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Clinical Benefit Potentially Evident with Immunopharmacodynamic Responses in Prior-checkpoint Failed Metastatic Melanoma Patients Treated with Imprime PGG and Pembrolizumab

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Abstract

Background: Checkpoint inhibitor (CPI) monotherapy has revolutionized the treatment of melanoma, yet most patients are primary non-responders or develop secondary resistance. Lack of antigen-specific T cell priming and/or immunosuppressive mechanisms leading to T cell exhaustion are critical cancer-extrinsic factors contributing to CPI resistance mechanisms. Immunotherapeutic agents capable of sparking *de novo* anti-tumor T cell responses or reinvigorating pre-existing exhausted T cell immunity could help reinstate the activity of CPI .

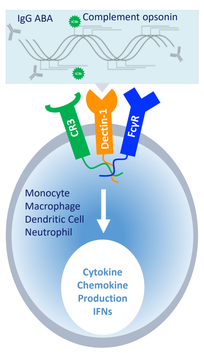
Methods: Our Phase 2, multi-center, open label study, NCT02981303 in collaboration with Merck & Co., Inc., is evaluating Imprime PGG (Imprime), a novel yeast derived, Dectin-1 agonist, β-glucan PAMP in combination with pembrolizumab (KEYTRUDA®, pembro) in heavily CPI pre-treated melanoma patients (20 patients; 65% had ≥2 prior CPI regimens with 17/20 having previously progressed on pembro). Patients received Imprime (4 mg/kg) + pembro (200 mg) intravenously in a 3-week cycle. Here, we present the immunopharmacodynamic (IPD) responses elicited by Imprime and pembro in the peripheral blood of 19 patients .

Results: In the intent-to-treat population (ITT; N=20), the disease control rate was 45% (1 CR and 8 SD), 6-month and 12-month OS rates were 65% and 45% respectively, and median OS (mOS) was 8.8 months. In the patients showing disease control, a significant increase in CH50, the classical pathway complement function (~0.7-2.6-fold), HLA-DR expression on classical monocytes (~0.61-1.94-fold) and reduction of frequency of PD-1$^+$Tbet$^-$EOMES$^+$ exhausted CD8 T cells (~0.9-4-fold) was observed. Stimulation of peripheral blood mononuclear cells from a subset of patients by CD3/CD28 beads showed enhanced production of IL-2 and IFN-gamma in the CD8 T cells. Some of these IPD responses were also associated with 6-month landmark OS analyses. Additionally, whole blood gene expression analyses showed >2-fold upregulation of several myeloid and T cell activation genes including IFNg, CD83, IP-10, and IL-2RA. Enhanced OS was observed in patients with >1.3 fold increase in CH50 (8/19; HR 0.385; p=0.1) or >1.5-fold reduction in the frequency of exhausted CD8 T cells (8/19; HR 0.102; p=0.001). The IPD responses observed in the ITT population included formation of circulating immune complexes (peak levels ranging from ~4.5-16.1-fold) and production of complement activation protein SC5b9 (~3.4-25.6-fold), and increase in the frequency of HLA-DR+ myeloid cells (~0.43-3.71-fold).

Conclusions: Overall, these data, albeit in a small population, demonstrate that Imprime/pembro combination can drive the innate/adaptive IPD responses that are critical for providing clinical benefit to the patients who have progressed through prior CPI treatments.

Background

Imprime PGG: a Novel Mechanism of Action



Imprime PGG binds the Dectin-1 Receptor instigating an *integrated* anti-cancer immune response involving both innate and adaptive immunity

1. **Imprime PGG forms an immune complex**
   - Anti-β glucan antibodies (ABA)
     - patient selection biomarker, identifies ~50% of patients
   - Complement fragment (iC3b)
2. **Immune Complex binds Dectin-1 and Co-Receptors**
   - This immune complex is the "active" drug
3. **Innate Immune Cell Activation**
   - Alleviates immunosuppression
     - Promotes shift from immunosuppressive "M2" state
     - Drives infiltration of PD-L1+, CD80+ immune cells (M1)
   - Activates antigen presenting cells
     - Dendritic Cells, M1 APCs
     - Increases expression of co-stimulatory CD80, CD86 markers
   - Increases tumor specific T Cell activation and infiltration

Imprime PGG-mediated changes to the innate immune system and the tumor microenvironment potentiate the activity of CPIs

Phase2 Clinical Study- Imprime in Combination with Pembrolizumab

Hyopothesis*: Imprime PGG may awaken (or re-awaken) the immune response in patients whose disease has progressed through single agent CPI therapy, activating macrophages and dendritic cells, promoting repolarization of the immune microenvironment, immune cell infiltration and T cell-based anti-cancer immunity to restore clinical benefit*

Study Design:
- *Imprime PGG (IV, 4mg/kg, weekly) + pembrolizumab (IV, 200mg, q3W).*
- *Patients pre-selected for biomarker (IgG ABA ≥ 20μg/ml). N = 20.*
- *Optional tumor biopsy pre-Tx and 6 wks on Tx, assessed by high content immunoprofiling*

Clinical Endpoints: *ORR, Safety, DCR, PFS, OS, TTR, DoR, PK/PD Profiling*

Translational Endpoints: *Tumor Biopsy analysis for immune infiltration and activation*

Patient Population: *The intended patient population were those failing a single regimen of CPI therapy.* ***For this cohort, 13/20 patients entered trial with 2 or more CPI lines of therapy, some having 6 or 7 prior CPI-based lines of therapy****. There is no comparator trial in such heavily CPI pre-treated patients.*

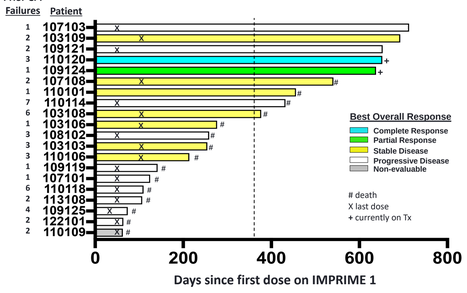
Efficacy in Metastatic Melanoma Patients with Prior Progression on ICI Therapy

IMPRIME-1 Metastatic Melanoma Patients with Prior Progression on IC (N=20)	
Overall Response Rate (ORR)	5% (1/20)
Stable Disease (SD)	40% (8/20)
Progressive Disease (PD)	50% (10/20)
Adverse Event/Not Evaluable	5% (1/20)
Duration of Response (DoR)	21.6 months+
Time to Response (TTR)	9.5 months
Progression-Free Survival (PFS)	1.6 months
Disease Control Rate (DCR)	
CR + PR + any SD	45% (9/20)
CR + PR + SD > 24 weeks	10% (2/20)
Median Overall Survival (mOS)	8.8 months
Landmark Survival Rate	
6 month OS	65%
12 month OS	45%
18 month OS	25%

CR = Confirmed Complete Responder
PR = Confirmed Partial Responder
Latest IMPRIME 1 data from October 16, 2019.
Median follow up time 21.9 months.

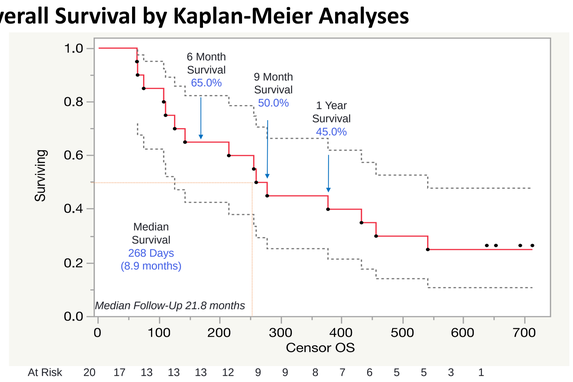
Extended Overall Survival

Prior CPI Failures / Patient



The swimmer's plot represents, on each bar, the data for each patient over time starting with the 1st dose on trial. Bars are color-coded to represent the patient's best overall response as noted. The number of prior CPI lines of therapy is shown to the left of each patient identifier. Patient 110109 is ineligible and non-evaluable but included here to show the full ITT. Data updated 9.5.19.

Overall Survival by Kaplan-Meier Analyses



Latest IMPRIME 1 data from August 28, 2019 – ITT population, n = 20.

Acknowledgements

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BIOTHERA pharmaceuticals

Multispectral Tissue Imaging Analyses of a Melanoma Patient Pre- and On-treatment Biopsies

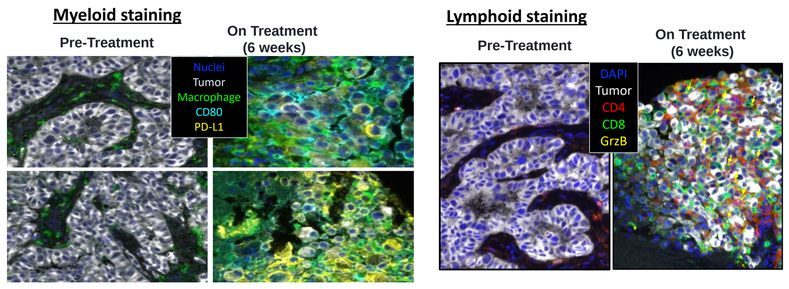
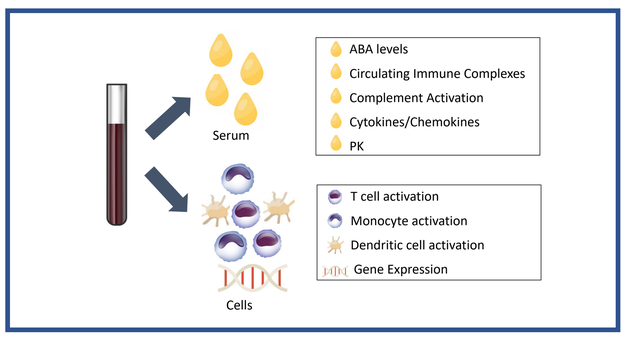


Figure 1. Increased myeloid infiltration and activation on treatment. (**Left**) Representative tissue images from 2 different regions of the pre-treatment and on-treatment tumor samples. Tumor is shown in white with prominent blue nuclei (DAPI-stained). Darkened tracts between tumor sheets represent stroma. Note that macrophages (green) are evident in the stroma for the pre-treatment images but not within the tumor tissue. Pre-treatment, these macrophages are devoid of the activation markers CD80 and PD-L1. By contrast, the on-treatment tumor tissues show heavy infiltration by macrophages that carry the marks of activation (CD80 in blue, PD-L1 in yellow). (**Right**) Representative tissue images from pre-treatment and on-treatment tumor samples. Tumor is shown in white with prominent blue nuclei. Darkened tracts between tumor sheets represent stroma. Note that for the pre-treatment tumor, there is little evidence for CD8 (green) T cells. A few CD4 (red) T cells are evident in the stroma. By contrast, the on-treatment tumor tissue is heavily infiltrated by both CD8 (green) and CD4 (red) T cells. These T cells express the activation marker, Granzyme B (punctate yellow stain highlighted by yellow arrows).

Serum and Cellular IPD Evaluations in Melanoma



In vivo Evidence of Immune Complex Formation

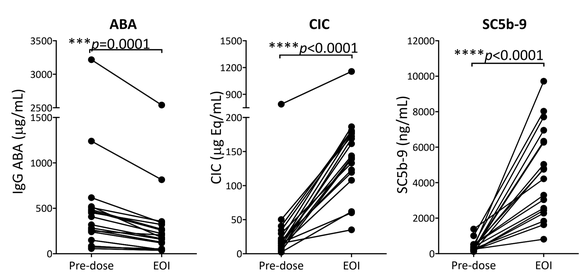


Figure 2. Imprime administration resulted in drop of free IgG ABA concentration, increase in CIC and SC5b-9 level. Shown here are serological changes in IgG ABA, CIC and SC5b-9 levels tested at pre-dose and ~end of infusion (EOI) of Imprime and Pembro of cycle 2 using an ELISA method.

Complement Activation and Cytokine Production

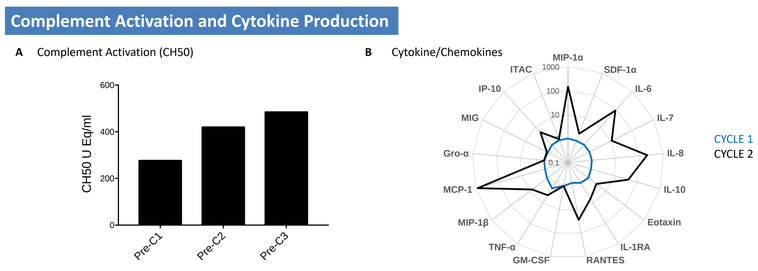


Figure 3. Complement activation and cytokine/chemokine production observed in melanoma patients. CH50, a measure of activation of the classical complement pathway, and cytokine production are measured in serum collected at pre-dose and end of infusion (EOI) of various dosing cycles. Shown here are **(A)** CH50 measured by commercially available EIA kit at pre-dose time points of cycles 1, 2 and 3 and **(B)** fold change over pre-dose in chemokine/cytokine production measured at EOI of cycles 1 and 2 using a commercial multiplex Luminex assay.

Imprime Binding to Immune Cells and Evidence for Target Engagement (Dectin-1)

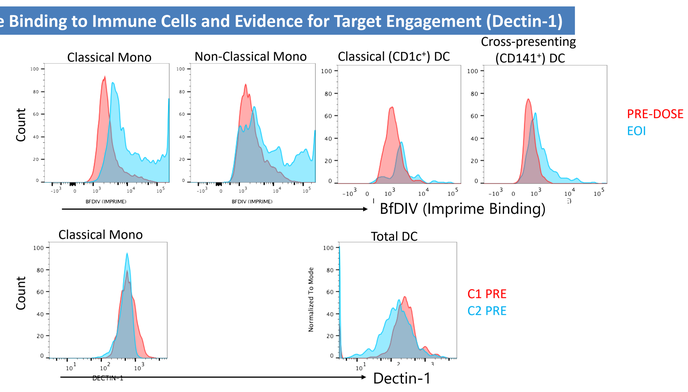


Figure 4. Imprime binding to monocytes/DC and modulation of Dectin-1 expression in a Melanoma patient. PBMC were isolated from whole blood of a subset of patients at pre-dose and EOI of various dosing cycles. PBMC were stained with anti-beta glucan antibody (BfDIV) and markers for monocytes/DC subsets. Imprime binding and modulation of Dectin-1 expression on different monocytes and DC subsets were analyzed by flow cytometry.

Monocyte and DC Activation

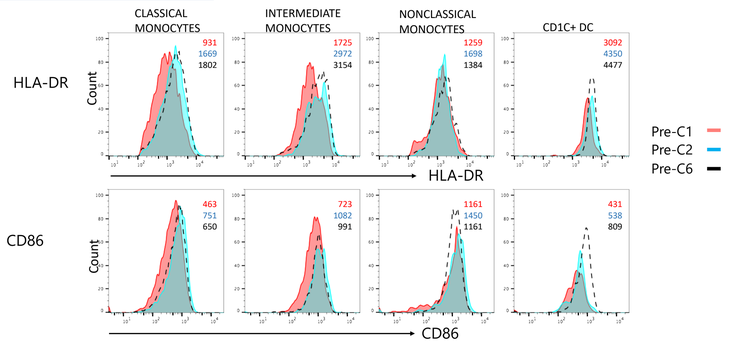


Figure 5. Monocyte and DC activation in Melanoma patients. PBMC were isolated from whole blood of patients at pre-dose and EOI of various dosing cycles. PBMC were stained with identification markers for monocytes or DC subsets and analyzed by flow cytometry. Each of the populations were assessed for expression of activation markers HLA-DR and CD86. Data shown here is for one of the RECIST responders.

Results

Alteration of Frequency and Functionality of Exhausted CD8 T cells (PD-1+ Tbet- EOMES+) Post-Imprime/Pembro Administration

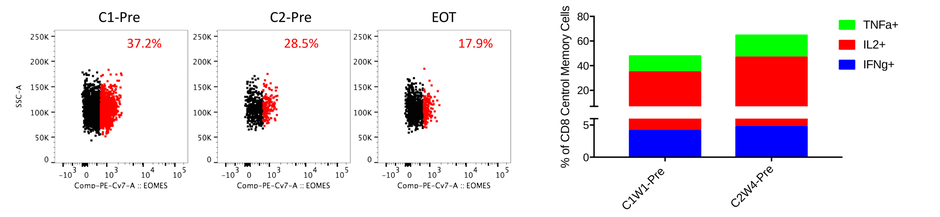


Figure 6. A reduction in the percentage of exhausted CD8 T cells concomitant with increase in functional responsiveness was observed post-Imprime and Pembro treatment. PBMC were stained with markers for T cells and analyzed by flow cytometry. The frequency of exhausted T cells (Tbet- EOMES+) as a percentage of total PD-1+ CD8 T cells at different time points are shown in the scatter plot (EOT: end-of-treatment). Functionality was assessed by stimulating PBMC with CD3/CD28 dynabeads at 1:1 ratio for 24 hrs and stained for intracellular effector cytokines along with CD8 T-specific cell surface markers.

IPD Responses and Association with Clinical Benefit

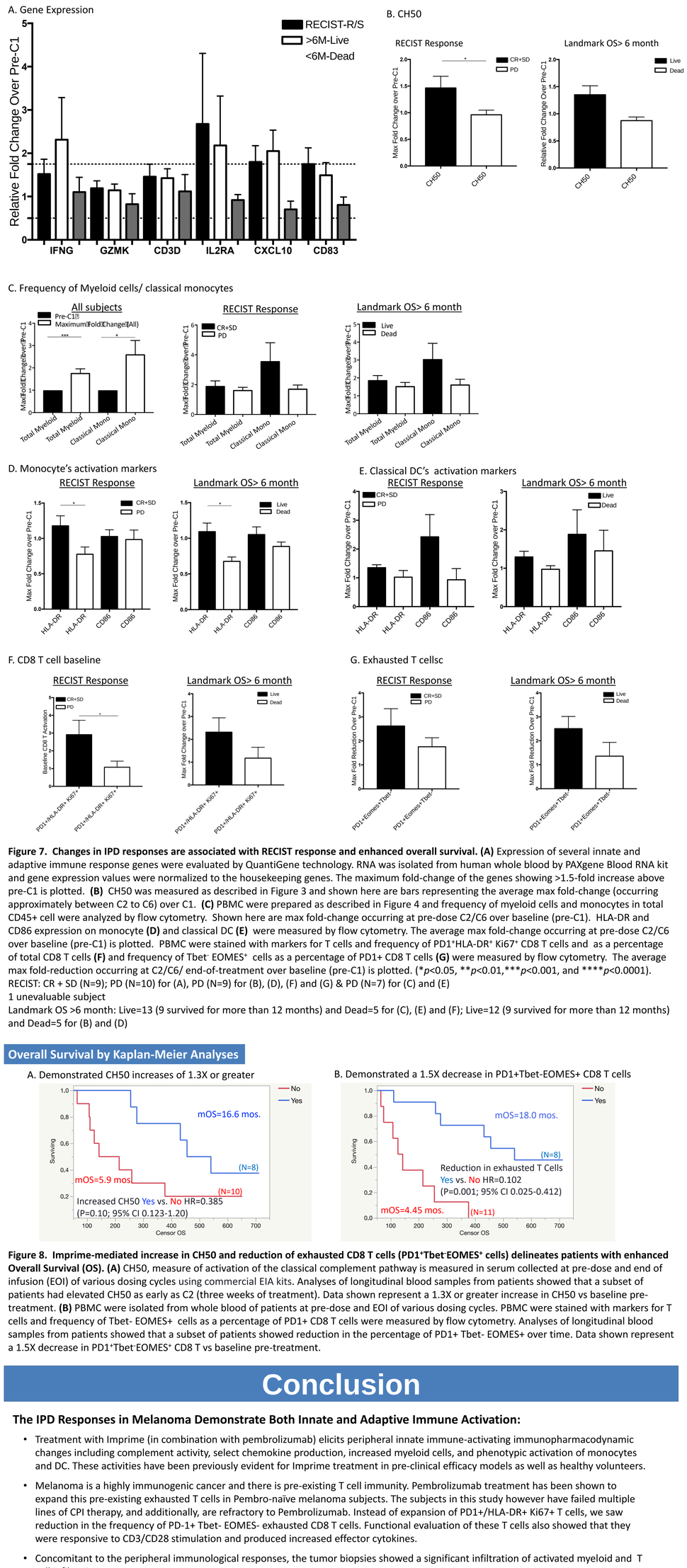


Figure 7. Changes in IPD responses are associated with RECIST response and enhanced overall survival. (A) Expression of several innate and adaptive immune response genes were evaluated by QuantiGene technology. RNA was isolated from human whole blood by PAXgene Blood RNA kit and gene expression values were normalized to the housekeeping genes. The maximum fold-change of the genes showing >1.5-fold increase above pre-C1 is plotted. **(B)** CH50 was measured as described in Figure 3 and shown here are bars representing the average max fold-change (occurring approximately between C2 to C6) over C1. **(C)** PBMC were prepared as described in Figure 4 and frequency of myeloid cells and monocytes in total CD45+ cell were analyzed by flow cytometry. Shown here are max fold-change occurring at pre-dose C2/C6 over baseline (pre-C1). HLA-DR and CD86 expression on monocyte **(D)** and classical DC **(E)** were measured by flow cytometry. The average max fold-change occurring at pre-dose C2/C6 over baseline (pre-C1) is plotted. PBMC were stained with markers for T cells and frequency of PD1$^+$HLA-DR$^+$ Ki67$^+$ CD8 T cells and as a percentage of total CD8 T cells **(F)** and frequency of Tbet$^-$ EOMES$^+$ cells as a percentage of PD1+ CD8 T cells **(G)** were measured by flow cytometry. The average max fold-reduction occurring at C2/C6/ end-of-treatment over baseline (pre-C1) is plotted. (*p<0.05, **p<0.01,***p<0.001, and ****p<0.0001).
RECIST: CR + SD (N=9); PD (N=10) for (A), PD (N=9) for (B), (D), (F) and (G) & PD (N=7) for (C) and (E)
1 unevaluable subject
Landmark OS >6 month: Live=13 (9 survived for more than 12 months) and Dead=5 for (C), (E) and (F); Live=12 (9 survived for more than 12 months) and Dead=5 for (B) and (D)

Overall Survival by Kaplan-Meier Analyses

Figure 8. Imprime-mediated increase in CH50 and reduction of exhausted CD8 T cells (PD1$^+$Tbet$^-$EOMES$^+$ cells) delineates patients with enhanced Overall Survival (OS). (A) CH50, measure of activation of the classical complement pathway is measured in serum collected at pre-dose and end of infusion (EOI) of various dosing cycles using commercial EIA kits. Analyses of longitudinal blood samples from patients showed that a subset of patients had elevated CH50 as early as C2 (three weeks of treatment). Data shown represent a 1.3X or greater increase in CH50 vs baseline pre-treatment. **(B)** PBMC were isolated from whole blood of patients at pre-dose and EOI of various dosing cycles. PBMC were stained with markers for T cells and frequency of Tbet- EOMES+ cells as a percentage of PD1+ CD8 T cells were measured by flow cytometry. Analyses of longitudinal blood samples from patients showed that a subset of patients showed reduction in the percentage of PD1+ Tbet- EOMES+ over time. Data shown represent a 1.5X decrease in PD1$^+$Tbet$^-$EOMES$^+$ CD8 T vs baseline pre-treatment.

Conclusion

The IPD Responses in Melanoma Demonstrate Both Innate and Adaptive Immune Activation:

- Treatment with Imprime (in combination with pembrolizumab) elicits peripheral innate immune-activating immunopharmacodynamic changes including complement activity, select chemokine production, increased myeloid cells, and phenotypic activation of monocytes and DC. These activities have been previously evident for Imprime treatment in pre-clinical efficacy models as well as healthy volunteers.
- Melanoma is a highly immunogenic cancer and there is pre-existing T cell immunity. Pembrolizumab treatment has been shown to expand this pre-existing exhausted T cells in Pembro-naïve melanoma subjects. The subjects in this study however have failed multiple lines of CPI therapy, and additionally, are refractory to Pembrolizumab. Instead of expansion of PD1+/HLA-DR+ Ki67+ T cells, we saw reduction in the frequency of PD-1+ Tbet- EOMES- exhausted CD8 T cells. Functional evaluation of these T cells also showed that they were responsive to CD3/CD28 stimulation and produced increased effector cytokines.
- Concomitant to the peripheral immunological responses, the tumor biopsies showed a significant infiltration of activated myeloid and T cell infiltration.
- The association between the clinical responses and the innate/adaptive immune responses are suggestive of interplay between the therapeutic mechanisms of Imprime and pembrolizumab.