INVESTIGATING THE TUMOUR IMMUNE MICROENVIRONMENT OF BREAST IMPLANT ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA USING SINGLE CELL RNA SEQUENCING

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Background Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a newly recognised T cell lymphoma that occurs in women with textured implants and is mostly limited to the periprosthetic seroma space. However, it is not clear how this ALCL forms and whether the tumour microenvironment (TME) plays a key role in ALCL survival and proliferation in breast tissue. To address these issues, we investigated the immunobiology of both the ALCL and endogenous immune cells within the breast seroma from a cohort of patients.

Methods We established a multi-centre national cohort study, and received breast implant seroma samples [benign (n=10) and BIA-ALCL (n=23)] via cosmetic and reconstructive surgeons. We performed single cell CITEseq, RNAseq to characterise the endogenous immune cells and ALCL cells; and abTCRseq and TCRVb deep sequencing to explore their clonal relationship. Finally, we analysed the microenvironment by investigating the seroma cytokine and chemokine levels, and performed an interactome analysis to provide putative cell-cell interactions (figure 1). We also performed targeted sequencing of BIA-ALCL samples to define mutations present.

Results scRNAseq data showed ALCL cells clustered together for individual patients, however their gene expression profile was distinct between patients. ALCL cells expressed common gene signature genes such as BATF3, SERPIN family genes, TNFSFR8, and IL2RA; along with genes unique to each patient. Endogenous T cells from the BIA-ALCL seroma displayed an activated/exhausted phenotype, immune checkpoints and endogenous T cell clonal expansion. In contrast, benign seroma endogenous T cells had a Th1/Th17 profile and limited T cell clonal expansion. Myeloid clusters were abundant in the BIA-ALCL seroma, but not the benign seroma. TME studies showed a significant increase in BIA-ALCL seroma for cytokines IL13, TNF, IL10 and secretory-PDL1. The single cell interactome analysis described potential unique cell-cell interactions in the BIA-ALCL TME including tumour-tumour, tumour-myeloid DC and tumour-CD8+ T effector memory cells. Mutational analysis revealed novel JAK1 mutations and STAT3 mutations along with chromosome 20 loss consistent with previous studies.

Conclusions BIA-ALCL cells are different between patients and from the endogenous immune cells. Furthermore, BIA-ALCL seroma is characterised by endogenous T cells with an exhausted profile and clonal expansion, increased IFN-g and TNF levels suggesting these T cells are responding to TME antigens. In addition, the BIA-ALCL TME has high levels of IL-13 and IL-10 which are generated via tumour-tumour and tumour-myeloid cell interactions.

Ethics Approval This study was approved by Peter MacCallum Cancer Centre human research ethics committee (Reference no. 44068) or by Macquarie University human research ethics committee (Reference No. 5201600427). Informed consent was obtained from all participating patients.