CORRESPONDENCE

Monitoring of molecular responses to tirabrutinib in a cohort of exceptional responders with relapsed/refractory mantle cell lymphoma

To the Editor,

Inhibitors of Bruton's tyrosine kinase (BTK) in chronic lymphocytic leukemia (CLL) result in durable responses in nearly all patients [1]. In contrast, in more aggressive B-cell malignancies, including diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL), responses to single agent BTK inhibitors (BTKi) occur only in subsets of patients and are mostly of brief duration. However, exceptional responses may occur [2–6].

In relapsed/refractory (R/R) MCL, median progression-free survival (PFS) with single-agent covalent BTKi range from 12 (ibrutinib) to 20 months (acalabrutinib ACE-LY-004 NCT02213926 study) (Table S1) [7–9]. Analysis of responding patients in ACE-LY-004 showed that 8/29 evaluable patients eradicated minimal residual disease (MRD) using the quantitative ClonoSEQ next-generation sequencing assay of cellular peripheral blood DNA [10]. However, molecular determinants of responsiveness to BTKi in MCL, and clinical significance of attaining MRD negativity in this setting remain unknown.

Like acalabrutinib, tirabrutinib is a highly selective BTKi, binding covalently to BTKC481 via a reactive acyl alkyne group. Although only 16 MCL patients were treated with single-agent tirabrutinib within the POE001 phase 1 trial (NCT01659255), extended follow-up showed an estimated median PFS of 25.8 months at 3 years [11]. Three patients from this cohort, described below, attained complete responses lasting over 72 months, despite adverse prognostic features at diagnosis including TP53 mutations, blastoid morphology, and refractoriness to immunotherapy. In these exceptional responders [12], we sought to determine common features or a tumoral mutational signature that might predict exceptional responsiveness. Secondly, we determined the depth of response using digital droplet PCR (ddPCR) in both plasma and cellular DNA samples and whether serial assessment of levels of cellular and plasma circulating tumor (ct) DNA samples might presage relapse.

Clinical and laboratory details of the three cases are given in Table 1. A schema of the treatment timelines is shown in Figure 1. Full materials and methods are found in the Supporting Information. The three patients (201-139, 201-162, and 201-170) were all treated at our center and received either 480 or 600 mg of tirabrutinib once per day. All entered a clinical and radiological remission. One patient (patient 201-162) with primary immunochemotherapy-refractory disease remained in complete remission (CR) > 108 months from trial initiation. However, in 2020, they developed estrogen receptor positive grade 2 invasive ductal breast cancer, necessitating temporary discontinuation of tirabrutinib for 4 months. The breast cancer was treated radically with surgical resection, post operative radiotherapy and tamoxifen. There remains no clinical or radiological evidence of recurrence. The other two patients relapsed 91 and 72 months after initiating tirabrutinib. Due to comorbidities, no further therapy was attempted in patient 201-170 and the patient died rapidly from uncontroll ed disease. Patient 201-139 received rituximab, bendamustine, cytosine arabinoside, and subsequently pirtobrutinib with the aim of proceeding to chimeric antigen receptor (CAR)-T cell therapy, but failed to respond adequately.

Whole exome sequencing showed t(11;14)(q13;q32) with breakpoints in CCND1 and IGH as anticipated, but there were no other common mutations (Table S2). Two patients exhibited TP53 mutation (p.L194R and p.R181H), one KMT2D mutation (p.S2773Lfs*72), and one patient had two ATM mutations. All three cases showed unmutated (> 99% homology to germline) IGHV gene segment usage: two cases utilized IGHV4-34, the other IGHV3-23. Unmutated IGHV4-34 is seen in 15% of MCL and in 15% of the non-germinal center subtype of DLBCL, including the MCD subgroup, sensitive to BTKi. Unmutated IGHV4-34 recognizes autoantigens on the surface of malignant B cells, shown to result in chronic active BCR signaling in activated B-cell-like DLBCL models [13]. At relapse, in patient 201-139 mutations in PLCG2 (p.M1141T, 54% VAF), previously described in relapsed CLL with ibrutinib, and in NFKB2 (p.G373_G374insEGVLC, 36% VAF) that activates the alternative nuclear factor kappa B pathway were seen. No BTK mutations were present, supporting prior findings [14].

Interestingly, in all 3 cases, we observed a complete absence of peripheral blood CD19+ B cells with tirabrutinib, consistent with B cell aplasia. In one case (201-139) bone marrow analysis confirmed a B-cell differentiation block at the CD19+ CD38+ slg-B-cell precursor stage (Figure S1A). In this patient, B cell aplasia resulted in hypogammaglobulinemia and recurrent encapsulated bacterial infections commencing 30 months following the initiation of tirabrutinib (Figure S1B). No hypogammaglobulinemia nor infections were observed in the other

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FIGURE 1  (A) Treatment timelines for the three exceptional responder patients. Red diamonds correspond to the time of clinical progression, (B–D) Droplet frequency plots corresponding to serial ddPCR analysis during treatment with tirabrutinib (timeline) performed for patient 201-139 detected with t(11;14) translocation breakpoint assay (B), for patient 201-162 with t(11;14) translocation breakpoint (top panel) and KMT2D (c.8315_8316insT) (bottom panel) assays (C), for patient 201-170 with t(11;14) translocation breakpoint (top panel), TP53 (c.581T>G) (middle panel) and ATM (c.8399A>C) (bottom panel) assays (D) with additional biopsy positive and human genomic DNA (hgDNA) negative controls. The pink line indicates amplitude thresholds. Mutant droplets are indicated in blue; negative droplets in grey. ctDNA, circulating tumor DNA; CTC, circulating tumor cells; NTC, no template control. ddPCR assays were run at different time points and figures were constructed for clarity.
two patients. This phenotype was not observed in this cohort in those with a shorter duration or depth of response.

From sequencing data, we derived patient-specific ddPCR assays to monitor both t(11;14)(q13;q32) translocation breakpoint and point mutations (Table S3). In serial cellular and plasma DNA samples, all cases initially showed clearance of tumor DNA from the peripheral blood either as ctDNA or genomic (g) DNA derived from circulating tumor cells (CTC) (Figure 1A–C and Figure S2). In the two relapsing patients it was possible to detect recurrent tumor DNA in peripheral blood either as plasma ctDNA or CTC genomic DNA (gDNA), 3 months, and 14 months, prior to radiological and clinical relapse. In patient 201-162, the disease remains undetectable.

**AUTHOR CONTRIBUTIONS**

Abdullah N. M. Alqahtani, Sandrine Jayne, Christopher S. Trethewey, Sai S. Duraisingham, and Susann Lehmann performed research. All authors were involved in data analysis and interpretation. Harriet S. Walter, Sandrine Jayne, and Martin J. S. Dyer drafted the article that was revised and approved by all authors (article writing). Martin J. S. Dyer and Harriet S. Walter designed and supervised the study.

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**CONFLICT OF INTEREST STATEMENT**

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DATA AVAILABILITY STATEMENT
Data are available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT
This study was approved by the local Research Ethics Committee and the University Hospitals of Leicester National Health Service Trust (06/Q2501/122). All patients were consented to this.

REFERENCES

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.