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Imprime PGG Drives Innate Immune Activating Pharmacodynamic Changes in a Phase I Clinical Study in Healthy Human Volunteers

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Abstract

Background

Imprime PGG (Imprime) is currently in clinical development as combination therapy with checkpoint inhibitors. Imprime, a yeast-derived, soluble β-1,3/1,6 glucan is a Pathogen Associated Molecular Pattern (PAMP) that complexes with endogenous anti-beta glucan antibodies (ABA) and activates innate immune effector cells to trigger the anti-cancer immunity cycle. In this study, we sought to investigate the immunopharmacodynamic (IPD) responses of Imprime in healthy human subjects.

Methods

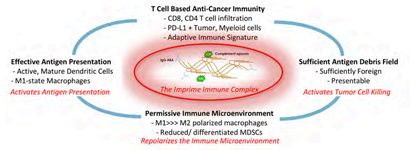
Cohort 1 (n=12) received a single IV infusion of Imprime 4 mg/kg, Cohort 2 (n=12) received three weekly infusions of 4 mg/kg Imprime. Cohort 3 (n=12) received infusions of 4 mg/kg or 2 mg/kg Imprime on weeks 1, 2 and 5. In cohorts 1 and 2, six subjects each received premedication with diphenhydramine 50 mg IV and dexamethasone 8 mg IV. IPD changes were measured at various times before, during and after Imprime administration.

Results

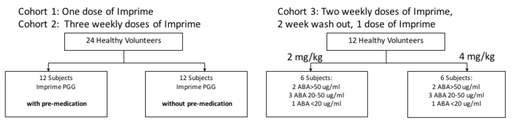
Peak levels of complement activation products, C5a and SC5b-9, were detected at the end of infusion (EOI). A 2 to 3-fold increase in neutrophil and monocyte numbers were seen 4 hours post-Imprime infusion. Chemokines, especially IL-8 and MCP-1, were consistently detected at EOI. Cellular analyses showed Imprime binding to neutrophils, monocytes, subsets of DC and B cells. 24 hrs after EOI, a population of intermediate monocytes expressing higher levels of the activation markers CD86, PD-L1 and HLA-DR, was observed. Approximately one week post-Imprime, increased switched memory B cells and plasmablasts were detected. Consistent increase in expression of innate immune activation marker genes was evident as well. A substantial drop in free ABA and a concomitant increase in circulating immune complexes were seen at the EOI. Adverse events (AE) were limited to infusion related reactions. Importantly, the IPD changes and the AE were seen in subjects with higher ABA levels. The effect of pre-medications on some of the IPD will also be presented.

Background

Imprime PGG Impacts Multiple Points of the Cancer Immunity Cycle



Objective: Evaluate IPD Changes Induced by Imprime in a Phase I Healthy Donor Trial



PHASE I EVALUTIONS:

- AE and Complete Blood Counts
- Whole Blood and serum at various time points post-infusion:
  - Imprime Binding to Monocytes, Neutrophils, B-cells, DC subsets
  - Complement activation- C5a and Sc5b9
  - Serum cytokines/chemokines
  - Anti-beta glucan (ABA) analysis
  - Flow cytometry of immune cells
  - Circulating immune complex formation
  - Quantigene analysis of transcriptional profile

Premedication: low-dose corticosteroids (4 mg of dexamethasone PO) and low-dose H1 antagonist (50 mg diphenhydramine IV)

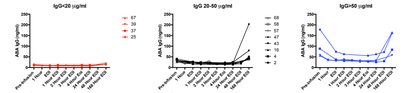
COHORT 1 (4 mg/kg)			COHORT 2 (4 mg/kg)			COHORT 3		
subject	IgG (µg/ml)	Pre-med	subject	IgG (µg/ml)	Pre-med	subject	IgG (µg/ml)	dose
02	32.9	NO	25	10.9	NO	51	62.4	2 mg/kg
04	35.7	NO	29	10.5	YES	52	21.9	2 mg/kg
06	158.9	NO	30	84.5	YES	54	64.5	2 mg/kg
07	143.2	YES	32	11.8	YES	55	56.2	4 mg/kg
09	12.9	YES	36	31.8	NO (YES)	57	37.3	4 mg/kg
10	183.2	NO	37	10.8	NO	58	34	4 mg/kg
13	39	YES	38	114.0	YES	59	26.6	2 mg/kg
16	29	NO	39	12.3	NO	60	25	2 mg/kg
17	18.2	YES	41	25.0	YES	67	15	4 mg/kg
18	30.7	YES	43	33.5	NO	68	26.1	4 mg/kg
20	41.9	YES	45	22.5	YES	70	58.7	4 mg/kg
22	87.9	NO	47	16.4	NO	71	14.6	2 mg/kg

Subjects highlighted in grey did not receive a full dose of Imprime on all weeks

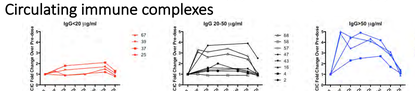
Results

IPD Changes Induced by Single Dose of Imprime in Subjects with Different ABA Levels - Serum & Cellular Changes

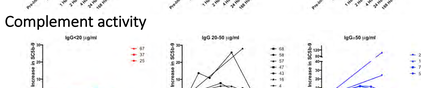
A. ABA levels



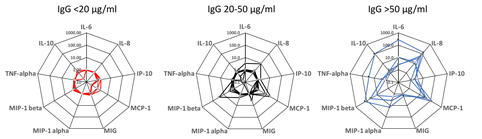
B. Circulating immune complexes



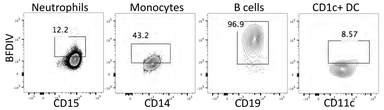
C. Complement activity



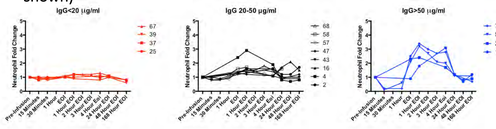
D. Cytokine/chemokine production



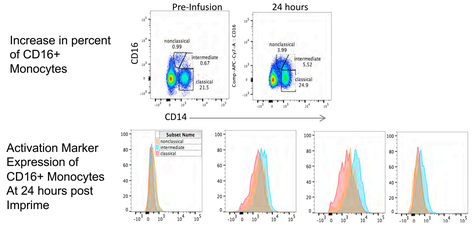
E. Binding of Imprime to immune cell subsets



F. Mobilization of neutrophils and monocytes (monocyte data not shown)



G. Appearance of nonclassical/intermediate monocytes



H. Gene expression

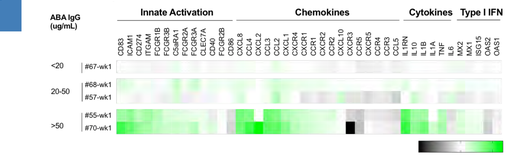
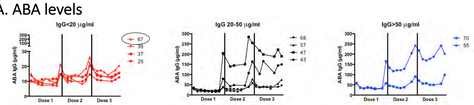


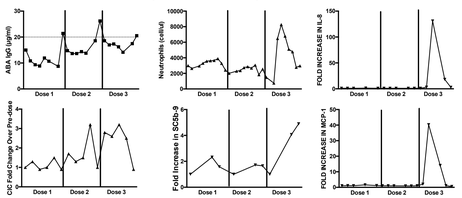
Figure 1. Intravenously administered Imprime induces several IPD changes in the peripheral blood of healthy human subjects. Whole blood or serum was drawn from healthy volunteers at various time points before and after a single dose (cohort 1) or multiple weekly doses (cohorts 2 and 3) of Imprime infusion (See Objective). **(A)** ABA were measured in serum by ELISA. Subjects were grouped by ABA levels and indicated by red (<20 µg/mL), black (20-50 µg/mL) or blue lines (>50 µg/mL). **(B)** Total circulating immune complex formation was measured in serum by ELISA (Quidel). **(C)** Complement activation was measured by ELISA (Quidel) in plasma. **(D)** Cytokine/chemokine measurement in serum was performed using Novex magnetic multiplex assay (Life Technologies) the Luminex XMAP technology. End of Infusion relative to pre-dose values are plotted. **(E)** *In vivo* immune cell binding was assessed by flow cytometry from whole blood of subjects dosed with Imprime at the end of infusion. Gates were set based on BFDIV staining (Anti-Imprime MAb) on blood cells prior to infusion. **(F)** Cell mobilization was measured by complete blood cell counts, plus differentials. Neutrophil numbers only are represented. Monocyte and lymphocyte numbers were also collected. **(G)** PBMC were stained with indicating markers and analyzed by flow cytometry. Each of the populations were assessed for expression of activation markers CD86, ICAM-1, HLA-DR and PD-L1 24 hours after infusion. **(H)** Gene expression in blood of subjects dosed with Imprime (3 hr post vs. pre-dose levels). Whole blood was collected in PaxGene blood collection tubes (BD Biosciences) at various time points pre and post-Imprime administration. RNA were isolated and assayed by Quantigeneplex (Affymetrix). Relative fold changes were log 2 transformed. Data presented are from subjects who were treated with Imprime without pre-medications.

Imprime Induces IPD Changes with Increasing ABA levels - Multiple doses

A. ABA levels



B. Representative example of IPD changes in a subject with increase in ABA levels after multiple doses of Imprime



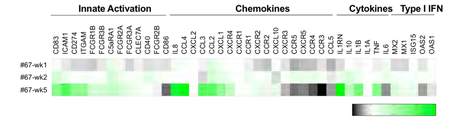


Figure 2. Imprime induces IPD changes in low ABA subjects if the ABA level increases beyond 20 µg/mL. Whole blood or serum was drawn from healthy volunteers at various time points before and after a single dose (cohort 1) or multiple weekly doses (cohorts 2 and 3) of Imprime infusion (See Objective). **(A)** ABA were measured in serum by ELISA. Subjects were grouped by ABA levels and indicated by red (<20 µg/mL), black (20-50 µg/mL) or blue lines (>50 µg/mL). **(B)** ABA, CIC, Monocyte mobilization, Complement activation, fold increase in cytokines/chemokines and gene expression (3 hr) are represented for one individual (subject 67) that converted from low ABA to mid level. All assays performed as described in figure legend 1.

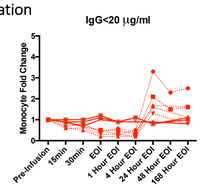
ABA and AE (Single Dose)

S/N	Age	Gender	ABA		Adv. Events (CTCAE grade)
			Screening	Pre-dose	
025	64	M	11.3	9.9	None
037	29	M	11.3	10.4	None
039	50	M	13.3	13.6	Gr 1 headache Gr 1 drowsiness
067	31	M	17.7	15.0	Gr 1 LE stiffness
047	63	M	28.5	33.6	None
068	27	F	28.5	26.1	None
016	21	F	30.3	27.6	Gr 1 headache
002	19	F	34.2	34.4	None
058*	52	M	35.9	34.0	Gr 1 dysuria (delayed) Gr 2 myalgia (delayed) Gr 2 arthralgia (delayed)
043	30	F	38.4	32.6	Gr 2 headache
057	55	F	39.2	37.3	Gr 1 diarrhea
004	33	M	41.5	39.7	None
055	64	M	66.2	56.2	Gr 1 nausea Gr 1 flushing Gr 2 chest pressure Gr 2 dyspnea Gr 2 low back pain Gr 2 headache (delayed) Gr 2 back pain (delayed) Gr 2 neck/shoulder pain (delayed)
070	26	M	68.7	58.7	Gr 1 headache
034	31	F	78.4	68.0	Gr 1 chest pressure Gr 1 warm sensation Gr 1 light headedness Gr 1 nausea Gr 1 hand paresthesia Gr 2 chills Gr 3 headache
022	26	M	100.7	87.8	Gr 1 chest pressure Gr 1 light headedness Gr 1 abdominal cramping Gr 2 myalgia
006	34	M	132.5	127	Gr 1 chills Gr 2 nausea/emesis Gr 2 chest pressure
010	48	M	158.9	177.9	Gr 1 back pain Gr 2 chest pressure Gr 2 nausea

Table 1. Adverse Events observed with Imprime infusion.
* One subject (#058) developed delayed symptoms of generalized arthralgia and myalgia with elevated C-reactive protein and was later determined to have had elevated pre-dose circulating immune complex levels.

Effects of Pre-meds on IPD Changes

A. Cell mobilization



B. Cytokine production

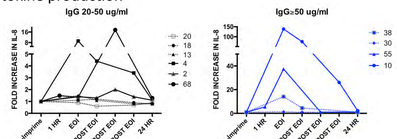


Figure 3. Pre-medications affect Imprime induced IPD changes. Whole blood or serum was drawn from healthy volunteers at various time points before and after a single dose (cohort 1) or multiple weekly doses (cohorts 2 and 3) of Imprime infusion (See Trial Design). Subjects were grouped by ABA levels and indicated by red (<20 µg/mL), black (20-50 µg/mL) or blue lines (>50 µg/mL). Filled lines represent subjects without pre-meds and dashed lines are pre-med subjects **(A)** Monocyte mobilization was measured by complete blood counts at various times before and after Imprime administration in the low ABA group. (B) Cytokine production (fold IL-8 over pre-dose levels) are shown. All assays performed as described in figure legends 1 and 2.

ABA and Clinical Response

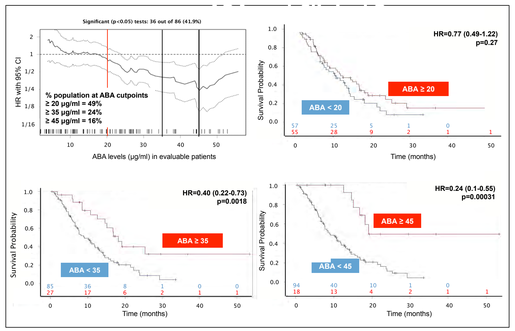


Figure 4. ABA levels could potentially be predictive of clinical response in Imprime-treated patients. IgG ABA were determined by ELISA for all evaluable patients in the Primus trial (third line CRC patients treated with Cetuximab or Imprime+Cetuximab). Upper left panel- Hazard Ratio (HR) vs ABA level is graphically represented. The ABA level for each patient is shown on the x axis. The solid line in the graph represents the HR while the dotted lines that bracket this line represent the 95% confidence intervals. Vertical lines imposed on the graph simply represent the cut-points chosen for Kaplan-Meier analyses. The inset shows the % of patients in this trial at the different ABA cut points. Other panels- each represents Kaplan-Meier analyses for OS at the indicated ABA cut-points (20, 35, or 45 µg/ml). HR for each, and statistical significance are noted in the inset.

Conclusions

- Imprime-induced IPD changes are ABA dependent
  - < 20 ug/ ml = no response
- Infusion reaction-related AE (CARPA; complement-activation related pseudoallergy) are observed in some, but not all, subjects with ABA > 20 ug/ml.
- Pre-medications (corticosteroids with antihistamine) dampen Imprime-mediated cytokine induction.
- Imprime-driven IPD responses are dose-dependent. Doses 2 and 4 mg/kg induced IPD responses were similar in subjects with ABA > 50 ug/mL. Subjects with mid ABA levels (20-50) showed minimal response to 2 mg/kg dose Imprime (data not shown).

Acknowledgements

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For additional questions, please contact: nbose@biothera.com

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