Micronuclei in Circulating Stromal Cells Correlated with PD-L1 Expression and Predicts Progression in Metastatic Breast Cancer

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ABSTRACT

Micronuclei (MN) are a result of biological DNA repair mechanisms forming due to internal chromosomal aberrations which indicate sub-clonal cancer populations with higher cell survivability and drug therapy resistance. MN are often observed as small fragments of nucleic acids excised from a primary nucleus in Circulating Stomal Cells (CStCs) as result of DNA damage[1,2]. CStCs with damaged DNA undergoing repair mechanisms, such as those that form MN, appear to have upregulated expression of programmed cell death ligand (PD-L1). We evaluated CStCs in metastatic breast cancer (mBC) patients for presence of MN and the cell's PD-L1 expression, to determine its prognostic significance to clinical outcomes.

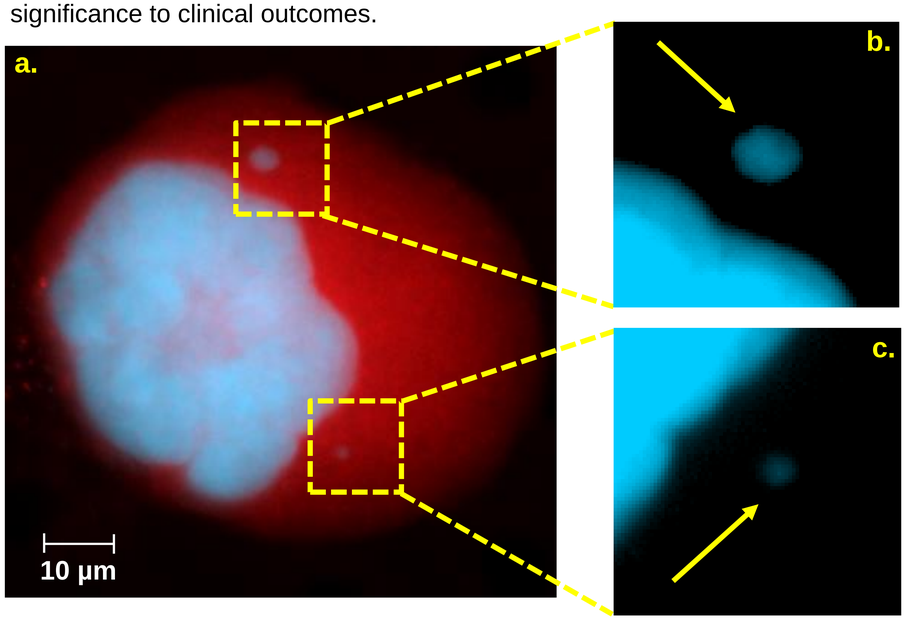

Figure 1. Micronuclei positive CStC (66µm diameter) stained with PD-L1 (red) and DAPI (blue). MN size varies from 4µm (Fig. b) to 2µm (Fig. c).

MATERIALS & METHODS

We enumerated MN formation in CStCs in a prospective pilot study using n=76 mBC patients starting new lines of treatment. Whole peripheral blood (7.5mL) was procured and filtered for CStCs and then stained for PD-L1[3]. DAPI was used to identify MN, defined by small (<3µm) DAPI+ circular formations within the cytoplasm, separate from the primary nucleus. We compared number of MN to PD-L1 expression of all CStCs, and MN presence to all available clinical variables. Patients' progression-free survival (PFS) and overall survival (OS) hazard ratios (HRs) were analyzed by censored univariate analysis based on RECIST v1.1 over two-years.

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Figure 2. PFS of Micronuclei in CStCs

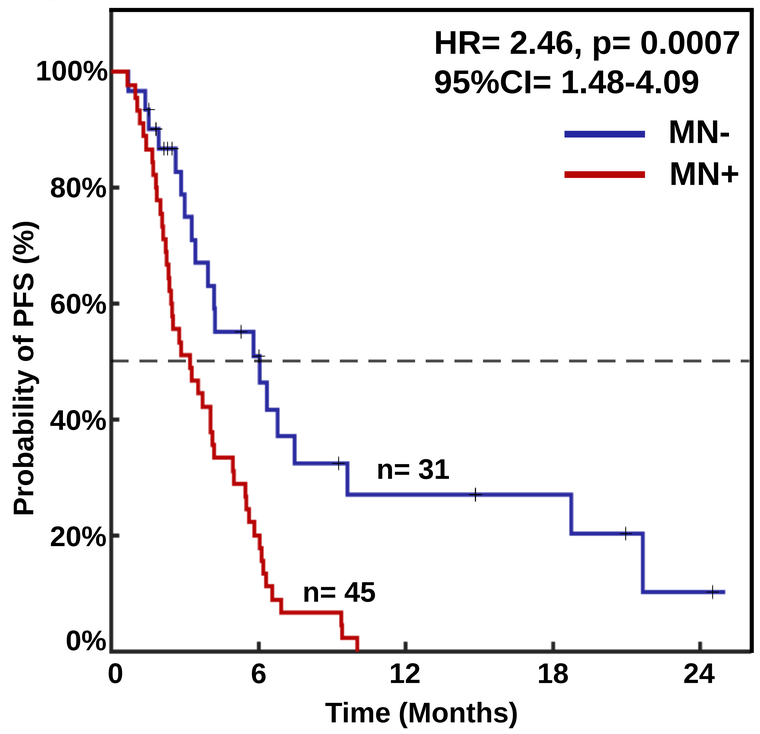
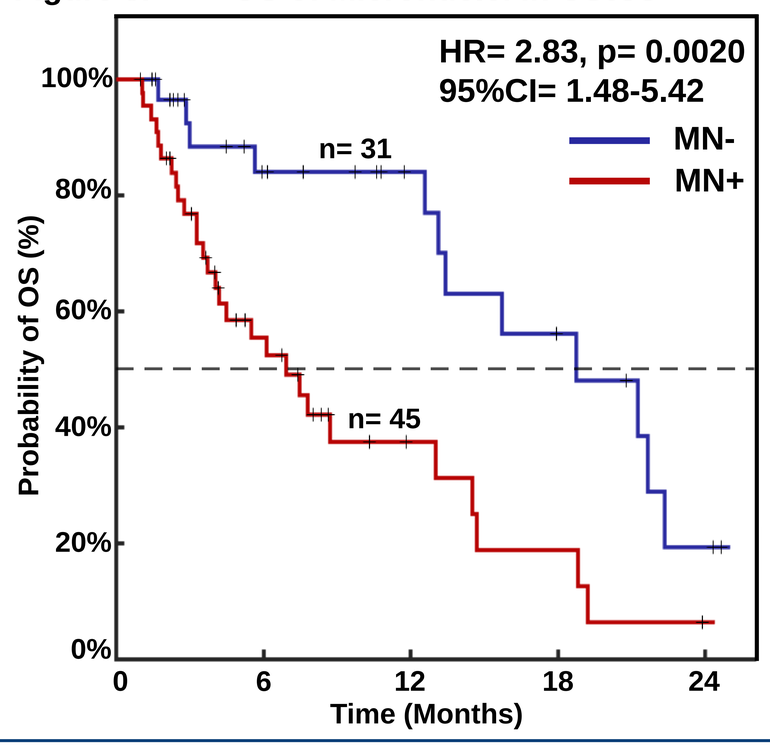


Figure 3. OS of Micronuclei in CStCs



RESULTS

- MN were identified in CStCs in 59% (n=45/76) of patients.
- MN positive CStCs had a significantly higher PD-L1 expression than MN negative CStCs **(p=0.0082), Figure 4**.
- Regression analysis identified a significant linear relationship between MN number and PDL-1 expression within CStCs (**R^2=0.9821, p=0.0089**).
- The presence of MN within CStCs was significantly prognostic for worse PFS and worse OS over 24 months (**Figures 2 & 3**).

CONCLUSIONS

- CStC MN formations in mBC are a type of observable biomarker that can represent an underlying DNA repair mechanism.
- MN formation may represent cellular survivability of sub-clonal cancer populations of more aggressive cancer subtypes which may have worse progression rates.
- Further studies to evaluate the effect of PD-1 immunotherapies in MN positive patients is ongoing.

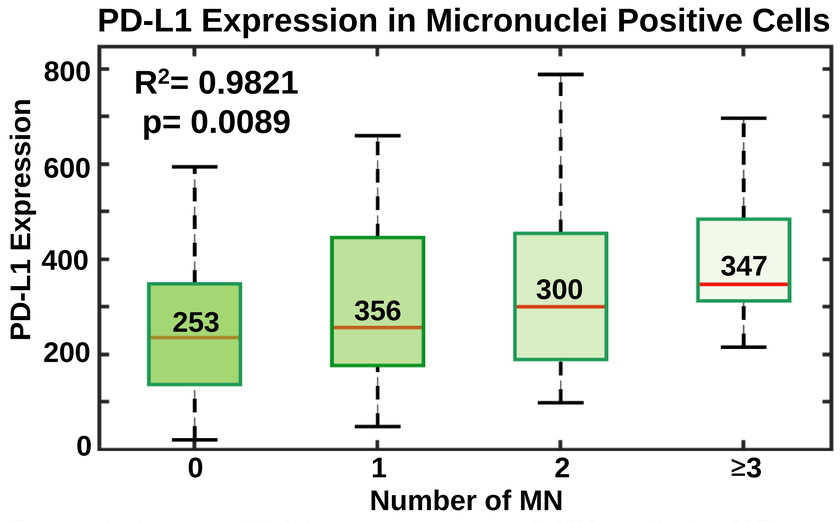


Figure 4. Average PD-L1 expression for all MN+ cells (n=185) was 338 while average expression in MN- cells (n=347) was 253. Median (red line), high error bar (max), low error bar (min).

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