**ENRICHMENT OF ATYPICAL MEMORY DOUBLE NEGATIVE (CD27⁻IgD⁻) TUMOUR INFILTRATING B CELLS FOLLOWING NEOADJUVANT CHEMOTHERAPY FOR EARLY-STAGE BREAST CANCER**

**Abstract**

Background: Humoral immune responses have previously been associated with improved outcomes, with B cell infiltrates able to independently predict pathologic complete response to neoadjuvant chemotherapy (NACT). B cells represent a diverse population of cells and the complex interplay between specific B cell subsets in the context of chemotherapy treated breast cancers remains unclear. Here, we investigate the dynamic changes in the B cell immune landscape before and after NACT treatment across different breast cancer subtypes.

Methods: Treatment naïve, mid-treatment and post-NACT breast tumour tissue samples were dissociated into single cells, stained with two panels of B cell-specific antibodies recognising a total of 24 target proteins, and analysed by flow cytometry. In addition, PBMC before and after NACT were also profiled. B cell subsets were classified as either naïve (CD27⁻IgD⁺), class-switched memory (CD27⁺IgD⁻), unswitched memory (CD27⁻IgD⁺) or double negative (DN) (CD27⁻IgD⁻). DN B cells were further characterised into DN1 (CXCR5⁺CD21⁺) and DN2 (CXCR5⁻CD21⁻) subsets.

Results: In both treatment naïve and chemotherapy treated samples, we observed a significant expansion in the DN B cell population within the tumour microenvironment (TME) compared to the periphery. DN B cells represented on average 40.96% of B cells in treatment naïve tumours vs 9.48% in PBMC (p<0.0001), and 71.80% vs 6.34% of B cells in post-NACT tumour vs PBMC samples respectively (p<0.05) (figure 1). Interestingly, in treatment naïve PBMC and tumour tissue samples, the largest proportion of the DN subset consisted of DN1 cells, 69.35% and 64.11% respectively. In contrast, following NACT, DN2 cells constituted the majority of the DN population both within the TME (86.30%) and in the periphery (50.44%) (figure 1). Although the specific functions of these B cell subsets remain unclassified, deeper phenotyping suggests DN1 cells more closely resemble the phenotype of class-switched memory cells, whilst DN2 cells are thought to have antibody-secreting properties and more closely resemble the plasmablast phenotype. scRNA sequencing of B cells pre- and post NACT is currently underway.

Conclusions: To our knowledge, this work is the first to identify an expanded population of DN B cells in breast tumour tissue and highlights the requirement for further investigation into these cells to decipher their role in the context of chemotherapy treatment and resistance in breast cancer.

Ethics Approval: Written informed consent was obtained from all individuals in accordance with the Declaration of Helsinki under the following research ethics committee; London-Chelsea approved study (REC ID 13/LO/1248).