Early detection of T-cell lymphoma with T follicular helper phenotype by *RHOA* mutation analysis

Contact details: Rachel Dobson: rd571@cam.ac.uk

Rachel Dobson[1], Peter Y Du[1], Lívia Rásó-Barnett[2], Wen-Qing Yao[1], Zi Chen[1], Calogero Casa[2], Hesham El-Daly[2], Lorant Farkas[2,3], Elizabeth Soilleux[1,4], Penny Wright[4], John W Grant[4], Manuel Rodriguez-Justo[5], George A Follows[6], Hala Rashed[7], Margarete Fabre[8], E Joanna Baxter[6], George Vassiliou[8], Andrew Wotherspoon[9], Ayoma D. Attygalle[9], Hongxiang Liu[2], and Ming-Qing Du[1,4].

[1]Division of Cellular and Molecular Pathology, Department of Pathology, University of Cambridge, Cambridge, UK;[2]The Haematopathology and Oncology Diagnostic Service, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; [3]Department of Pathology, Akershus University Hospital, Lorenskog, Norway;[4]Department of Histopathology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; [5]Department of Pathology, Royal Free and University College Medical School, London, UK; [6]Department of Haematology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; [7]Department of Cellular Pathology, University Hospitals of Leicester, East Midlands Pathology Services, Leicester, UK;[8]Wellcome-MRC Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Department of Haematology, University of Cambridge, Cambridge, UK;[9]Histopathology Department, The Royal Marsden Hospital, London, UK;

Background

- Angioimmunoblastic T-cell lymphoma (AITL) and peripheral T-cell lymphoma with T follicular helper phenotype (PTCL-TFH) originate from TFH cells and share a similar mutation profile. Unfortunately, they commonly display poor clinical outcome.
- Their diagnosis is often a challenge, particularly at an early stage of the disease with small core biopsies, due to a lack of specific histological and immunophenotypic features, paucity of neoplastic T cells and prominent polymorphous inflammatory background.
- *RHOA* Gly17Val (G17V) (c.50G>T) mutation is the most frequent somatic genetic change identified for AITL/PTCL-TFH (seen in 60-70% of cases).

Materials and Methods

Tissue materials and DNA extraction: Crude DNA was prepared from 78 FFPE tissue specimens from 37 patients with AITL (n=35) or PTCL-TFH (n=2), and 61 controls.

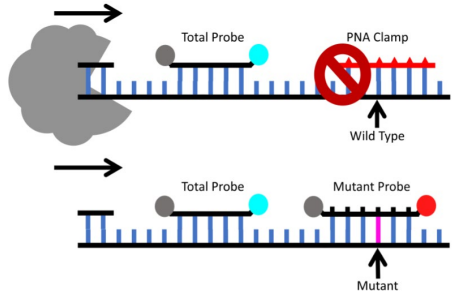

Figure 1: Schematic illustration of the PNA-LNA qPCR

Quantitative PCR with peptide nucleic acid and locked nucleic acid probes (PNA-LNA qPCR): This was performed as previously described by Nuhat *et al* 2018[1]. It uses an LNA probe specific to the mutation, and a PNA clamp oligonucleotide to suppress the amplification of the wild type allele (see **Figure 1**).

Aim

- To investigate whether *RHOA* G17V (c.50G>T) mutation is present in the early 'reactive' lesions which later develop AITL/PTCL-TFH and explore whether mutation analysis can help advance early lymphoma diagnosis.

Results

The assay is sensitive and specific for *RHOA* G17V

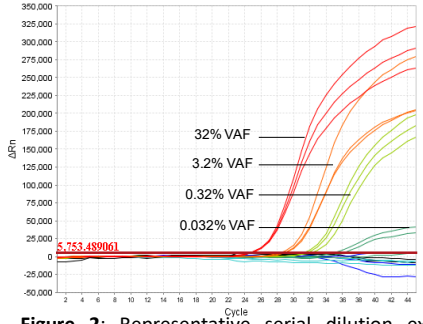

Figure 2: Representative serial dilution experiment for sample with known *RHOA* Gly17Val allele frequency of 32%

PNA-LNA qPCR sensitivity was tested using serial dilutions on purified DNA samples (n=3) with known *RHOA* G17V allele frequency **(Figure 2)**.

The assay was highly sensitive, detecting allele frequency of 0.03%. The assay was also highly specific, not detecting other changes in *RHOA* Gly17 and not detecting the G17V mutation in 61 control samples (including reactive lymph node samples, classical Hodgkin lymphomas and clonal hematopoiesis of indeterminate potential samples).

RHOA mutation is present in the initial biopsies that are not diagnostic for lymphoma by combined histological, immunophenotypic and clonality analyses.

- Among the 37 AITL/PTCL-TFH cases investigated, 23 (62.2%) were positive for *RHOA* G17V mutation by PNA-LNA qPCR.
- Of the 23 cases with *RHOA* G17V mutation, 19 cases had multiple biopsies. The mutation was detected in each of these biopsy specimens (including matched preceding and follow up).
- *RHOA* G17V was identified in 18 specimens that showed no evidence of lymphoma by combined histological, immunophenotypic and clonality analyses **(Figure 3)**. However, these cases were diagnosed to have AITL/PTCL from other biopsies.
- The mutation was seen in biopsies 0-26.5 months (mean=7.87 months) prior to lymphoma diagnosis.

All points (red outline/filled) in figure 3 are *RHOA* mutation positive.
Points filled in grey could not be tested

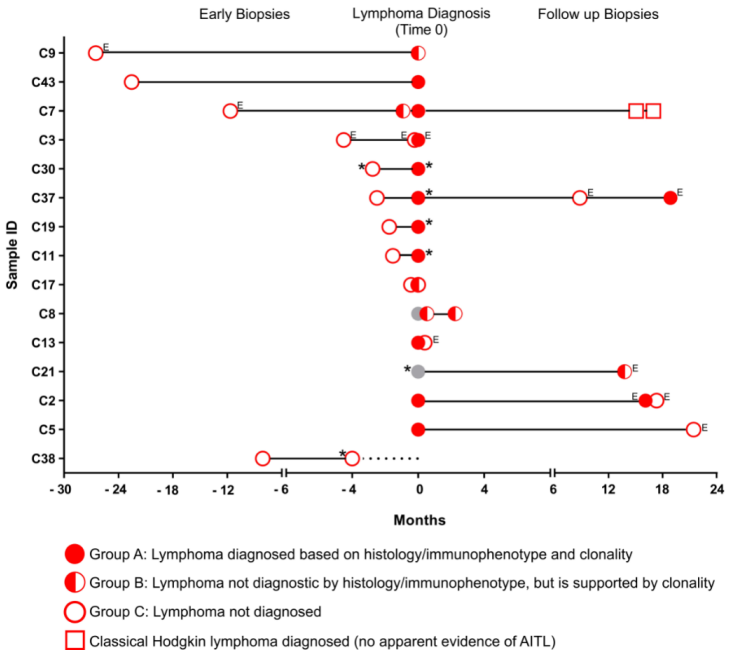

Figure 3: Analysis of *RHOA* G17V mutation in the *RHOA* positive cases with longitudinal biopsies in patients with AITL (only including cases with at least one biopsy classed as not diagnostic). E denotes extranodal biopsies. * denotes lymph node excision biopsies and all others are core biopsy specimens.

Conclusions:

- *RHOA* mutation is highly specific to AITL/PTCL-TFH and is not seen in any reactive lymph nodes or CHIP samples investigated.
- *RHOA* mutation is present in early lesions of AITL /PTCL-TFH, which is suggestive, but not diagnostic for T-cell lymphoma.
- *RHOA* mutation analysis is valuable in early detection of AITL/PTCL-TFH.

References:

1.Nuhat ST, et al. Droplet digital polymerase chain reaction assay and peptide nucleic acid-locked nucleic acid clamp method for *RHOA* mutation detection in angioimmunoblastic T-cell lymphoma. *Cancer Science* 2018; **109**: 1682-1689

This work has now been published: Dobson *et al*. Early detection of T-cell lymphoma with T follicular helper phenotype by RHOA mutation analysis. *Haematologica* 2021. doi: 10.3324/haematol.2020.265991.