



Heterogeneity of Epithelial, Mesenchymal, Progenitor, and Stem Cell Phenotypes on Circulating Tumor Cells Correlates to Clinical Outcomes in Metastatic Cancer Patients

Alexis B. Duffy¹, Massimo Cristofanilli², Carolina Reduzzi², Cha-Mei Tang³, Saranya Chumsri⁴, Susan Tsai⁵, Mohammed Aldakkak⁶, Daniel L. Adams¹

¹Creatv MicroTech, Monmouth Junction, NJ 08852, ²Weill Cornell Medicine, New York, NY 10065, ³Creatv MicroTech, Rockville, MD 20850, ⁴Mayo Cancer Clinic, Jacksonville, FL 32224, ⁵Ohio State University, Columbus, OH 43221, ⁶Medical College of Wisconsin, Milwaukee, WI 53226

ABSTRACT

Circulating tumor cells (CTCs) are cancer cells that detach from primary tumors, enter the blood and seed distant metastatic sites. CTCs spread as single cells, or cluster aggregates, and can be heterogenous with differing biological phenotypes, i.e. epithelial, mesenchymal, progenitor, and stem cell, or phagocytosed by tumor macrophages. Three clinically relevant circulating subtypes are often described in patients 1) CTCs with normal epithelial traits (strong filamentous cytokeratin (CK+) & CD45-/CD14-), 2) Epithelial-mesenchymal CTCs (EMTs), cancer cells with downregulated epithelial & upregulated mesenchymal traits (low diffused CK+, CD45-/CD14-) and 3) tumor-macrophage fusion cells (TMFCs), cancer cells that phagocytose/fuse with macrophages (polynucleated DAPI+, diffuse CK+ and CD14+/CD45+) (**Figure 1**). To better elucidate the biological and clinical importance of the 3 subtypes, we isolated them by a marker agnostic method from the blood of n=49 metastatic cancer patients. We then enumerated and phenotypically subtyped the 3 cell populations comparing their clinical utility of clustering and heterogeneity over 2 years.

Figure 1. CTC Subtypes show varying levels of biological phenotypes

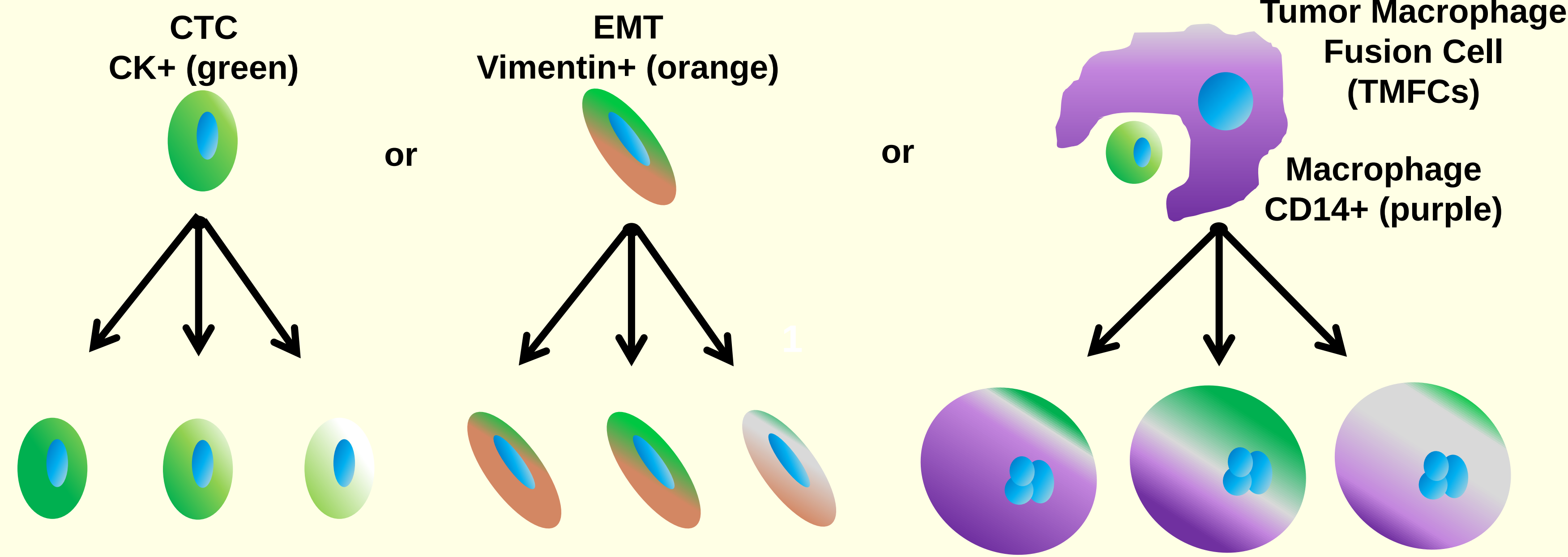


Table 1: Biomarkers on Cell Types

+: Expressed
+/-: Mixed Expression
-: Not Expressed

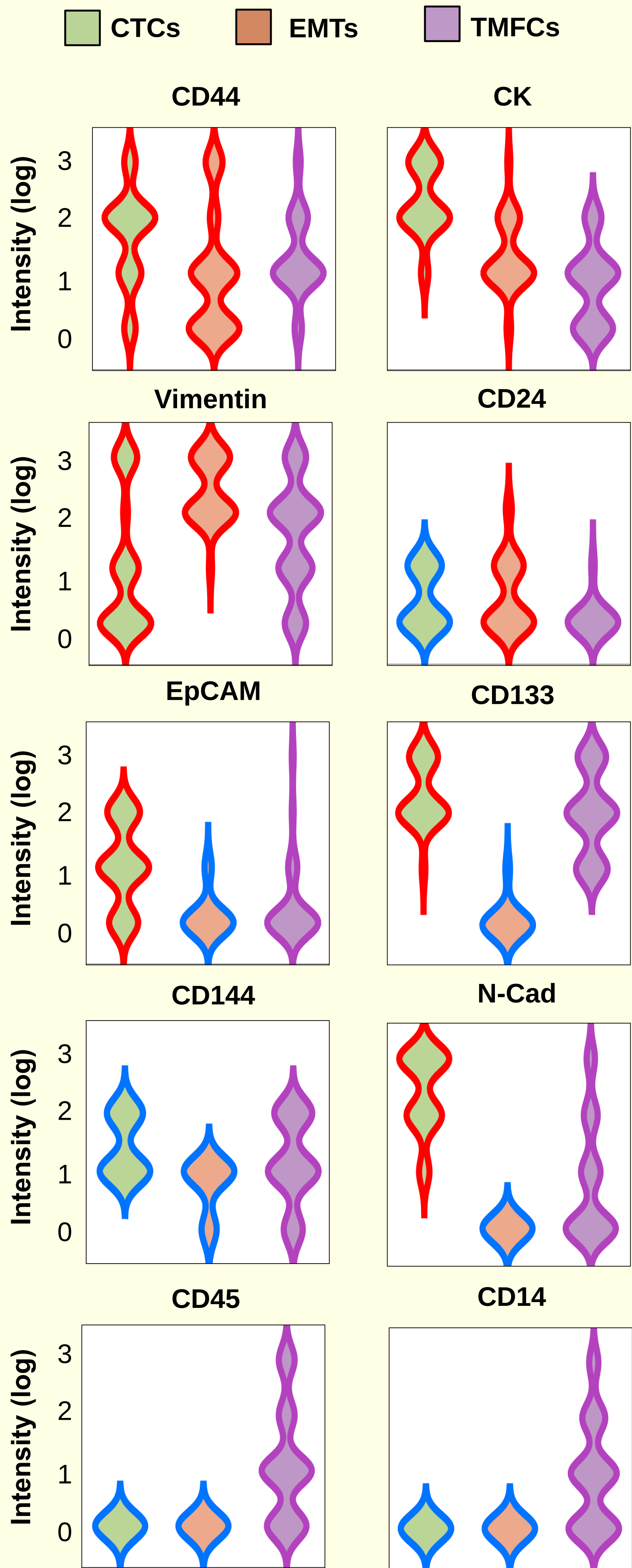
	Macrophage	Stem Cell	Epithelial	Mesenchymal	WBC
CD45	++	+/-	-	+/-	++
CD14	++	+/-	-	-	+/-
CD44	+	++	+/-	+	+
CD24	-	-	-	+	+
CD133	+/-	++	-	+	-
CK	-	+/-	++	-	-
EpCAM	-	+/-	++	-	-
CD144	-	+/-	-	-	-
Vimentin	+	+	-	++	+
N-Cad	-	+	-	++	-

MATERIALS & METHODS

52 peripheral blood samples were collected from 49 metastatic cancer patients, breast (n=35), pancreatic (n=9), colon (n=3), lung (n=1), and prostate (n=1). Whole blood (7.5mL) was filtered by size exclusion using CellSieve™ microfilters and fluorescently stained for CD45, CD14, CK, and DAPI to identify CTCs, EMTs and TMFCs. Cells were imaged, enumerated and fluors quenched by the QUAS-R technique; followed by re-stained for CD44, CD24, CD133, CD144, N-Cadherin, EpCAM, and Vimentin (**Table 1**). All markers were categorized as negative, low, medium, or high. A heterogeneity biomarker score was developed as high, 2-3 log deviations between marker expression, or low 0-1. Patient progression free survival (PFS) and overall survival (OS) were analyzed over 2 years.

Figure 2. Biomarker Heterogeneity of Cells

Violins with 0-1 log division shows low heterogeneity, while ≥ 2 log division shows high heterogeneity. Outlines: Blue=Low CTC heterogeneity, Red=High CTC heterogeneity. Purple=TMFC heterogeneity



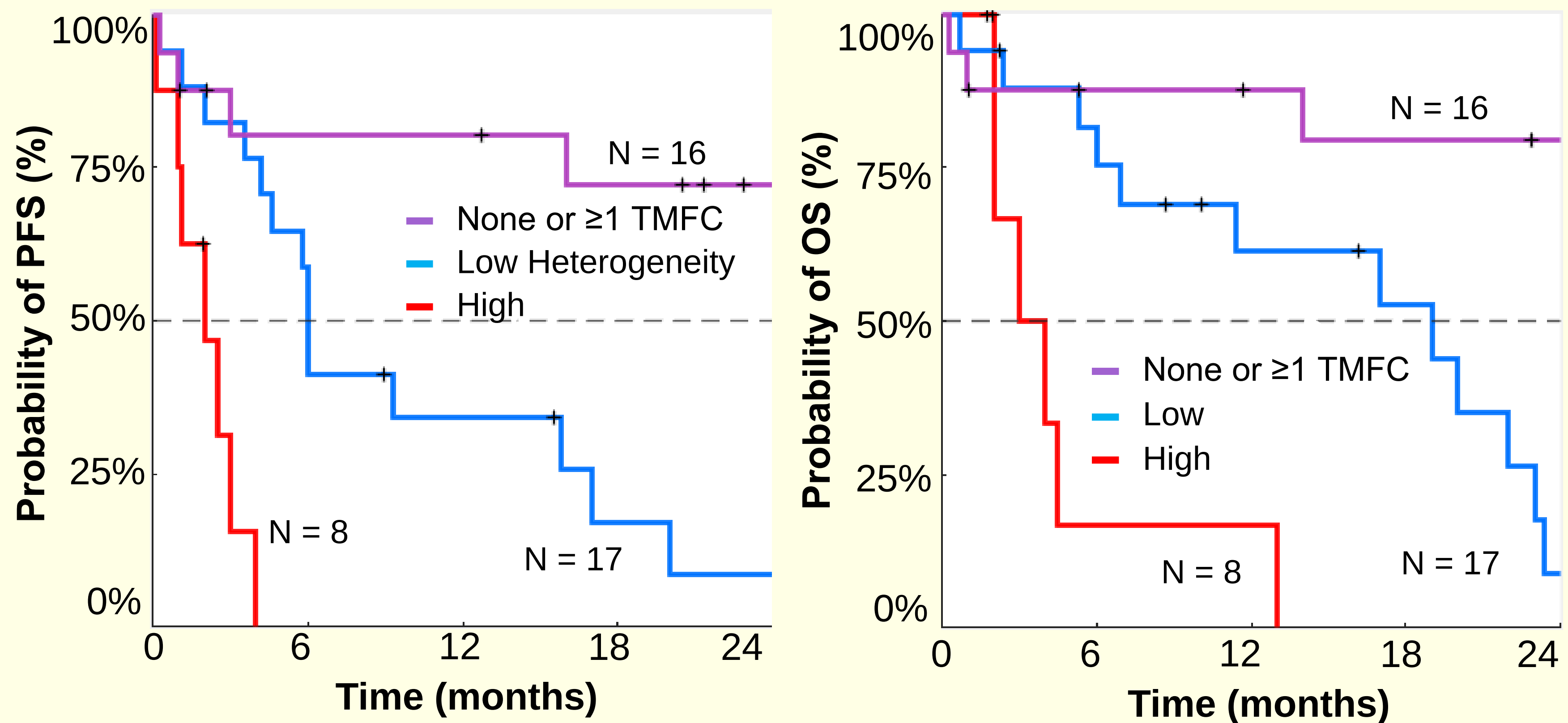
RESULTS

- CTCs were found in 42% of samples (n=22/52), EMTs in 54% (n=28/52), CTCs or EMTs in 75% (n=39/52), and TMFCs in 92% (n=48/52)
- All populations showed high levels of heterogeneity in biomarkers (**Figure 2**).
- Patients with any CTCs or EMTs correlated with poor PFS and OS
- Patients with highly heterogeneous CTCs or EMTs correlated with even poorer PFS (HR 15.4) and OS (HR 12.4) than homogeneous CTCs or EMTs (**Figure 3**)
- Highly heterogeneous TMFCs did not correlate with PFS and OS

CONCLUSIONS

- Presence of CTCs or EMTs correlates with poor outcomes
- Some biomarkers showed notable expression variations across cell types and patients
- Large variation in CTCs or EMTs correlated with even worse clinical outcomes than cells with low deviations, while large deviations in TMFCs did not correlate to worse clinical outcomes
- Expanded, ongoing studies are being conducted comparing treatment types in these cell populations

Figure 3. PFS and OS of varying Levels of Heterogeneity in CTCs and EMTs



Groups	HR	95% CI	P-Value	HR	95% CI	P-Value
Low vs None	4.3	1.6-11.3	0.0070	5.1	1.8-14.7	0.0054
High vs None	14.6	3.3-65.2	0.0018	19.0	3.6-101.2	0.0024
High vs Low	15.4	3.4-68.9	0.0014	12.4	2.6-59.1	0.0056

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