Imaging mass cytometry uncovers cellular makeup of proliferative regions and non-proliferative regions in CLL

Paul R. Buckley[1,2], Stella Bouziana[1,2,3], Timothy Woo[1], Hanna Mohamed[1], Aamina Ahmed[1], Julie Chan[1], Liron Barnea Slonim[3], Cynthia Bishop[4], Piers EM Patten[1,2,3]
1) Comprehensive Cancer Centre, King's College London, UK, 2) Department of Haematology, King's College London, UK, 3) King's College Hospital London UK, 4) Advanced Cytometry Platform, Guy's and St Thomas Hospital, London UK

Introduction

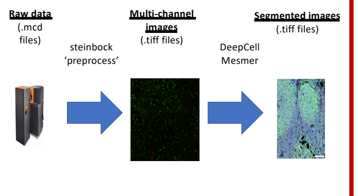
- Chronic lymphocytic leukaemia (CLL) is a heterogeneous disease in both tissue architecture and clinical course.
- The development and progression of CLL is dependent on permissive interactions between CLL cells and the tumour microenvironment (TME).
- The CLL TME is incompletely understood and not yet evaluated at high-plex single cell spatial resolution. Proliferation centres (PCs) in CLL lymph nodes (LNs) are drivers of disease progression. Unravelling their biology in more detail could identify pathways for targeting with novel therapeutics.
- Imaging mass cytometry (IMC) permits multiplexed analysis of up to 40 markers simultaneously, providing a platform to study complex spatial cell interactions within the TME.
- We hypothesise that PCs in CLL LNs are spatially organised hubs of tumour–immune–stromal interaction with distinct phenotypes that can be systematically mapped using IMC.

Aim

- To characterise the spatial architecture and immune microenvironment of CLL LNs using IMC, and to explore how these tissue phenotypes relate to disease biology and immune interactions, with a special focus on PCs.

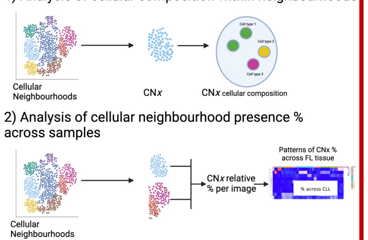
Method

Tissue microarrays from fixed formalin paraffin embedded (FFPE) **lymph node biopsies** were constructed and imaged by IMC.



In total, **314,668 cells were segmented from 15 images**. We employed consensus clustering to phenotype cells based on marker expression.

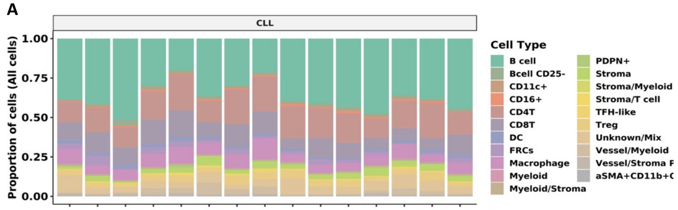
2) Analysis of cellular neighbourhood presence % across samples



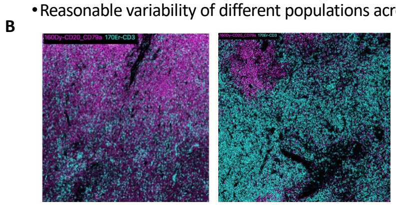
CNs are defined as unique sites of local processes within tissue[4]. Cellular composition within CNs have been associated with outcomes in colorectal cancer[4]. CNs were computed using *imcRtools* which adopts the methodology proposed in[4]

Analysis focused on **Ki67+CD20+CD79a+** regions as surrogate of PCs.

Figure 1: Variable abundance of cell populations across CLL lymph nodes

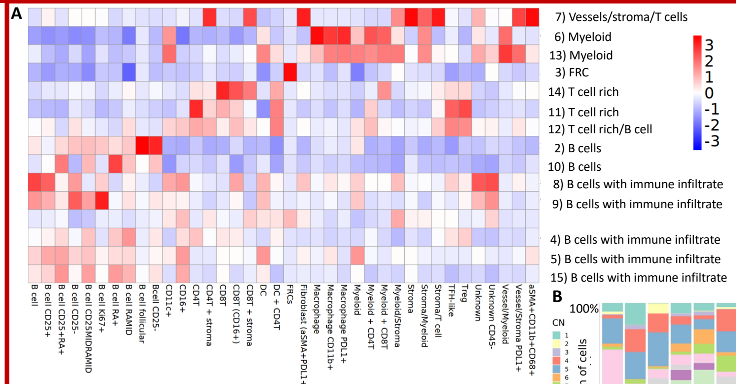

A
CLL
Proportion of cells (All cells)
1.00
0.75
0.50
0.25
0.00
Cell Type
B cell
Bcell CD25-
CD11c+
CD16+
CD4T
CD8T
DC
FRCs
Macrophage
Myeloid
Myeloid/Stroma
PDPN+
Stroma
Stroma/Myeloid
Stroma/T cell
TFH-like
Treg
Unknown/Mix
Vessel/Myeloid
Vessel/Stroma F
aSMA+CD11b+C

- Clustering has identified a range of key immune and stromal subsets
- B cell presence: range (~31%-52%), mean= approx. 38%
- As expected second most common cell typically are CD4+ T cells
- Reasonable variability of different populations across the CLL TME

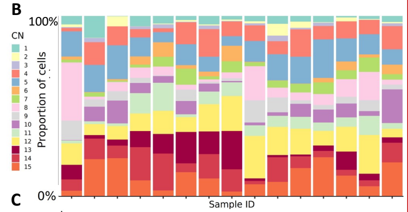


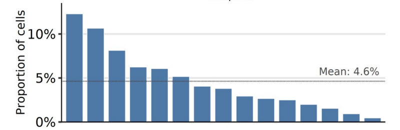
- Example images show differential abundances of T/B cells across CLL tumours

Figure 2: Diverse cellular neighborhoods in the CLL lymph node TME


A
7) Vessels/stroma/T cells
6) Myeloid
13) Myeloid
3) FRC
14) T cell rich
11) T cell rich
12) T cell rich/B cell
2) B cells
10) B cells
8) B cells with immune infiltrate
9) B cells with immune infiltrate
4) B cells with immune infiltrate
5) B cells with immune infiltrate
15) B cells with immune infiltrate
3
2
1
0
-1
-2
-3
B cell
B cell CD25+
B cell CD25+RA+
B cell CD25-
B cell CD25MDRAMID
B cell Ki67+
B cell RA+
B cell RAMID
B cell follicular
Bcell CD25-
CD11c+
CD16+
CD4T
CD4T + stroma
CD8T
CD8T (CD16+)
CD8T + stroma
DC
DC + CD4T
FRCs
Fibroblast (aSMA+PDL1+)
Macrophage
Macrophage CD11b+
Macrophage PDLL1+
Myeloid
Myeloid + CD4T
Myeloid + CD8T
Myeloid/Stroma
Stroma
Stroma/Myeloid
Stroma/T cell
TFH-like
Treg
Unknown
Unknown CD45-
Vessel/Myeloid
Vessel/Stroma PDLL1+
aSMA+CD11b+CD68+

Wider neighbourhoods observed include Myeloid rich regions, T cell zones and B cells mixed with diverse immune infiltrate.



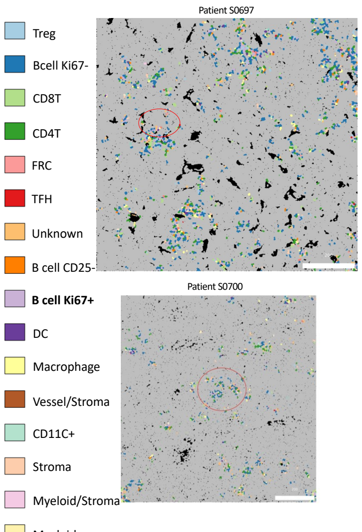


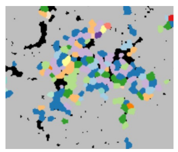
A) We found 15 distinct CNs with diverse cellular composition. There are 7 B cell rich regions, with CN2 and CN10 almost exclusively containing B cells. Ki67+B cells are present across multiple tissue regions/CNs, but predominate in **CN9**. **CN9** is enriched with wider B cell subsets and wider array of immune cells, including CD8/CD4 T subsets, Tregs and CD11c+ cells. Regions enriched in proliferative tumour cells are distinct from those enriched in CD45RA+ tumour cells.

B) Variation in cellular neighbourhood presence across CLL TME

C) Considerable variation in **CN9** (proliferative region) presence across CLL samples range: (0.46% - 12.29%) mean: 4.6%

Figure 3: Visualisation of proliferative tumour regions (CN9)

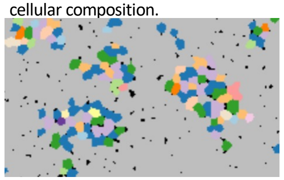




Wider cell subsets observed in these regions include Tregs, Ki67- B cells, CD4+ and CD8+ T cells and macrophages

Higher abundance Ki67+ regions (CN9). They are small scattered across samples. There is diverse cellular composition in proliferative regions of CLL tissue.

Similar patterns were found across patients but with variability in the presence of these regions and their cellular composition.

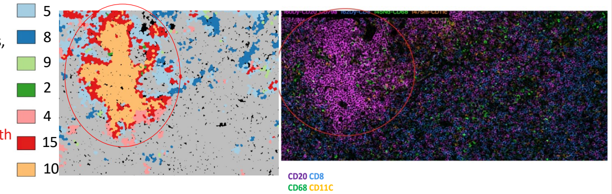


Computer generated plots using cytomapper in R.
Only CN9 cells are colour coded, all other cells are labelled in grey.

Figure 4: Non-proliferative tumour regions (CN10 & CN15) exhibit a distinct cellular composition

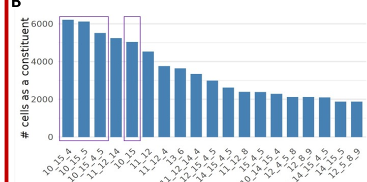
A

An additional commonly observed feature are CD20++ Ki67-CD45RA+ regions across CLL patients, although with variability in their abundance.
CN10: almost exclusive B cells. Reflects the core of the structures. Contrasted to the proliferative regions there is lower immune infiltrate.
CN15: surrounds the core and comprises B cells with numerous CD8T, DCs, CD68+/CD16+ cells.



Neighbourhoods 10-15 spatially interfacing is highly common across CLL TME samples.

B



Barplot illustrating cells constituent in an interface between neighborhoods 10 and 15 as pictured above are of the most commonly identified spatial contexts in CLL TME samples

These neighbourhood interfaces are termed spatial contexts. We now hypothesise that differential presence of these Ki67+ vs Ki67- structures may be associated with clinical features of the disease.

Conclusions and Future Directions

- IMC identified distinct proliferative Ki67+ and non-proliferative Ki67- regions with different cellular composition, size and abundance in CLL TME. These differential spatial phenotypes may be associated with clinical disease features.
- A better understanding of the CLL TME and its spatial features will drive the development of novel prognostic and therapeutic strategies.

References

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Contact

Dr Paul R. Buckley (DPhil) – paul.buckley@kcl.ac.uk
Dr Piers EM Patten (FRCP FRCPath PhD) – piers.patten@kcl.ac.uk

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