**Abstract**

**Background:** Though efficacious, checkpoint inhibitor (CPI) monotherapy fails to elicit response in the majority of patients. TNBC is one such cancer type where CPI antibodies (pembrolizumab, avelumab, atezolizumab) have demonstrated only a ~5-10% response rate, irrespective of PD-L1 expression. We are developing Imprime PGG (Imprime), a novel yeast derived β-glucan PAMP in combination with pembrolizumab, to enhance the benefit that TNBC patients derive from CPI-based therapy.

**Methods:** In this analysis, we present the serum and cellular IPD responses elicited by Imprime and pembrolizumab in the peripheral blood of 12 TNBC subjects who previously failed front-line chemotherapy, enrolled as part of a Phase 2 study (NCT02981305). Subjects received Imprime (4 mg/kg qw) + pembrolizumab IV (200 mg qw) in 3 week cycles. Anti-beta glucan antibodies (ABA), circulating immune complexes (CIC), complement activation, cytokine production, gene expression changes, and phenotypic changes on immune cells were evaluated.

**Results:** As Imprime is known to complex with serum IgG ABA, a drop in the free ABA levels and a concomitant increase in the CIC was observed at the end of infusion (EOI) of every Imprime dose. Interestingly, 11 of 12 subjects showed increased ABA levels between cycles 1 and 2, with peak levels increasing ~1.5 to ~35 fold over baseline. In line with this ABA increase, peak levels in serum CIC levels (range ~3 to 22-fold) and complement protein SC5b-9 (~1.4 to 41-fold) were also observed at cycle 2 EOI. In a subset of patients, a maximum increase of ~30,000-fold in several chemokines was detected at cycle 2 EOI. Gene expression analyses of whole blood indicated peak activation of several genes at cycle 2 associated with activation of innate immune cells and T-cells. In 8 of 12 subjects, an increased frequency up to 11-fold in the CD16+ monocytes, cells known for their enhanced cytotoxicity as well as M1-polarizing functions, was observed between cycles 1 and 2. We also observed an increase, up to 2-fold, in CD16+ inflammatory DC in 8 of 12 subjects. The maximal increase (~4 to 20-fold) in newly proliferating (Ki67+/-), activated CD8 T cells (PD-1+ CD38+ HLA-DR+) was observed at cycle 2 in 4 subjects. Of all these immunological responses, robust cytokine production together with an increased frequency of activated CD8 T cells, correspond with objective tumor responses.

**Conclusions:** These data provide the first evidence in cancer patients that Imprime can drive the critical IPD changes known to be associated with efficacy in preclinical cancer models.

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**Results**

**Evidence of Imprime-ABA Immune Complex Formation In Vivo**

**Peripheral Immunological Responses Associated with Clinical Response**

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**Summary**

- For the first time, this study provides evidence for Imprime-ABA immune complex formation and the downstream peripheral innate and adaptive immune activation responses in cancer subjects.
- For the first time in TNBC patients, treatment with Imprime (in combination with Pembrolizumab) elicits peripheral innate immune-activating immunopharmacodynamic changes including complement activity, select chemokine production, and phenotypic activation of monocytes and DC. These activities have been previously evident in pre-clinical efficacy models as well as healthy volunteers.
- The strong association between the clinical responses and the innate/adaptive immune responses are suggestive of interplay between the therapeutic mechanisms of Imprime and pembrolizumab.

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