EFFECTS OF CHEMOIMMUNOTHERAPY ON THE PERIPHERAL BLOOD: INSIGHTS FROM IMMUNE MONITORING OF A PHASE IB TRIAL OF PEMBROLIZUMAB AND PACLITAXEL OR CAPECITABINE FOR TRIPLE-NEGATIVE BREAST CANCER (TNBC)

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Background Pembrolizumab plus curative-intent dose-dense anthracycline-based chemotherapy (ddAC) is associated with improved outcome in PD-L1-negative TNBC,¹ whereas in the metastatic setting, clinical benefit of chemoimmunotherapy (taxane or gemcitabine/carboplatin) is restricted to PD-L1-positive patients.² We hypothesize that this discordance could be related to immunomodulatory differences of the various chemotherapies. On-treatment serial monitoring of peripheral blood and tumoral T cells can be used to compare the effects of various regimens. We also hypothesize that T cell clonal expansion may differ across the regimens, and that tumor-enriched T cell clones are more likely to be tumor-reactive and expand following chemoimmunotherapy.

Methods Blood and tumor samples were collected from patients enrolled in a phase Ib clinical trial of palliative pembrolizumab and paclitaxel or capecitabine for metastatic TNBC, and from a contemporaneous cohort of patients treated with ddAC. T-cells were characterized using fresh whole blood flow cytometry and T-cell receptor (TCR) immunosequencing (immunoSEQ, Adaptive Biotechnologies) of DNA digests. Longitudinal regression was used to test the hypothesis that tumor-enriched T-cell clonotypes are more likely to expand in peripheral blood following therapy.

Results When combined with pembrolizumab, paclitaxel versus capecitabine had similar effects on T-cells, resulting in a time-dependent lymphodepletion across all major T cell subsets (average CD3+ T cell fold-change capecitabine: -0.42, paclitaxel: -0.56, $p = 0.80$ t-test), whereas ddAC was associated with more profound lymphodepletion (CD3+ average fold-change: -1.21). Notably, ddAC was associated with higher odds of novel clonotype detection compared to capecitabine (odds ratio (OR): 3.42, 95% CI: 3.34–3.5) as well as compared to paclitaxel (OR: 1.53, 95% CI: 1.47–1.60). Significant expansion of tumoral clonotypes occurred in five patients receiving chemoimmunotherapy (average 4.2 unique clonotypes per patient, range 2–11). These clonotypes did not significantly expand over time in the blood. Similarly, T-cell clonotypes that were enriched within tumor did not exhibit measurable differences in serial trend within the peripheral blood.

Conclusions Effects to T cell subsets and clonotypes are similar between capecitabine and paclitaxel when combined with pembrolizumab. ddAC was more profoundly lymphotoxic, but resulted in greater clonotype expansion. These findings offer mechanistic insight onto the differences in clinical activity observed with chemoimmunotherapy in early stage versus metastatic TNBC. We observed no strong association between tumor clonotype enrichment and peripheral clonotype expansion, highlighting the unmet need to develop methods of monitoring tumor-reactive T cell clones in the context of immunotherapy.

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Ethics Approval All patients provided written, informed consent. The study protocols for the collection of specimens from the early-stage breast cancer cohort and from the metastatic TNBC clinical trial were separately approved by independent review boards at Providence Portland Medical Center and Cedars Sinai Medical Center (mTNBC clinical trial only).

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