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High-dimensional Single-cell Characterisation of the Chronic Lymphocytic Leukaemia Tumour Microenvironment using Imaging Mass Cytometry

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INTRODUCTION

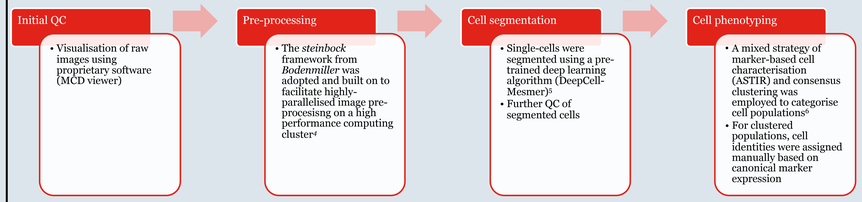
- Chronic lymphocytic leukaemia (CLL) represents a clinically-heterogeneous malignancy of CD5$^+$ B-cell clones.
- The tumour microenvironment (TME) within lymphoid compartments is critical in promoting survival and proliferation of neoplastic cells[1].
- However, a spatial and high-dimensional understanding of the CLL TME is lacking. Indeed, a systematic evaluation of the TME may facilitate improved prognostic and therapeutic stratification.
- Imaging mass cytometry (IMC) permits the highly-multiplexed assessment of >32 concomitant antigens on a tissue slide with single-cell resolution, and has been successfully applied in the spatial evaluation of solid and haematological malignancies, although not in CLL yet[2, 3].

AIM

- We therefore set out to apply IMC to evaluate the CLL TME landscape by deep phenotyping and characterisation of its cellular composition, with the intention of exploring how spatially resolved features associate with clinical outcome.

METHODS

- Formalin-fixed paraffin-embedded, CLL (n = 7) and non-malignant (n = 2) lymph node (LN) specimens were available for imaging.
- Tissue slides were stained with a previously-optimised panel of 32 metal isotope-conjugated primary antibodies designed to identify lineage-specific cell subsets, functional status and stromal elements.
- Images were acquired using the Hyperion Imaging System.
- An in-house workflow was utilised for downstream image analysis (Figures 1 and 2).



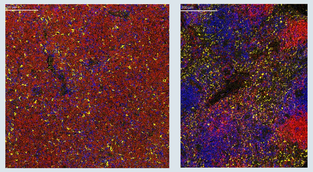
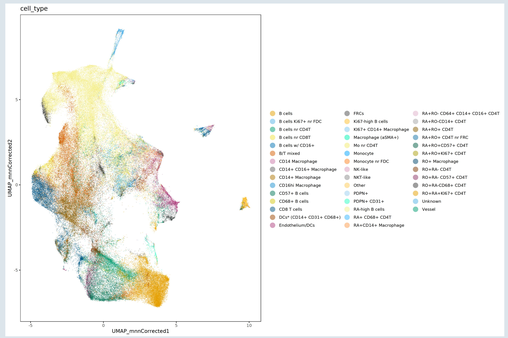


Figure 1 (above). IMC analysis pipeline.
Figure 2 (below). Representative IMC images visualised on MCD viewer, depicting LN tissues from a patient with CLL (left) and no malignancy (right). **Red – CD20_79a**; **blue – CD3**; **yellow – CD68**. In CLL there is effacement of follicular architecture typically seen in healthy LNs by malignant B-cells.

RESULTS

- A total of 163,212 single-cells were segmented across all slides.
- We identified diverse cell populations including B-cells, T-cell subsets, monocytes, macrophages, dendritic cells, NK-like-cells, and other stromal components (Figure 3).
- The median relative frequency of CLL cells was 66.4% (13.4% to 85.1%), with a median of 2.1% (0.2% to 8.4%) of the tumour bulk expressing Ki67 indicative of a proliferative state.
- Preliminary analysis revealed a negative correlation between the relative proportions of macrophages and total CLL cells (Spearman; R = -0.84, p = 0.024; Figure 4).



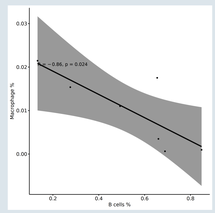


Figure 3 (left). UMAP demonstrating the diversity of cellular subpopulations observed in the LN microenvironment.
Figure 4 (right). Correlation plot between relative proportions of macrophages and B-cells in CLL LNs.

CELLULAR HETEROGENEITY IN THE TME

- Interestingly, we observed considerable differences between the cellular composition of malignant and non-malignant LNs, as well as substantial inter-patient heterogeneity between individuals with CLL (Figure 5).
- These data are consistent with the premise that CLL alters the TME[7].

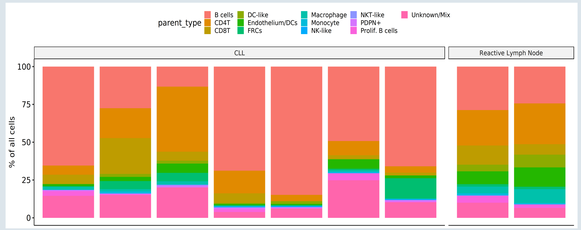


Figure 5. Barplot showing relative frequencies of key cell lineages across malignant and non-malignant LNs.

DEEP PHENOTYPING OF SUBPOPULATIONS

- Through subclustering key parent lineages, we uncovered heterogeneous cellular populations including previously-described CD4$^+$ CD45RO$^+$ T-cells as well as, intriguingly, CD45RA^{hi} and CD57$^+$ CLL B-cells which to our knowledge have not been commonly characterised in CLL (Figure 6).

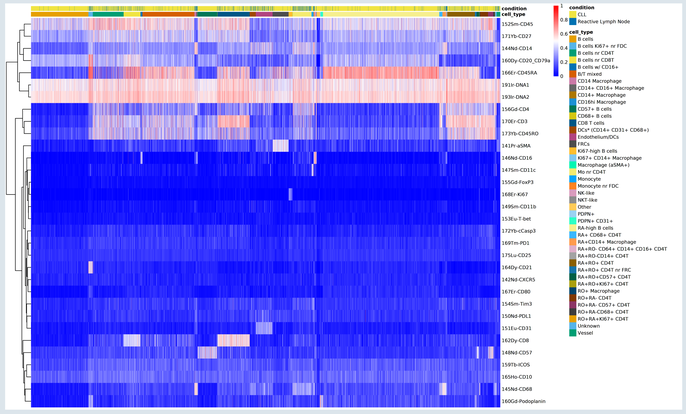


Figure 6. Heatmap with dendrogram demonstrating heterogeneous subclustered cell populations.

CONCLUSIONS

- Applying IMC, we have effectively characterised the non-spatial landscape of the CLL TME.
- Our study has thus far revealed an altered composition between malignant and non-malignant LNs, as well as considerable heterogeneity between CLL patients which mirrors the highly variable clinical and molecular paradigm observed in the disease.
- Confirmatory ablations are ongoing to enable expansion of sample size and evaluation for technical artefacts.
- In future, we will perform an in-depth characterisation of the spatial context of cellular neighbourhoods to gain insights into interactions between proliferating tumour cells and their microenvironment.
- We envision that such work will uncover associations with clinical outcome that may guide translational practice.

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