

Applying a mitotic index to circulating tumor cells and its prognostic significance: A cytological approach to patient stratification



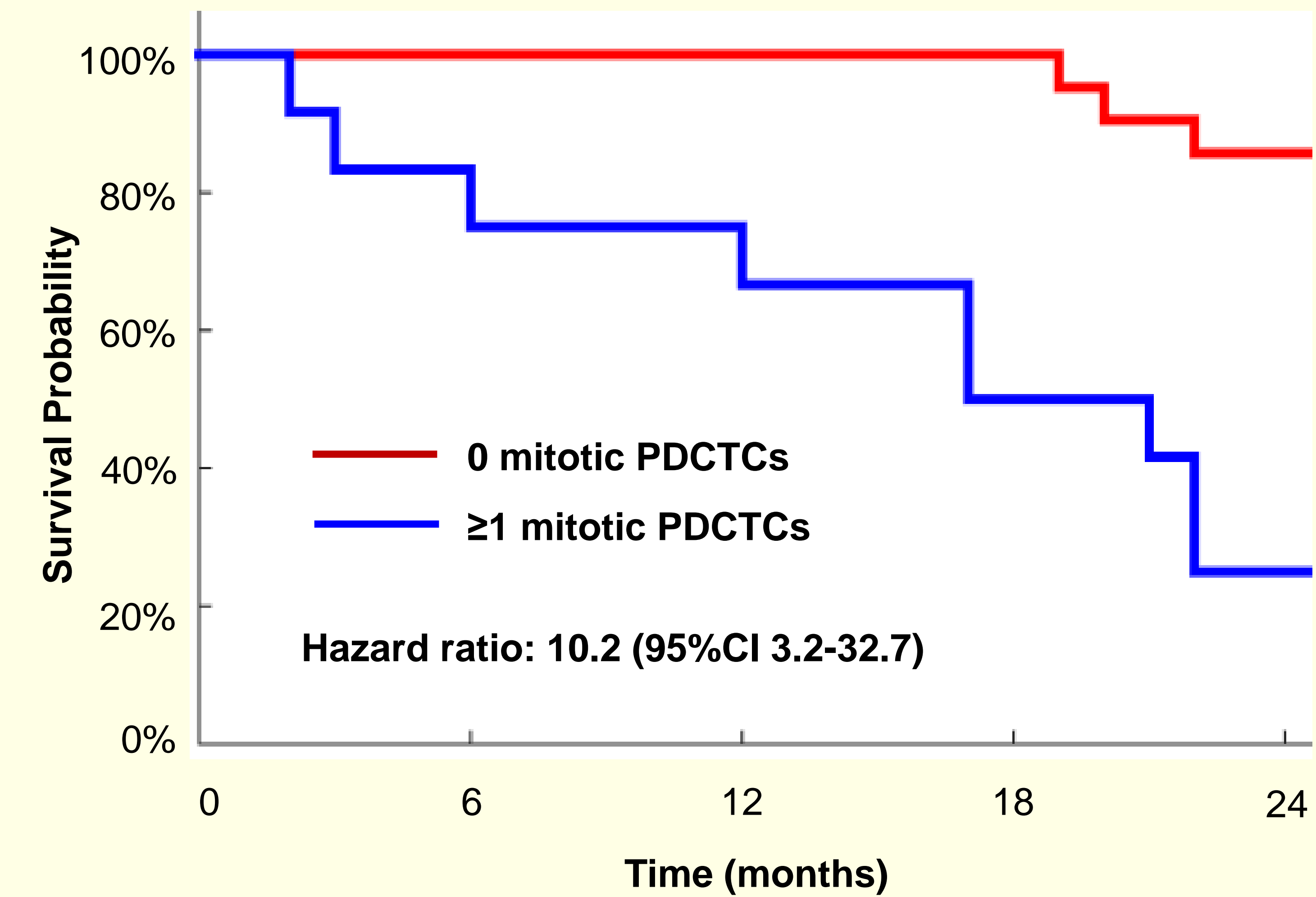
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ABSTRACT

It has been well documented that enumeration of Circulating Tumor Cells (CTCs) isolated from the peripheral blood of breast cancer patients can be used as a prognostic indicator of survival. Typically, CTC identification relies on immunohistochemical stains used in an absent/present method (i.e. CK+/EpCAM+/CD45-). However, the methodology for identification of CTCs is highly subjective, and histological cytology remains the standard identifier of cancer cells. We expand upon our work regarding the cytological criteria of CTCs to determine if pathological grading criteria can be applied to CTCs. We report the assessment for overall survival of late stage breast cancer patients in relation to CTC number and presence of active mitosis within the isolated CTCs.

Figure 3. Kaplan-Meier plot of patients with and without a mitotic CTC event (n=30).



RESULTS

- PDCTCs were found in 87% (26/30) of patient samples tested.
- 14 patients had ≥5 PDCTCs/7.5mL, of which 36% (5/14) survived 24 months: Hazard ratio:4.0 (95%CI 1.4-11.1)
- 16 patients had <5 PDCTCs/7.5mL, of which 81% (13/16) survived 24 months.
- 12 patients had ≥1 PDCTC with a mitotic event, of which 17% (2/12) survived 24 months: Hazard ratio:10.2 (95%CI 3.2-32.7)
- 18 patients had no mitotic PDCTCs, of which 89% (16/18) survived 24 months.

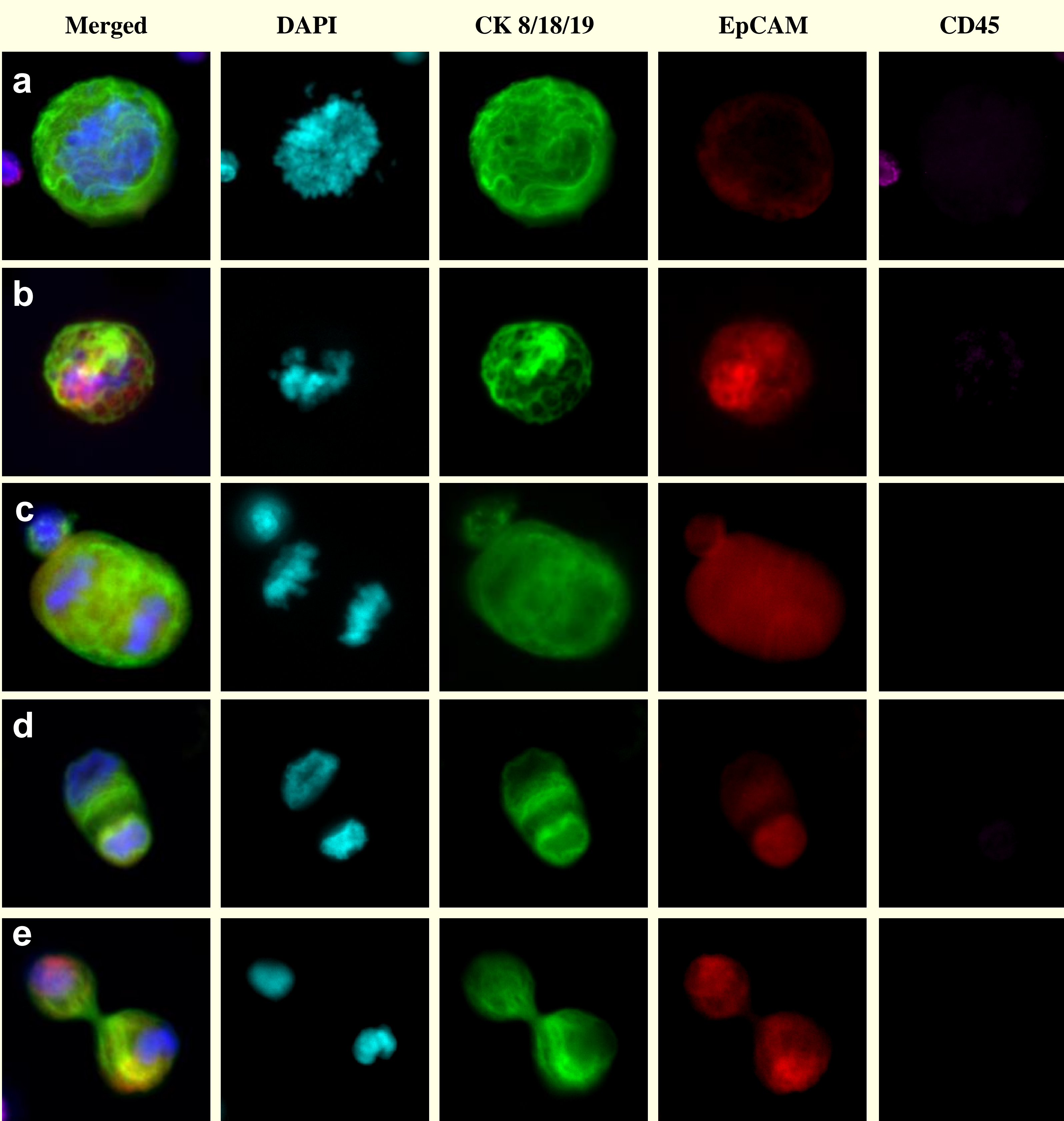


Figure 2. Various examples PDCTCs found in breast cancer patients identified as M phase: (a) Prophase, (b) Metaphase (beginning of anaphase), (c) Anaphase, (d) Telophase, (e) Cytokinesis (end of telophase).

CONCLUSIONS

- Low pressure microfiltration captures CTCs while retaining fine cellular architecture, such as mitotic events
- Stratification of breast cancer patients based on number of PDCTCs is a prognostic indicator of patient survival.
- Subtyping CTCs based on their mitotic index identifies a highly aggressive CTC subtype, as assessed by overall survival over a 24 month period
- CTC subtypes may indicate definable traits which may be exploited for personalized treatment of cancer patients

References

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2. Adams DL, et al "Circulating giant macrophages as a potential biomarker of solid tumors." *Proc Natl Acad Sci*, 111(9):3514-3519, 2014
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INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. CTC enumeration can be used to monitor therapy response and predict outcome.¹⁻⁴ However, CTC subtyping remains reliant on immuno-staining presence/absence, not the more standardized histopathological identification¹.

Low pressure microfiltration using CellSieve™ microfilters is a technique shown to isolate patient CTCs while retaining the fine architectural detail required for histopathology¹⁻². High resolution morphology can identify CTC subtypes, i.e. apoptotic CTCs, highly pleomorphic CTCs, and CTCs in active mitosis. That aggressive phenotypes are associated with these CTC populations, subtyping by phenotypic determinates may aid in identifying CTCs cellular status for diagnosis, prognosis and therapy determination.¹⁻⁴

MATERIALS & METHODS

A prospective pilot study of 30 single blinded Stage III/IV breast patient samples were provided by Fox Chase Cancer Center and University of Maryland Greenebaum Cancer Center. 7.5mL whole blood was diluted in pre-fixation solution and filtered by CellSieve™ microfiltration. Cells were fixed, permeabilized, and stained with DAPI, an antibody cocktail against CK 8/18/19, EpCAM, and CD45. CTCs were enumerated and identified as described by Adams et al.¹ CTCs were further subtyped by 1) number of pathologically definable CTCs (PDCTCs) (Figure 1), and 2) presence of mitotic events, identified by standard visual cues (e.g. prophase, anaphase, etc.) (Figure 2). Kaplan-Meier plots and Hazard ratios were determined at 24 months (Figure 3).