometimes associated with the deposition of the membrane form of the terminal complement complex (C5b-9) such as hemolytic anemia or pancytopenia were not observed. This is consistent with our earlier reported findings that the membrane bound-C5b-9 was not detected on innate immune cells, thus indicating that Imprime-induced complement activation is not overwhelming the complement regulatory processes (11).

In addition to the immunological changes in the serum, a single dose of Imprime induced rapid and robust cellular changes in the high-ABA group, including induced expression of innate immune response genes in the blood, systemic production of cytokine/chemokines, and expansion of neutrophils and monocytes. The gene expression profile included a range of innate immune activation targets, including modulation of innate receptors, dendritic cell maturation markers, dendritic cell and monocyte activation markers, chemokines and chemokine receptor family, adhesion molecule, and select proinflammatory and anti-inflammatory genes (Fig. 6). Consistent with the gene expression profile, Imprime stimulated production of several chemokines and only low levels of select proinflammatory cytokines such as IL-6 and TNF-α. The increase in anti-inflammatory cytokine IL-10 was sporadic (Fig. 4). Considering that Imprime induced key chemokines known to be involved in margination/demargination, transendothelial migration, and mobilization of myeloid and lymphoid cells (e.g., CXCL1, CXCL2, IL-8, MCP-1, MIP-1α, IP-10), it is not surprising that there was a rapid, transient reduction in the neutrophil, monocyte, and lymphocyte count followed by substantial expansion of the neutrophil and monocyte counts (Fig. 5) (16, 17). Moreover, the effect of β-glucans on mobilization of myeloid cells from the bone marrow is already well documented (18, 19). Thus, Imprime-induced pharmacodynamic serum and cellular changes were consistent with the early innate immune activation events elicited by a pathogen or a pathogen-associated molecular pattern (PAMP). However, unlike other PAMP agents, including TLR and/or STING, agonists that are precluded from systemic clinical administration because of the potential risk of inducing cytokine storm or immune cell death, Imprime’s unique cytokine profile allows it to be well tolerated when administered systemically (20–22). Although some of these PAMP-based innate immune modulators when administered in a biomarker-unselected patient population have been demonstrated to induce some of the systemic immune activity, translation to clinical efficacy is yet to be shown. As Imprime is currently being clinically developed in concert with the T cell checkpoint inhibitors, anti–PD-1 ( pembrolizumab) in metastatic melanoma and triple-negative breast cancer (NCT02981303) and anti–PD-L1 (atezolizumab) in metastatic colorectal cancer (NCT03555149), some of the innate immune changes in both peripheral blood and tumor tissue that are critical to evoke, expand, and sustain T cell immunity, including the production of select chemokines required for T cell migration, together with changes in the expression of costimulatory markers on the myeloid APC, are being extensively evaluated (23, 24). As ABA levels are being measured in these trials, it will be interesting to see the correlation between the ABA levels, the innate immune pharmacodynamic responses, and ultimately, the clinical responses.

Imprime-induced IPD changes were consistently not observed in the low-ABA subjects. Strikingly though, some of the low-ABA subjects showed responses to Imprime after their ABA levels increased and crossed the threshold of ~20 μg/ml upon multiple weekly dosing of Imprime (Fig. 7). As Ag–Ab ratio is one of the critical determinants of immune complex formation and downstream innate immune functions, it is not surprising that there may be an optimal threshold of IgG ABA required for Imprime to complex with and consequently bind to and activate the innate cells. However, our earlier study had shown that subjects with higher IgM did not produce some of the cytokines in response to Imprime because of lack of FcγRIIA engagement (11). So it is possible that other factors, including ratio of IgG/IgM ABA or the...
subclass of the IgG ABA that could preclude Imprime–IgG complex from interacting with the FcγRIIA receptor, are also important considerations for Imprime’s activity.

It is conceivable that naturally occurring Abs cross-reacting to Imprime or shared epitopes among structurally related β-glucans from different pathogens exist (25–27). ABA likely exists as component of the natural Ab repertoire or as a reflection of a humoral response to environmental Ags. The kinetics of increase in the IgG ABA levels (7 d postdose) and increased gene expression of CD138 are suggestive of secondary activation of pre-existing Ag-specific memory B cells or even release of CD138 positive plasma cells. Considering that the subjects showed no increase in the IgM ABA levels, it is likely that the increase in ABA levels is not a consequence of a primary de novo immunogenicity response (Fig. 7). The cellular and molecular mechanisms behind activation of the memory B cells remains to be investigated, but we have shown that complement receptor 2 (CR2) plays a vital role in binding of Imprime to B cells (28). As CR2 is the coreceptor known to lower the threshold of BCR signaling, it is possible that Imprime is activating the memory B cells through the bridging of β-glucan–specific BCR and CR2 (29–32). The subject-to-subject variability in this ABA increase could be

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<th>Group</th>
<th>Subject</th>
<th>1st Dose</th>
<th>2nd Dose</th>
<th>3rd Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG ABA (μg/mL)</td>
<td>AE</td>
<td>IgG ABA (μg/mL)</td>
<td>AE</td>
</tr>
<tr>
<td>Low-ABA (&lt;20 μg/mL)</td>
<td>25</td>
<td>9.9</td>
<td>None</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>10.4</td>
<td>None</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>13.6</td>
<td>Gr1 headache/drowsiness</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>15.0</td>
<td>Gr1 leg stiffness</td>
<td>21.4</td>
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<tr>
<td>Mid-ABA (20-50 μg/mL)</td>
<td>02</td>
<td>34.4</td>
<td>None</td>
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<tr>
<td></td>
<td>04</td>
<td>39.7</td>
<td>None</td>
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<tr>
<td></td>
<td>16</td>
<td>27.6</td>
<td>Gr1 headache</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>32.6</td>
<td>Gr2 headache</td>
<td>78.9</td>
</tr>
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<td>47</td>
<td>33.6</td>
<td>None</td>
<td>203.7</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>37.3</td>
<td>Gr1 diarrhea</td>
<td>38.9</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>34.0</td>
<td>Gr1 dysuria* Gr2 myalgia/arthralgia*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>26.1</td>
<td>None</td>
<td>37.5</td>
</tr>
<tr>
<td>High-ABA (&gt;50 μg/mL)</td>
<td>06</td>
<td>127.0</td>
<td>Gr1 chills/Gr2 nausea/emesis/chest pressure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>177.9</td>
<td>Gr1 lower back pain/chest pressure/nausea</td>
<td></td>
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<tr>
<td></td>
<td>22</td>
<td>87.8</td>
<td>Gr1 chest pressure/light headedness/abdominal cramping/Gr2 myalgia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>68.0</td>
<td>Gr1 chest pressure/warmth/nausea/light headedness/hand paresthesia/Gr2 chills/Gr3 headache</td>
<td>141.0</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>56.2</td>
<td>Gr1 nausea/flushing/Gr2 chest pressure/dyspnea/low back pain/headache* / back pain/neck &amp; shoulder pain*</td>
<td>165.4</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>58.7</td>
<td>Gr1 headache</td>
<td>57.8</td>
</tr>
</tbody>
</table>

Red indicates low-ABA, black indicates mid-ABA, and blue indicates high-ABA.

*Delayed response.
due to multitude of reasons that affect a secondary recall response, including the clonal frequency or BCR affinity of the memory B cells. Thus, although repeated dosing of Imprime could potentially be a strategy to overcome unresponsiveness to Imprime in patients with low baseline levels of ABA, it may not give consistent results. Instead, our previous work has shown that the administration of IVIG to low-ABA patients can reliably increase the ABA concentration to a level that restores Imprime-mediated innate pharmacodynamic responses (24).

Understanding the IPD changes elicited by Imprime provided the context for the infusion reactions observed with Imprime. The characteristics of the infusion reactions were suggestive of manifestations of complement activation-related pseudoallergy (33–35). The dynamics of the longitudinal changes in the ABA levels also delineated the progression of infusion reactions across multiple doses of Imprime. Increasing ABA levels were associated with heightened IPD responses to the second/third doses of Imprime (Fig. 7) as well as increased incidences of grade 2 and 3 infusion reactions (Fig. 9). It is important to note that even though infusion reactions were more common among subjects with higher ABA levels, high ABA levels alone did not necessarily predict the occurrence of a reaction. Further characterization of the infusion reaction, especially the type I hypersensitivity aspect, is warranted. Even though our ex vivo studies have already shown healthy volunteers to have a minimal IgE titer to Imprime (N. Bose and R.M. Walsh, unpublished observations), it would be interesting to assess longitudinal change in IgE and tryptase levels as markers of possible anaphylaxis.

Premedication or concomitant treatment with corticosteroids is commonly employed for managing some of the clinical immunotherapy-associated infusion reactions or autoimmune side effects of several classes of anticancer or immune agonistic agents. Corticosteroids were recently shown to significantly affect the efficacy of checkpoint inhibitors in both preclinical and clinical studies (36, 37). It is important to understand the effect of corticosteroid premedications on the pharmacodynamics of Imprime, as they have been the agent of choice to manage the infusion reactions. When used as premedications, corticosteroids substantially inhibited Imprime-mediated cytokine/chemokine production. Because cytokines/chemokines are paramount to mounting and coordinating an effective immune response, it is safe to assume that corticosteroids could hinder Imprime activity (Fig. 8). As an alternative to corticosteroids, several strategies have been employed to manage Imprime-related infusion reaction. In this trial, subjects who experienced a grade 3 or greater (CTCAE) infusion reaction or were unable to complete dosing during weeks 1 and/or 2 were, at the discretion of the investigator, allowed to be rechallenged in the subsequent week with double the length of the standard infusion time. In our ongoing cancer studies (NCT02981303), an informal trial of steroid-sparing premedication regimen containing antihistamine (diphenhydramine), antiemetic (ondansetron), nonsteroidal anti-inflammatory drug (ketorolac), and antipyretic/analgesic agents (acetaminophen) is recommended before commencing dosing of study medications on each treatment day and, as needed, postdose. To date, this premedication regimen appears to be relatively effective in both

Table III. AE in healthy volunteers administered Imprime with premedications

<table>
<thead>
<tr>
<th>Group</th>
<th>Subject</th>
<th>1st Dose</th>
<th>2nd Dose</th>
<th>3rd Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG ABA (µg/mL)</td>
<td>AEs (CTCAE grade)</td>
<td>IgG ABA (µg/mL)</td>
</tr>
<tr>
<td>Low-ABA (&lt;20 µg/mL)</td>
<td>09</td>
<td>21.4</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>15.2</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>10.6</td>
<td>None</td>
<td>30.2</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>11.3</td>
<td>Gr1 flushing</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>6.2</td>
<td>None</td>
<td>7.2</td>
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<td>Mid-ABA (20-50 µg/mL)</td>
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<td>20</td>
<td>41.8</td>
<td>Gr1 flushing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>23.3</td>
<td>None</td>
<td>26.4</td>
</tr>
<tr>
<td>High-ABA (&gt;50 µg/mL)</td>
<td>07</td>
<td>138.1</td>
<td>Gr3 chest pressure/dyspnea/abdominal cramping/sinus tachycardia</td>
<td></td>
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<tr>
<td></td>
<td>30</td>
<td>153.6</td>
<td>None</td>
<td>102.2</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>106.6</td>
<td>None</td>
<td>137.8</td>
</tr>
</tbody>
</table>

Red indicates low-ABA, black indicates mid-ABA, and blue indicates high-ABA.
managing the symptoms as well as preserving the Imprime-induced cytokine response in the treated subjects (24). These data suggest that the use of steroids as supportive premedication may compromise the potential benefit of some immune-oncology agents and, again, highlight the need for the medical community to explore nonsteroid alternatives wherever possible.

In summary, the data in this report provide in vivo evidence that Imprime’s pharmacological activity is dependent on its binding to naturally occurring ABA. The data presented demonstrate that there is a threshold of IgG ABA necessary for activation of the innate immune responses. These data suggest that although the acute pharmacodynamic responses to Imprime-based immunotherapy are predicated on ABA levels in individual healthy subjects, it is also likely that the high-ABA subjects will be more prone to infusion-related reactions. It is imperative that a more prudent use of corticosteroids be considered for Imprime-based therapy wherever possible. Ongoing phase 2 studies will provide an opportunity to understand the correlation of these pharmacodynamic responses to clinical responses in cancer patients. Furthermore, exploration of this threshold in the context of other factors, including the subclass of the IgG, the concentration of IgM ABA, the combination agent, the patient population, and the underlying target disease, will be essential. This study provides a strong rationale for selecting patients based on their pretreatment ABA levels as a means to enrich for patients most likely to elicit a response to Imprime-based immunotherapy.

Disclosures


References


Supplemental Fig. 1. Imprime induces cytopenia in high-ABA subjects early into the infusion. CBC with differentials were obtained at various time points and a fold change over the pre-dose value was calculated for neutrophils (top panels), monocytes (middle panels), and lymphocytes (bottom panels). Each line represents an individual subject in each of the ABA groups.
Supplemental Fig 2. Imprime-induced IPD changes with repeated dosing. Serological changes in ABA levels and IPD changes with repeated dosing of Imprime were measured using the methods previously described. (A) A representative example of a mid-ABA subject showing, a) increasing ABA levels with repeated dosing of Imprime and thereby enhanced IPD responses as measured by increased b) CIC levels, c) SC5b-9 levels, d) cytokine production, e) fold change in neutrophil counts, and f) gene expression changes. (B) A representative example of a high-ABA subject showing, a) increasing ABA levels with repeated dosing of Imprime and thereby enhanced IPD responses as measured by increased b) CIC levels, c) SC5b-9 levels, d) cytokine production, e) fold change in neutrophil counts, and f) gene expression changes.
Supplemental Fig 3. Dexamethasone inhibits Imprime-induced cytokine production *ex vivo*. Whole blood from high ABA individuals were treated *ex vivo* with vehicle alone or Imprime at 25 µg/mL in the presence of dexamethasone (0.2 and 2 µM) over 24 hours. Plasma was collected by centrifugation and assayed by Luminex X-MAP multiplex platform. Representative data shown from n = 2 experiments using different donors.
Supplemental Fig 4. Imprime-induced IPD changes in pre-medicated subjects. Serological changes in ABA levels and IPD changes were measured using the methods previously described. Shown here are the maximum fold changes at ~EOI for (A) IgG ABA, (B) CIC, (C) SC5b-9, and (D) CBC in low-, mid-, and high-ABA groups. Each point represents an individual subject in each of the ABA groups.