

Structured Reports for the Pathological Society Grant Awards: Career Development Fellowships (CDF), Path-Soc / J Shanks Fellowship, PhD Studentships, International Collaborator Awards, Small Grant Scheme (several types) Awards, CRUK/Path-Soc Pre-Doctoral Research Bursary & Early Career Pathology Researcher (Hodgkin & Leishman) Grants.

Recipients of grant awards from the Pathological Society of Great Britain and Ireland should submit a scientific report detailing the work undertaken with support from this award and any outputs arising from this. The reports should be set out using the following subheadings and should consist of:

Annual Reports: Final report on ICA

Title: Angioimmunoblastic T-Cell Lymphoma: Evidence For More Than One Clonal Neoplastic T-Cell Clone

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Background and aims:

Angioimmunoblastic T-cell lymphoma (AITL) and peripheral T-cell lymphoma with T follicular helper phenotype (PTCL-TFH) are a group of clinicopathological entities which originate from T follicular helper cells and share a similar genetic profile. Based on the oncogenic function, the diverse genetic changes identified in AITL may be divided into two classes. Class I genetic changes include mutations in DNA methylation regulators namely *TET2*, *DNMT3A* and *IDH2*, and these mutations are found in a range of haematological malignancies, with *TET2* and *DNMT3A* mutations occurring in hematopoietic stem cells. Class II genetic changes include mutation in *RHOA*, *PLCG1*, *TNFRSF21* and *CD28*, as well as the *CTLA4-CD28* and *ITK-SYK* fusion, and these genetic changes affect proteins critical to T-cell biology, thus most likely promote T-cell differentiation as well as malignant transformation, consequently generating the malignant phenotype of AITL. In an ongoing investigation, we have investigated the above genetic changes as well as TCR usage in 75 cases of AITL by targeted sequencing using our recently established Fluidigm multiplex PCR and Illumina MiSeq protocol. We have discovered evidence supporting that there is more than one neoplastic T cell clone in a proportion of AITL. To further investigate whether these multiple neoplastic T-cell clones are driven by the same *TET2* mutations, we aim:

- 1) to confirm the presence of more than one clonal neoplastic T-cell population in angioimmunoblastic T-cell lymphoma with multiple *TET2* mutations and TCR rearrangements;
- 2) to investigate their clonal evolution in cases with multiple post-treatment follow up biopsies.

Results:

1) Targeted sequencing of clustered tumour cells microdissection: We have investigated 3 cases of AITL with multiple *TET2* mutations and TRB rearrangements. The data obtained were subjected to both unsupervised hierarchical clustering and principal component analyses, followed by bootstrap evaluation of the clusters. In 2 of the 3 cases investigated, our preliminary data indicated the presence of two distinct neoplastic T-cell clones in AITL (Figure 1).

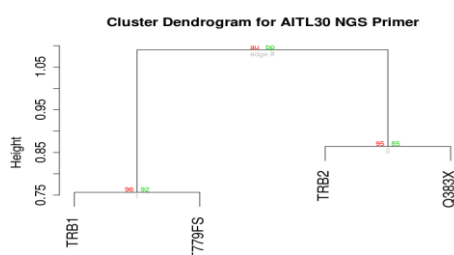


Figure 1. Bootstrap evaluation of unsupervised hierarchical clustering analysis of multiple *TET2* mutations and TRB productive rearrangements in a case of AITL by microdissection and next generation sequencing.

2) BaseScope *in situ* hybridisation using specific oligonucleotide probes: We performed BaseScope ISH using specific probes targeting the unique TRB VDJ junctional sequence in two cases (one AITL and one PTCL-TFH). As shown in case AITL30, the V7-J1 probe identified a diffuse cell population, while the V27-J2 probe revealed only scattered cells, confirming that they represented two independent clonal T-cell populations. To examine whether both clonal T-cell populations carry the same *TET2* mutations, we further designed the BaseScope probes for the two *TET2* mutations in AITL30, and performed double BaseScope ISH by combining the TRB VDJ probe with each of the *TET2* mutation probes. The results showed co-localization of the TRB-VDJ and each of the two *TET2* mutation probe signal in both T-cell populations (Figure 2), indicating that both clonal T-cell populations harbored the same *TET2* mutations.

To further investigate the immunophenotype, particularly that of the minor V27-J2 clone, we combined BaseScope ISH with immunohistochemistry, and demonstrated that both the T-cell populations are CD4 positive, but CD8 negative.

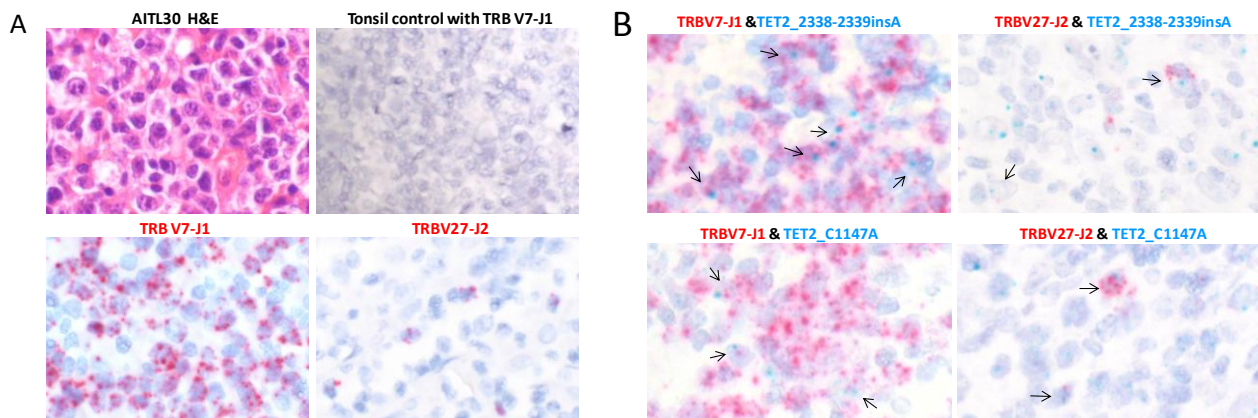


Figure 2: A) Confirmation of two clonal T-cell populations by BaseScope ISH with TRB clone specific probes; B) Double BaseScope *in situ* hybridization shows the presence of both *TET2* mutations in the two clonal T-cell populations of AITL30.

Conclusions:

We showed evidence of bi- or oligoclonal functional TRB rearrangements in 26% of these AITL and PTCL-TFH, and confirm two independent clonal T-cell populations on selected cases. We demonstrated that the two independent T-cell clones in the same case share the same *TET2* mutations, suggesting derivation from a common precursor cell population.

How Closely Have the Original Aims been Met:

We have made excellent progresses to achieve our original aims.

Outputs (including meeting abstracts, oral presentations, original papers, review articles) from the study in which the Pathological Society has been acknowledged:

1. Yao W, Zhang W, Thompson JS, Zhang SW, Clipson A, Wang M, Liu H, Bibawi H, Grant JW, Wright P, El-Daly H, Rásó-Barnett L, Farkas L, Gao Z, Chuang SS, Ashton-Key M, Liu W, Du MQ. Angioimmunoblastic T-cell lymphoma contains multiple clonal T-cell populations derived from a common *TET2* mutant progenitor cell. The 19th Meeting of the European Association of Haemathopathology. October 2018, Oral presentation
2. Yao W, Zhang W, Thompson JS, Zhang SW, Clipson A, Wang M, Liu H, Bibawi H, Grant JW, Wright P, El-Daly H, Rásó-Barnett L, Farkas L, Gao Z, Chuang SS, Ashton-Key M, Liu W, Du MQ. Angioimmunoblastic T-cell lymphoma contains multiple clonal T-cell populations derived from a common *TET2* mutant progenitor cell. PathSoc 2019 Winter meeting, Oral presentation
3. Yao WQ, Wu F, Zhang W, Chuang SS, Thompson JS, Chen Z, Zhang SW, Clipson A, Wang M, Liu H, Bibawi H, Huang Y, Campos L, Grant JW, Wright P, El-Daly H, Rásó-Barnett L, Farkas L, Follows GA, Gao Z, Attygalle AD, Ashton-Key M, Liu W, Du MQ. Angioimmunoblastic T-cell lymphoma contains multiple clonal T-cell populations derived from a common *TET2* mutant progenitor cell. (in revision with J Path)