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Identifying and Stratifying Circulating Tumor Cell Cluster Subtypes in Metastatic Breast Cancer Patients and Evaluating their Clinical Outcomes

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ABSTRACT

Circulating tumor cell clusters (CTCCs) are aggregated groups of tumor cells that detached from primary tumors and circulate in the bloodstream. However, while Circulating Tumor Cells (CTCs) are a well studied phenomenon, CTCCs remain relatively unexplored and ill-defined, with only initial studies evaluating their clinical utility. Adding to the CTCC complexity is that various subtypes exist (**Fig 1**), including homotypic clusters made of only tumor cells and heterotypic CTCCs made of CTCs attached to immune/stromal white blood cells (WBCs). Furthermore, CTCs can undergo Epithelial-Mesenchymal Transition (EMT), a process where tumor cells downregulate epithelial traits and upregulate mesenchymal traits, and also form clustered EMTs (CEMTs). Further, CTCs can fuse with macrophages forming Tumor Macrophage Hybrid Cells (TMHCs), aka Cancer-Associated Macrophage-Like cells (CAMLs) when in circulation. We enumerated single CTCs, EMTs, and CAMLs, as well as homotypic CTCCs, heterotypic CTCCs and CEMTs from the blood of metastatic breast cancer (mBC) patients to quantify these CTC populations and assess their clinical utility by median progression free survival (mPFS) and median overall survival (mOS) over 24 months.

MATERIALS & METHODS

We enumerated the 6 populations from a prospective pilot study of n=79 mBC patients. Whole peripheral blood (7.5mL) was filtered and stained with cytokeratin (CK) & CD45/CD14 to identify CTCs. CTCs were defined as having an intact DAPI nucleus and strong filamentous CK. Homotypic CTCCs were defined as ≥2 CTCs attached together. Heterotypic CTCCs were defined as ≥1 CTC attached to ≥1 WBC. EMTs were defined as having DAPI nuclei and weak non-filamentous CK. CEMTS were defined as ≥2 EMTs. CAMLs were defined as having an enlarged polynucleated DAPI, and positive for CD45/CD14 or non-filamentous CK.

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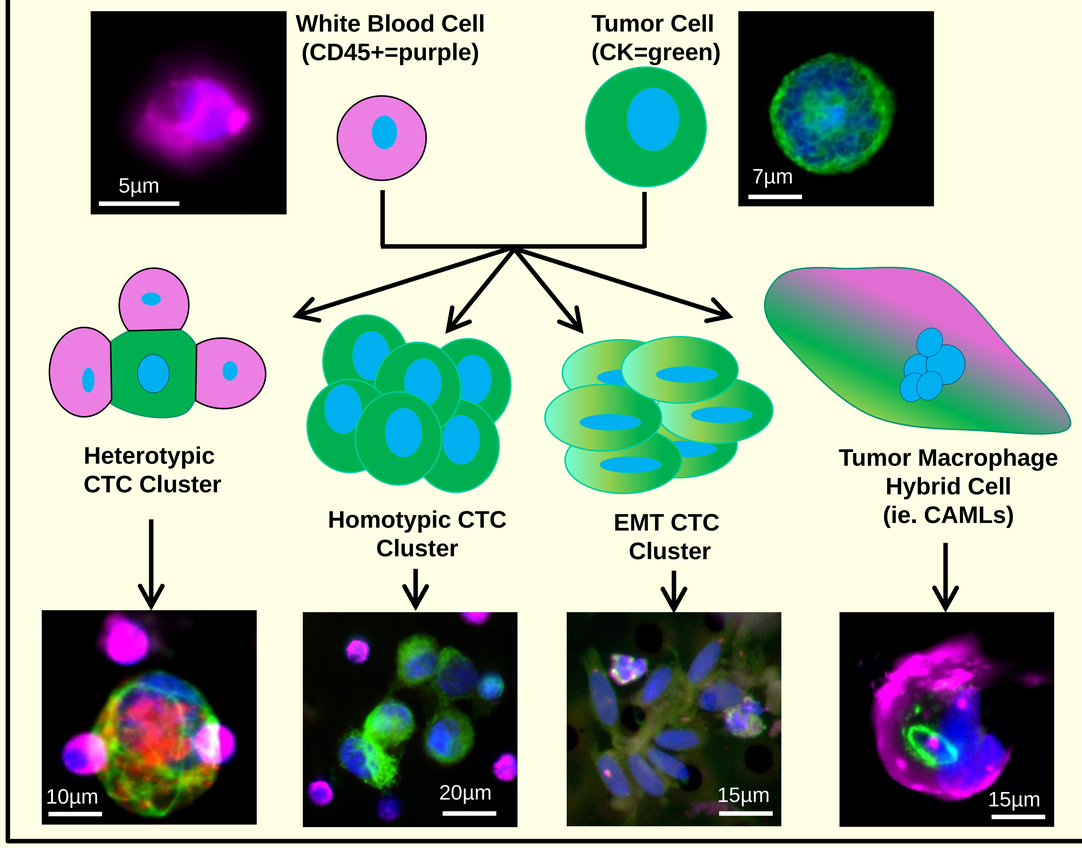
Figure 1. Diagram of the Different CTC Subtypes and Images from Metastatic Breast Cancer Patient's Blood



RESULTS

- Single CTCs were found in 57% of patients (n=34/79), homotypic CTCCs 15% (n=12/79), heterotypic CTCCs 66% (n=27/79), EMTs 56% (n=44/79), CEMTs 23% (n=18/79), any CAML in 97% (n=77/79), and Giant ≥50µm CAMLs in 84% (n=66/79) (**Table 1**)
- Over 24 months, patients with heterotypic CTCCs, homotypic CTCCs and Giant ≥50µm CAMLs had the worst PFS, followed by any CTCs, EMTs, and EMT clusters (**Figs 2 & 3**).
- Both CTCCs and CEMTs were rare in HER2+ patients at 8.7% (n=2/23) and 17.4% (n=4/23), respectively.

Table 1. Hazard ratio comparisons of CTCs and CTC Cluster Types

HR(95%CI) p value	Any CTC	Homotypic Cluster	Hetertypic Cluster	EMTCTC	EMTCTC Cluster	TMHC (ie ≥50µm CAML)
Present vs Absent	34 vs 45	12 vs 67	27 vs 52	35 vs 44	18 vs 61	66 vs 13
PFS	**2.0 (1.2-3.4) p=0.0202**	**5.5 (2.0-15.8) p=0.0032**	**2.7 (1.5-4.9) p=0.0027**	1.3 (0.7-2.2) p=0.4942	0.9 (0.5-1.7) p=0.8949	**2.3 (1.2-4.3) P=0.0151**
OS	2.0 (1.0-4.0) p=0.0849	2.0 (0.6-6.3) p=0.4139	**3.8 (1.7-8.3) p=0.0019**	1.4 (0.7-2.9) p=0.4025	2.0 (0.9-4.7) p=0.1502	**3.3 (1.5-7.5) P=0.0069**

Figure 2. PFS of Homotypic vs Heterotypic CTC Clusters

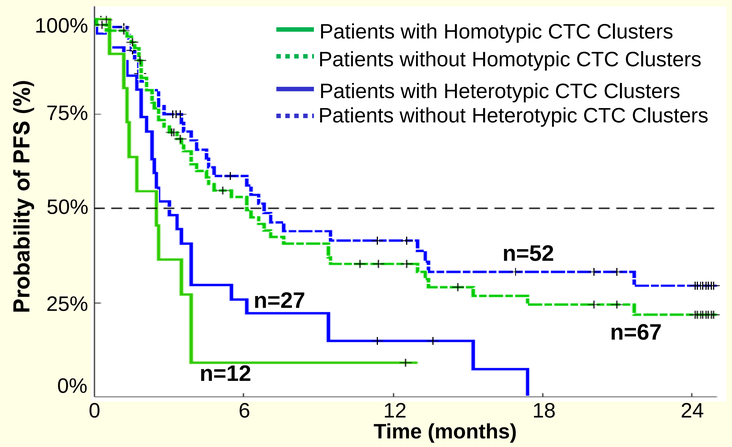
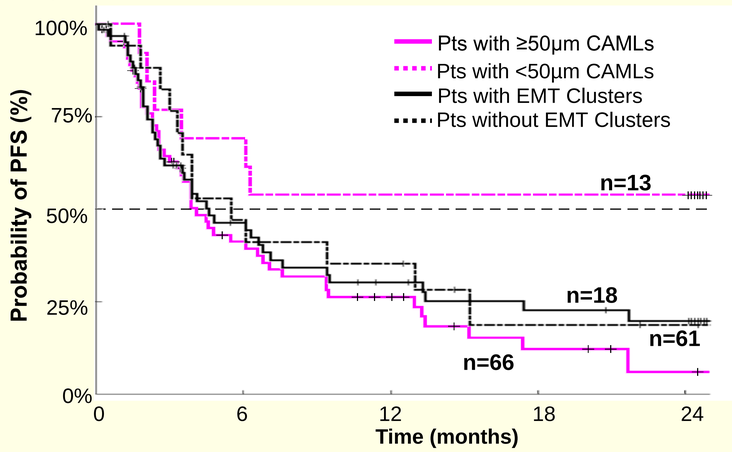


Figure 3. PFS of EMT Clusters vs ≥50µm CAMLs



CONCLUSIONS

- Despite no established definition, CTC Clusters appear to represent an array of subtypes with different biological and clinical meanings.
- We stratified and enumerated CTC cluster subtypes from the blood of mBC patients and compared them to clinical outcomes.
- CAML hyperploidy and CTC Clustering appears to indicate poor prognosis, though further understanding of their biology in tumor pathogenesis is needed.

REFERENCES

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