896 CHARACTERIZING MACROPHAGE HETEROGENEITY IN HR+/HER2- METASTATIC BREAST CANCER REVEALS NOVEL MECHANISMS OF RESISTANCE

[1]Kelly F Zheng*, [2,3]Kenichi Shimada, [4]Madeline G Townsend, [5]Tianyu Li, [5]Nabihah Tayob, [6]Molly DiLullo, [6]Eileen Wrabel, [7]Elizabeth A Mittendorf, [6]Tanya Keenan, [6]Romualdo Barroso-Sousa, [8,9]Sandro Santagata, [6]Sara M Tolaney, [9]Peter K Sorger, [1,6,9]Jennifer L Guerriero. *[1]Brigham and Women's Hospital, Boston, MA, USA; [2]Harvard Medical School, Boston, MA, USA; [3]Division of Breast Surgery, Department of Surgery, Brigham and Women's Hospital, Boston, MA, USA; [4]Brigham and Women's Hospital, Brookline, MA, USA; [5]Department of Data Science, Dana-Farber Cancer Institute, Boston, MA, USA; [6]Breast Oncology Program, Dana-Farber Cancer Institute, Boston, MA, USA; [7]Dana-Farber Brigham Cancer Center, Boston, MA, USA; [8]Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA; [9]Laboratory of Systems Pharmacology, Department of Systems Biology, Harvard Medical School, Boston, MA, USA*

Background Few patients with hormone receptor positive (HR+) metastatic breast cancer (MBC) experience therapeutic benefit from inhibitors against programmed cell death 1 (PD-1) or its ligand (PD-L1). Using whole transcriptome and whole exome sequencing of tumor samples from a randomized phase 2 trial of eribulin +/- pembrolizumab for patients with HR+/HER2- MBC, we showed that patients who derived clinical benefit had increased antigen presentation machinery and IFNg-response genes compared to patients that did not achieve clinical benefit, which did not differ between treatment arms. This data suggests that baseline subsets of immune cells may predict primary response in patients with HR+ MBC.

Methods We utilized single cell, multiplex cyclic immunofluorescence (CyCIF) on pretreatment, formalin-fixed paraffin-embedded tumor samples (n=29; table 1) from the trial of eribulin +/- pembrolizumab in HR+/HER- MBC to identify tumor cells, fibroblasts, and immune cells and to also characterize their states. We calculated cell type frequencies and associated them with measures of clinical outcome such as progression free survival (PFS) and overall survival (OS). Cox proportional hazard models were used to analyze PFS and OS in SAS 9.4.

Results Spearman correlation between cell type frequencies across all samples revealed that T cells (Thelper, Treg, CD8T), B cells, and macrophages (CD163+, CD68+/CD163+ cells), were strongly correlated, indicating that these cell types are, on average, more abundant in samples with high immune infiltration. Survival analysis using cell type numbers as continuous variables showed higher baseline levels of immune cells in patients who experienced longer PFS and OS. Specifically, T cells were associated with better clinical outcome as those characterized as CD8+ or CD8+PD1- were associated with longer PFS, TregPD1- T cells were associated with longer OS and ThelperPD1- frequency was associated with longer PFS and OS independent of treatment arm (table 2). Macrophages were generally associated with worse clinical outcome. Macrophage subset CD68+CD163-PDL1+DPB1- was associated with shorter PFS and the CD68+CD163+PDL1-DPB1+ and CD68-CD163+PDL1-DPB1- subsets were associated with shorter OS, whereas the CD68-CD163+PDL1+DPB1+ population was marginally associated with longer PFS (table 3).

Conclusions Our work confirms that infiltration of T cells into metastatic HR+ breast cancers is a key feature associated with those who benefit from either chemotherapy or chemotherapy plus immunotherapy. The analysis also suggests that the presence of specific macrophage subsets is associated with worse clinical outcomes (figure 1). Deep phenotyping of the myeloid compartment on serial sections by CyCIF is ongoing.

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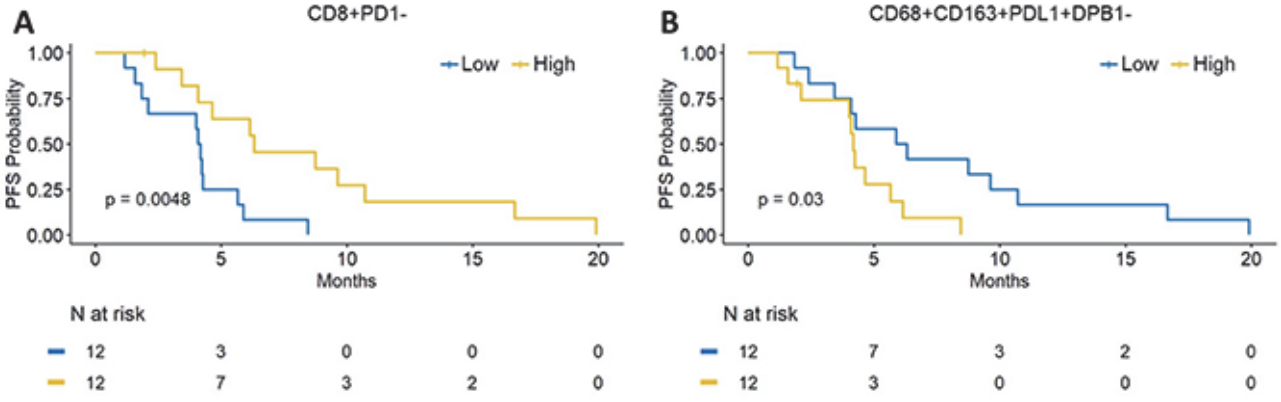
Trial Registration ClinicalTrials.gov Registration: NCT03051659.

Ethics Approval This study was approved by DFCI institution's Ethics Board; approval number 16–577.

Abstract 896 Table 1 Pretreatment formalin-fixed paraffin-embedded tumor samples were collected retrospectively and prospectively as archival primary, metastatic or baseline prior to initiating protocol therapy.

	Primary	Metastatic	Baseline	Total
Eribulin	7	3	2	12
Eribulin + Pembro	11	2	4	17
Total N (%)	18 (62%)	5 (17%)	6 (21%)	29 (100%)



Abstract 896 Figure 1 Kaplan-Meier curves of PFS stratified by biomarkers, median values as cutoff points

Abstract 896 Table 2 T cell association with PFS and OS. n.s., not significant; Treg, Tregulatory cell.

	PFS (p-value)	OS (p-value)	prognosis
CD8+	0.041	n.s.	good
CD8+PD1-	0.028	n.s.	good
TregPD1-	n.s.	0.039	good
ThelperPD1-	0.065	0.097	good

Abstract 896 Table 3 Macrophage association with PFS and OS. n.s., not significant.

	PFS (p-value)	OS (p-value)	prognosis
CD68+CD163-PDL1+DPB1-	0.025	n.s.	poor
CD68-CD163+PDL1+DPB1+	0.071	n.s.	good
CD68+CD163+PDL1-DPB1+	n.s.	0.020	poor
CD68-CD163+PDL1-DPB1-	n.s.	0.037	poor

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