The multi-switching activity of circulatory neutrophils in patients with Early Breast Cancer

A. Ramessur[1], B. Ambasager[1], R. C. Coombes[1*], I. Malanchi[2*]

[1]Imperial College London, [2] Francis Crick Institute, London.

*co-corresponding authors

MAP2021_138_Ramessur

Background

Neutrophils play a crucial role of the innate immune system in fighting bacterial infections and facilitating wound healing. Patients with Breast Cancer (BC) often display elevated levels of circulating neutrophils and a high neutrophil to lymphocyte ratio is associated with a worse disease-free survival in patients [1]. Pre-clinical evidence from mouse models of Early Breast Cancer has shown there are pro and anti-tumourigenic roles for neutrophils which implies that there may be heterogenous subsets of neutrophil populations [2]. CD62L (L-selectin) is a cell adhesion molecule found on the surface of neutrophils which helps them bind to the endothelium and migrate into tissues and is also rapidly shed upon neutrophil activation. Our laboratory found a lower proportion of neutrophils expressing CD62L in mouse models of Breast Cancer, which implies that these neutrophils have less endothelial- adhesive capacity and may remain in the circulation for longer. There is limited knowledge whether different circulatory neutrophil subsets are present in patients with Breast Cancer and if this is influenced by Breast Cancer subtype. In order to address these questions, we phenotyped neutrophils from Breast Cancer patients.

Aims and Methods

Aim: We used flow cytometry to investigate changes in neutrophils as a proportion of the total live cells in the circulation and differences in CD62L expression in neutrophils in patients with Early Breast Cancer (EBC) compared to healthy volunteers (HVs). Since kinases govern many key aspects of intracellular signalling, we assessed the kinase activity (n = 340 kinases) within neutrophils using a Pamgene kinase assay.

Method:

We recruited 37 patients with treatment naïve EBC (localised to the breast) and paired HVs (age matched) to compare the phenotype of neutrophils isolated from blood. 26 Patients with Oestrogen receptor positive (ER+) and 12 with Oestrogen receptor negative (ER –) EBC were recruited between June 2018 and September 2021. HER2 status was a variable in both groups (5 patients were ER+ HER2 + and 4 patients were ER- HER2+). No patient had overt metastatic disease and all were screened for exclusion factors.

Neutrophils were isolated from blood using Histopaque for flow cytometry and immunomagnetic negative selection for the kinase assay. Below is a schematic for the Pamgene kinase assay protocol (Figure 1a and 1b).

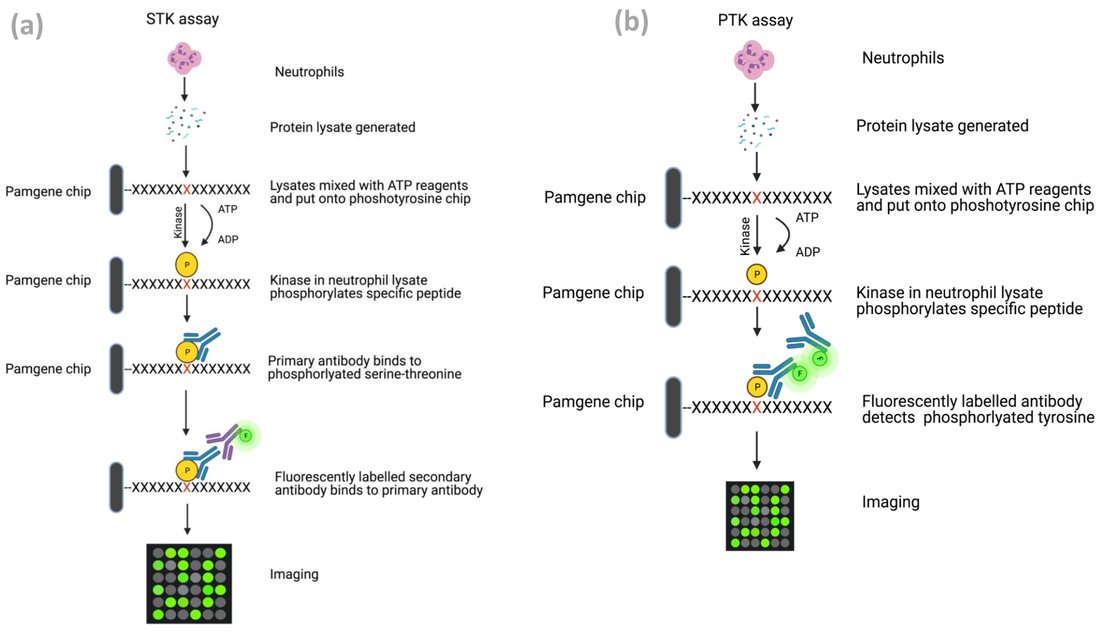
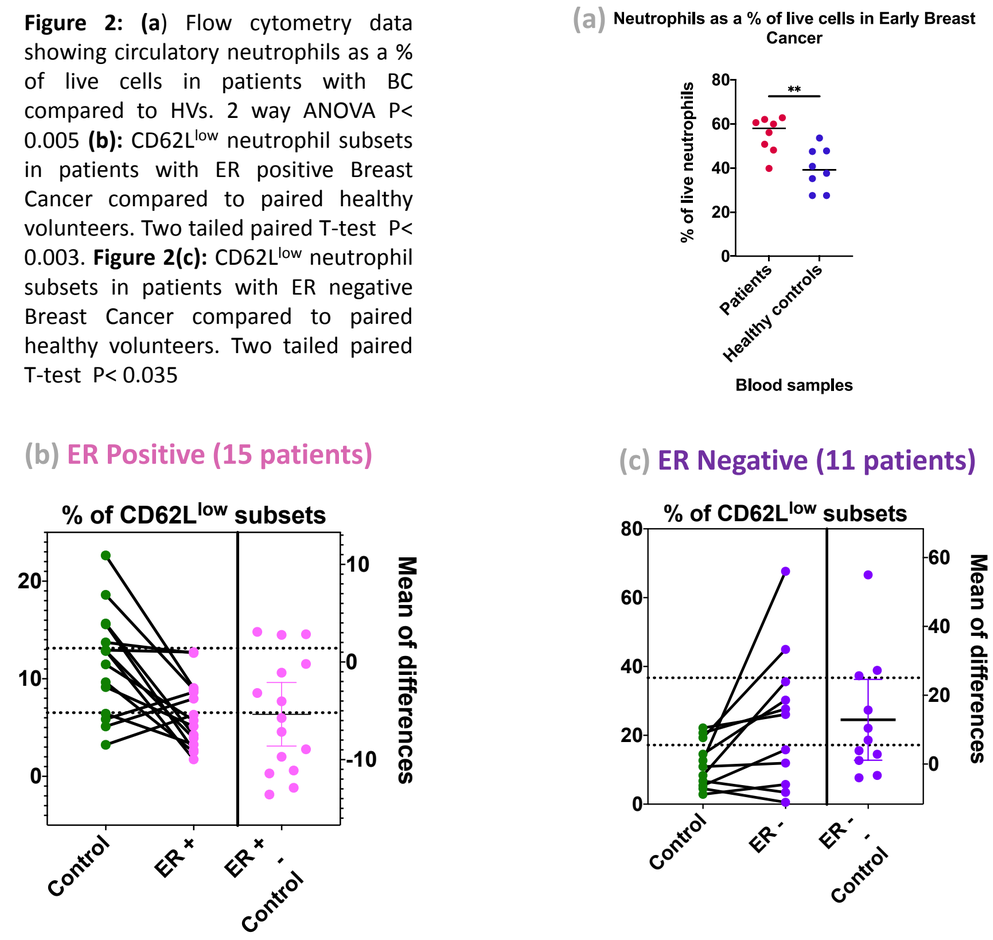


Figure 1a: Pamgene STK assay. Neutrophil lysates containing kinases are mixed with various reagents including ATP and primary antibodies which bind to phosphorylated serine/threonine residues. Fluorescently labelled secondary antibodies then bind to the primary antibodies and the signal is detected via imaging. **Figure 1b**: Pamgene PTK assay. Neutrophil lysates are mixed with reagents including ATP and fluorescently labelled antibodies which bind to phosphorylated tyrosine residues.

Results

The results show there is an increase in neutrophils as proportion of total cells within the circulation in patients with EBC compared to HVs (Figure 2a). ER+ patients had lower levels of $CD62L^{low}$ neutrophil subsets compared to HVs ($P< 0.003$) (Figure 2b). In contrast, there was a significant increase in $CD62L^{low}$ neutrophil subsets for ER- patients compared to HVs ($P< 0.035$) (Figure 2c). There was a general upregulation in activity within the Tyrosine Kinase (TK) family in neutrophils from patients with ER+ HER2- BC compared to HVs and a downregulation in TK activity for ER+ HER2+ BC patients compared to HVs. Neutrophils from patients with ER- HER2- BC had upregulation in the TK family activity and downregulation in the AGC family of kinase activity compared to paired HVs. A downregulation of TK kinase activity is seen in neutrophils from patients with ER- HER2+ BC compared to HVs. The functional implications of these differences in kinase activity are being actively investigated.

Figure 2: **(a)** Flow cytometry data showing circulatory neutrophils as a % of live cells in patients with BC compared to HVs. 2 way ANOVA $P< 0.005$ **(b):** $CD62L^{low}$ neutrophil subsets in patients with ER positive Breast Cancer compared to paired healthy volunteers. Two tailed paired T-test $P< 0.003$. **Figure 2(c):** $CD62L^{low}$ neutrophil subsets in patients with ER negative Breast Cancer compared to paired healthy volunteers. Two tailed paired T-test $P< 0.035$



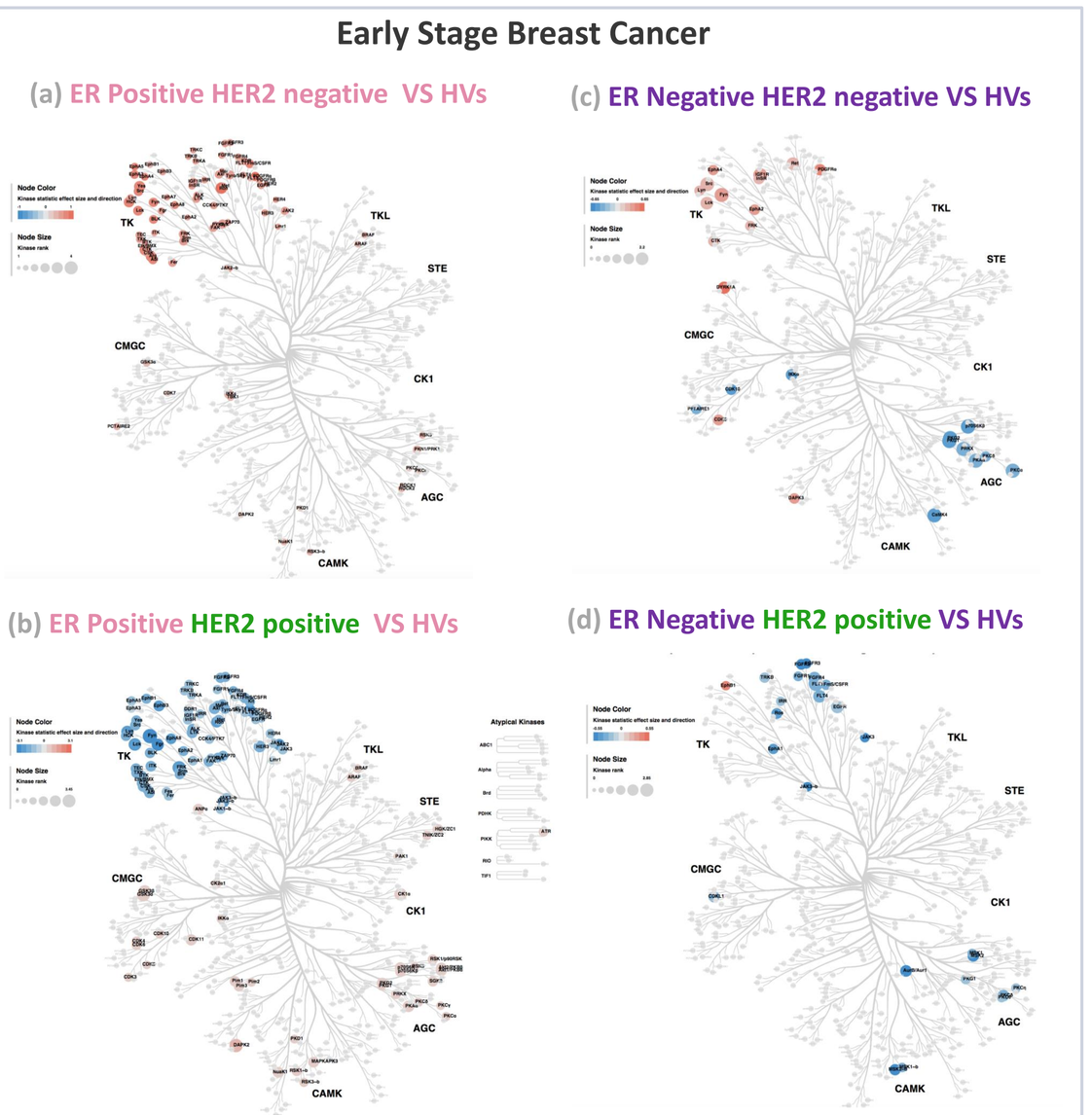


Figure 3: Kinome trees showing kinases which are upregulated (red) or downregulated (blue). **(a)** Patients with ER Positive HER2 negative BC Vs HVs **(b)** Patients with ER Positive HER2 positive BC Vs HVs **(c)** Patients with ER negative HER2 negative BC Vs HVs **(d)** Patients with ER negative HER2 positive BC Vs HVs

Conclusions

We have detected an expansion of neutrophils as a proportion of live cells within the circulation. We also found changes in CD62L expression and kinase activity within circulatory neutrophils in patients with Early Breast Cancer and the nature of these changes appear to be intrinsically linked to ER subtype and HER2 status. These findings may have important implications for use as part of an early diagnostic multi-omic platform.

References

[1] Wei B, Yao M, Xing C. et al (2016). The neutrophil lymphocyte ratio is associated with breast cancer prognosis: an updated systematic review and meta-analysis. *Onco Targets Ther*. 9, 5567–5575.

[2] Fridlender ZG, Sun J, Kim S et al. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell*. 16 (320), 183-94

Acknowledgements

Many thanks to the Breast cancer Research team at Imperial College London for sample collection and all the patients and HVs wh participated in the study. Thank you to additional supervisor Professor Edwin Chilvers at Imperial College London and the Malanchi lab members and for their support and encouragement over the study.

Email: anisha.ramessur@crick.ac.uk