Successful Employment of Spatial Imaging Mass Cytometry in Follicular Lymphoma Confirms a Central Role of Follicular Helper T-Cells in Disease Pathogenesis

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INTRODUCTION

- Follicular lymphoma (FL) is a germinal centre (GC) B-cell neoplasm that is dependent on its non-malignant tumour microenvironment (TME).
- Follicular helper T-cells (T_{FH}) are essential for normal GC B-cell development and play a key role in supporting FL survival.[1,2]
- Other T-cell subsets and immune cells within the TME can either support tumour growth or promote anti-tumour immunity.
- However, the precise role of these cells in FL pathogenesis is unclear, partly due to the inability of conventional *in vitro* or histology studies to assess the complex interplay between immune subsets *in situ.*
- Imaging Mass Cytometry (IMC) is a novel technology that has been used for highly-multiplexed, *in situ* analyses of the TME in other cancers.[3]

AIM

To deep phenotype and map cell interactions between FL B-cells, T-cell subsets and other immune cells within the FL TME by IMC, in order to understand disease pathogenesis and identify predictors of outcome.

METHODS

Archival formalin-fixed, paraffin-embedded (FFPE) tissue from 11 FL patients was selected from extremes of clinical outcome:: poor-risk: progression of disease (POD) within 24 months of 1st line immunochemotherapy (N=5).Good-risk: Untreated for > 5years or no POD > 60months after treatment (N=6).

Tissue sections were stained with metal isotope-tagged primary antibodies and imaged by Hyperion™ Imaging System (Fluidigm). A bespoke automated image analysis pipeline was developed using proprietary and in-house analysis tools (Fig 1).

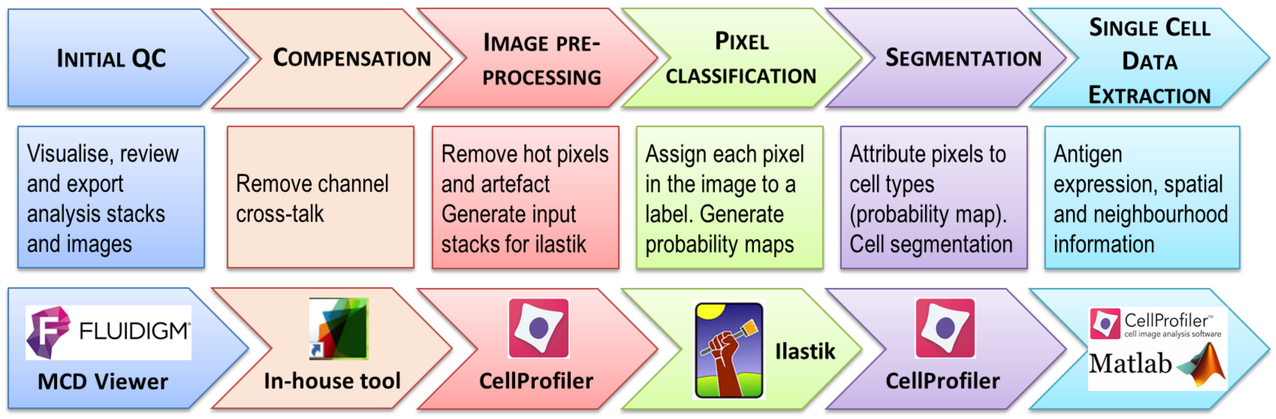
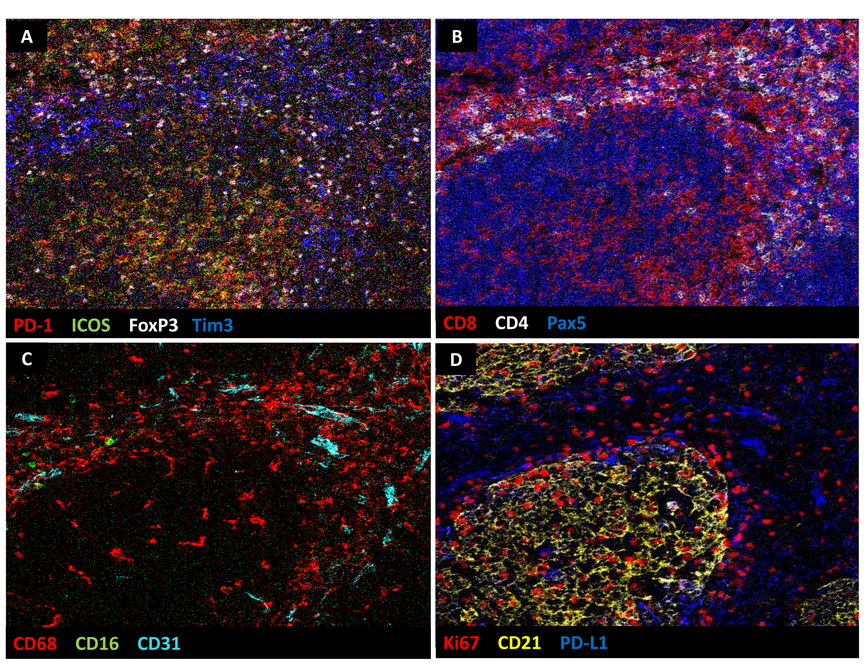


Figure 1: *IMC analysis pipeline*

RESULTS

A 20-antigen panel (Table) was used to identify T-cell subsets (Fig 2A/B), B-cells (2B), other TME cells (2C/D), proliferation (Ki67; 2D) and malignant follicles (CD21; 2D).

T_{FH} (CD3+CD4+PD-1+ICOS+) were present in all samples, and can be seen where PD-1 and ICOS are co-expressed within malignant follicles (yellow; Fig 2A). These cells did not express regulatory (FoxP3) or exhaustion markers (Tim3).



Target	Isotope
Pax5	142Nd
BCL2	146Nd
BCL6	147Sm
ICOS	148Nd
PD-L1	150Nd
CD31	151Eu
CD16	153Eu
Tim3	154Sm
FoxP3	155Gd
CD4	156Gd
CD68	159Tb
CD20	161Dy
CD8	162Dy
CD21	164Dy
PD-1	166Er
Ki67	168Er
CD3	170Er
cCaspase-3	172Yb
Tbet	176Yb
DNA/RNA	191Ir

Figure 2: *IMC images acquired simultaneously from the same FL tissue section show selected FL, T_{FH} and other TME markers*

T-cell subsets and FL proliferation

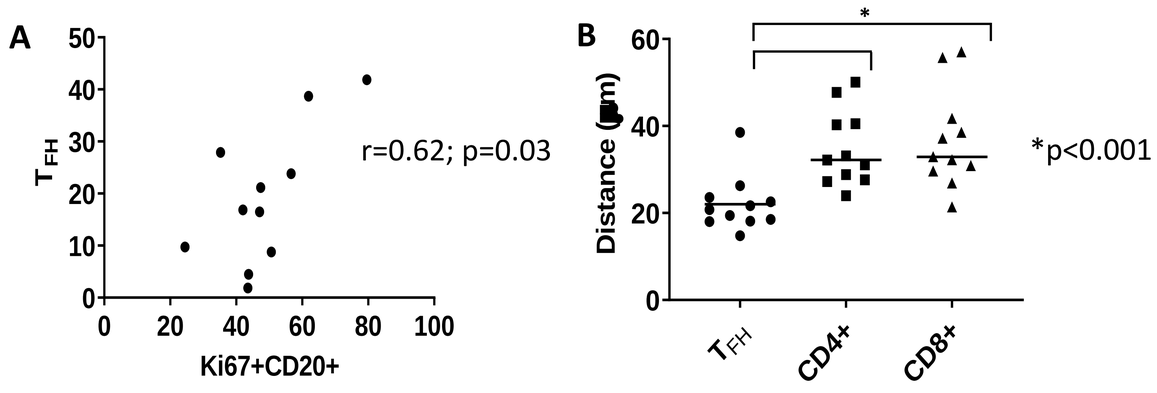


Figure 3: *T_{FH} and FL proliferation. A) shows the correlation between the area of T_{FH} and proliferating B-cells by IMC. B) shows the mean distance (µm) between T-cell subsets and the nearest proliferating B-cell. 'CD4+' represents non-T_{FH} helper T-cells*

IMC confirmed a correlation between the number of T_{FH} and proliferating FL cells (Fig 3A), consistent with previous results using confocal microscopy.[2] Neighbourhood analysis highlighted a close spatial relationship between T_{FH} and FL proliferation (3B).

There was no correlation between the number of Ki67+ B-cells and either CD8+ (r=0.07, p=0.83) or non-T_{FH} CD4+ T-cells (r=0.27, p=0.41; data not shown).

Together, these data suggest that T_{FH} may influence FL B-cell proliferation.

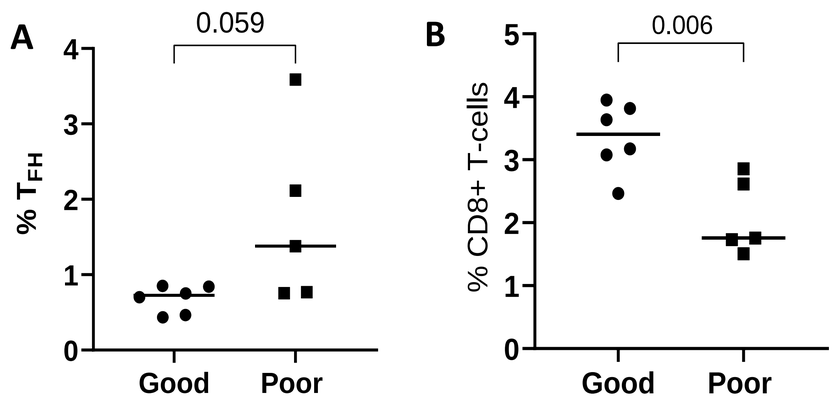
TME immune cells and disease phenotype

T_{FH} were more prevalent in poor-risk patients (N=5) vs good-risk cases (N=6; Fig 4A), whilst CD8+ T-cells were more prevalent in good-risk cases (4B).

Other CD4+ T-cell subsets, including Tregs (FoxP3+), Th1 (T-bet+) and exhausted (Tim3+) cells, did not differentiate good- and poor-risk cases (data not shown).

There was higher expression of PD-1 ligand (PD-L1) by CD68+ macrophages and CD31+ vessels in good-risk disease (Fig 5A/B), which may inhibit T_{FH} activity. These changes were predominantly within the extrafollicular TME (defined by CD21+ FDC networks), whereas T_{FH} were mostly intrafollicular.

Figure 4: *T-cell subsets in the FL TME. Graphs show the proportional area of T_{FH} (A) and CD8+ T-cells (B) by IMC, according to disease phenotype.*



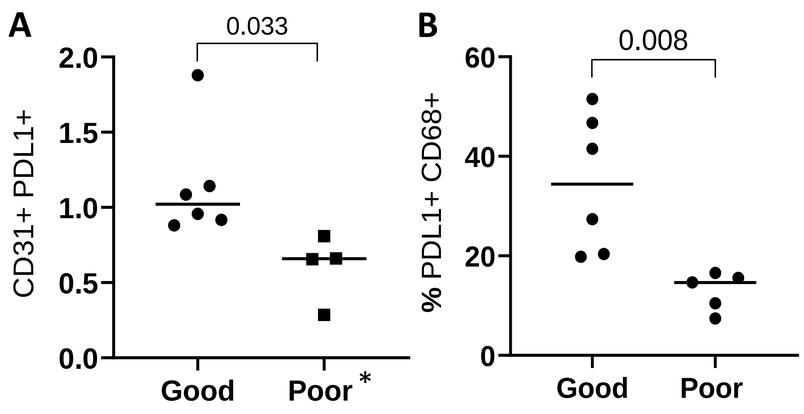


Figure 5: *PD-L1 expression according to disease phenotype: A) area of PD-L1+ vessels and B) percentage of CD68+ macrophages that expressed PD-L1.*

** 1 poor-risk case excluded due to poor CD31 stain quality (N=4)*

CONCLUSIONS

- IMC can be effectively employed on FFPE tissue to deep phenotype and analyse the TME in FL.
- Spatial characterisation of the T-cell subsets in FL confirms a close link between T_{FH} and proliferation, confirming a central role for T_{FH} in driving FL growth.
- T-cell subsets and other immune markers are associated with prognosIs, although larger studies are required to confirm these findings.
- In the immunotherapy era, a better understanding of these interactions will suggest future targets and pathways for therapeutic intervention in FL.

REFERENCES: 1) P Ame-Thomas, *Blood* 2015;125:2381-5. **2)** W Townsend, *Haematologica* 2019, DOI:10.3324/haematol.2019.220160. **3)** C Giesen, *Nature Methods* 2014;11:417-422.

ACKNOWLEDGEMENTS: This work was supported by the UK Medical Research Council (award MR/T005106/1) and unrestricted educational grants from the British Society for Haematology and F.Hoffman-La Roche

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