Received: 11 June 2024 | Accepted: 2 September 2024

DOI: 10.1111/bjh.19765

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

LETTER TO THE EDITOR

Telomere length and DNA methylation epitype both provide independent prognostic information in CLL patients; data from the UK CLL4, ARCTIC and ADMIRE clinical trials

Patients with chronic lymphocytic leukaemia (CLL) exhibit varied clinical paths; some have indolent courses, while others experience aggressive disease.[1] Despite numerous molecular and cellular biomarkers being discovered, only *TP53* aberrations (*TP53ab*: *TP53* deletion and/or mutations) and unmutated IGHV status (U-CLL) are used for treatment stratification.[2,3] Other biomarkers have not been adopted due to a lack of independent prognostic or predictive value. Validating new biomarkers in large phase II/III trials with extensive molecular characterization and long-term follow-up is crucial. This study aims to validate the clinical relevance of two CLL biomarkers, methylation-based epitype (DME) and telomere length (TL), using large trial cohorts.

CLL patients can be classified into three DME subgroups: naïve-like (n-CLL), intermediate (i-CLL) and memory-like CLL (m-CLL), based on similarities with normal B-cell maturation states.[4,5] These subgroups correlate with IGHV mutational status and clinical outcomes, reflecting the influence of cell of origin signatures on disease behaviour.[4] TL in CLL cells, which correlates with DME, also has clinical utility.[6,7] TL, measured by quartile cut-offs and fusogenic range, is associated with reduced progression-free (PFS) and overall survival (OS) in patients treated with (immuno)chemotherapy.[8–10] Notably, 59% of n-CLL and 85% of m-CLL patients exhibit short and long telomeres respectively.[9] This study aims to distinguish the clinical correlation of DME and TL in prospective trials, quantifying their individual association with survival to enhance the risk-adapted stratification of CLL patients.

Our study focused on 519 patients from three UK clinical trials based on the availability of DNA for biomarker profiling: UK LRF CLL4 (NCT00004218, $n = 304$), UKCRN ARCTIC ('ARC', ID7136, $n = 107$) and UKCRN ADMIRE ('ADM', ID6897, $n = 108$; Table S1). Due to similarities in inclusion criteria and follow-up data, ARC and ADM trials were combined for survival analysis.[9] We used pre-existing DME data and supplemented published TL data with new data from 60 patients selected based on DNA availability and pre-existing DME data (Table S2), using the MMQ-PCR assay with established TL cut-offs (TL-Short <2.92 kb, TL-Intermediate 2.92–3.57 kb, TL-Long >3.57 kb).[9,11,12] The analysed cohorts were representative of the wider clinical trials for an extensive biomarker panel (Tables S3 and S4). Informed consent was obtained from all patients, adhering to the Declaration of Helsinki and regional ethics committee approvals. For data inquiries, contact Jonathan C Strefford at jcs@soton.ac.uk.

Initially, we confirmed the association between DME and TL, consistent with previous findings: 73% of m-CLL and 50% of n-CLL patients exhibited TL-L and TL-S respectively ($p < 0.001$; Figure 1A,B). We also showed that n-CLL and TL-S were linked with other poor-risk features, including U-CLL, biallelic *ATM* lesions and *TP53ab* (Figure 1C,D). Next, we verified the association of each clinico-biological feature, including DME and TL, with PFS and OS in the CLL4 and ARC/ADM trials using univariate and multivariate analyses.[9,11] In univariate analysis, we found significant associations for TL and DME with OS and PFS in both trials (Figure 2Ai, ii). Specifically, TL-S had the worst PFS and OS in CLL4 (median 1.95 and 4.95 years respectively), compared to TL-I and TL-L (Tables S5 and S6). n-CLL had the highest hazard ratios (HR) for both PFS and OS in ARC/ADM (Tables S7 and S8). In CLL4, n-CLL had the shortest median PFS and OS among the DME subgroups, but *TP53ab* had a greater HR (Tables S5 and S6). Importantly, stratification of i-CLL patients in only the ARC/ADM cohort showed significantly longer PFS in TL-L patients (median PFS: 6.12 years) compared to those in the TL-S (median PFS: 3.8 years) or TL-I groups (median PFS: 4.35 years; PFS $p < 0.01$, Figure 2Bi, ii).

We compared the predictive performances of three biomarkers (DME, TL and *TP53ab*) for PFS and OS in both cohorts. Predictive performance was assessed using likelihood ratios (LR+/LR–). Due to limited *TP53* data, cohorts were restricted to 250 and 176 patients for CLL4 and ARC/ADM respectively. TL and DME emerged as the strongest predictors of PFS events in the CLL4 (LR+/LR–: 7.30) and ARC/ADM (LR+/LR-: 5.42) cohorts respectively (Table S9). For OS, DME showed the highest predictive potential in

Jane Gibson, Chris Pepper and Jonathan C. Strefford share senior authorship.

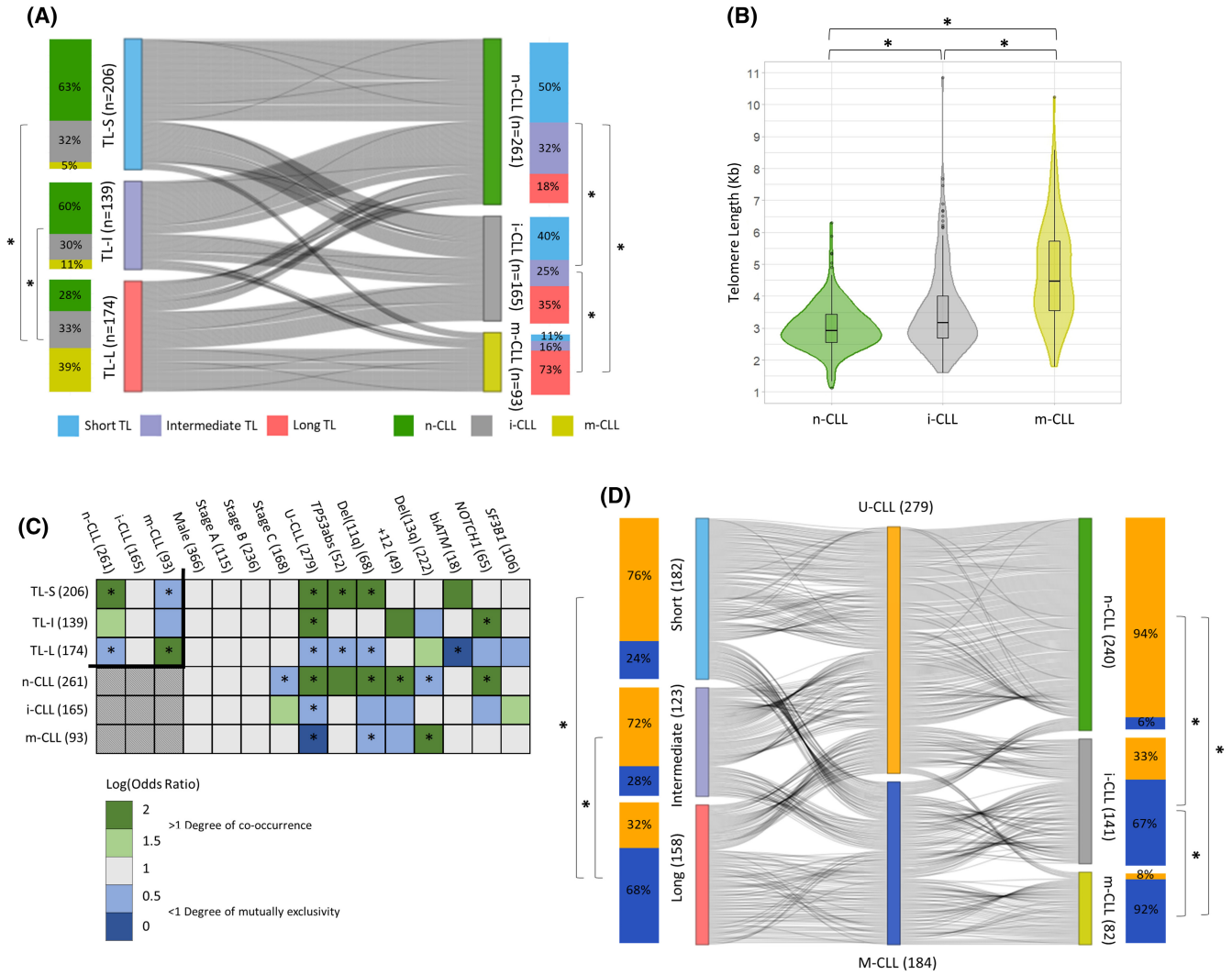

FIGURE 1 Clinico-biological associations between DME, TL and other biomarkers in 519 patients from CLL4, ARC/ADM. (A) Sankey plot showing the relationship between DME and TL subgroups ($n = 519$). A chi-squared test indicated significant differences (*$p < 0.01$). (b) Violin plot showing TL as a continuous variable across DME subgroups. A pairwise Wilcoxon test indicated significant differences (*$p < 0.01$). (C) TL and DME subgroups (columns) compared against all variables (rows) in a pairwise fashion. (D) Sankey plot showing the relationship between DME, TL subgroups and IGHV mutation status ($n = 463$). A chi-squared test indicated significant differences (*$p < 0.01$). bi*ATM*, Biallelic *ATM* inactivation; Chl, Chlorambucil; FC, Fludarabine+Cyclophosphamide; M-CLL, Mutated-CLL; TL-S / -I / -L, Telomere length-Short / -Intermediate / -Long, n- / i- / m-CLL: Naïve- / intermediate- / mature-CLL; *TP53*ab, *TP53* deletion and/or mutations; U-CLL, Unmutated-CLL.

ARC/ADM (LR+/LR−: 3.69), followed by *TP53ab* (LR+/LR−: 3.30), while TL had a lower predictive potential (LR+/LR-: 1.87; Table S10).

Given our analysis suggesting that both DME and TL predict outcomes, we constructed a multivariate Cox regression model to assess their independent associations with PFS and OS. Eight significant predictors from univariate analysis were included in the initial model, followed by stepwise backwards elimination to reach the final model. For CLL4, the final models included 246 subjects, 221 PFS events and 205 OS events. The ARC/ADM PFS model was based on 138 subjects and 86 events, while the OS model included 176 subjects and 45 events. Both DME and TL provided independent prognostic information. TL-S independently predicted poorer PFS (CLL4 HR:2.14, 95% CI:1.39–3.3, $p < 0.001$; ARC/ADM HR:2.18, 95% CI:1.17–4.05, $p < 0.01$) and inferior OS in CLL4 (CLL4 HR:2.4, 95% CI:1.51–3.81, $p < 0.001$; ARC/ADM HR:2.26, 95% CI:1.09–4.67, $p = 0.08$). DME remained significant in all models except for PFS in ARC/ADM. *TP53ab* showed the highest increase in risk of progression (CLL4 HR:3.38, 95% CI:2.13–5.37, $p < 0.001$; ARC/ADM HR:4.94, 95% CI:2.58–9.48, $p < 0.001$; Figure 2C, Tables S11–S14). TL-S and n-CLL epitype were strongly associated with early patient death. Limited data availability prevented analysis of the clinical importance of IGLV3-21R110 in our cohort.[13,14]

In summary, our analysis is the first direct comparison of the prognostic impact of TL and DME in a large cohort of CLL patients enrolled in a prospective clinical trial, with extensive follow-up and molecular characterization, and confirmation in an independent cohort (ARC/ADM). Our

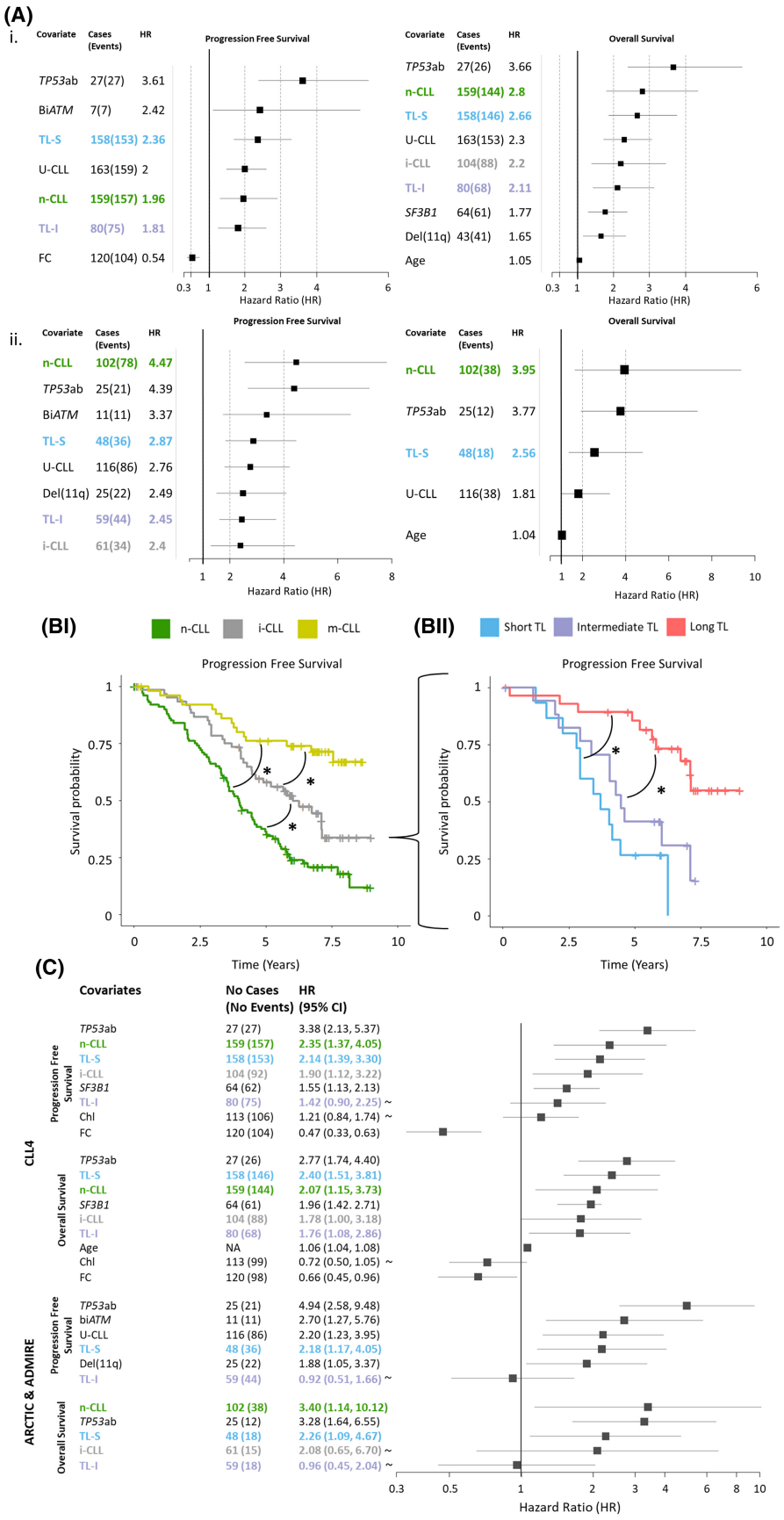

FIGURE 2 Clinical impact of DME and TL in CLL4 and ARC/ADM patients. (A) Forest plot of variables significant in univariate analyses for CLL4 (Ai) and ARC/ADM (Aii) cohorts with PFS or OS outcomes. (B) Kaplan–Meier plots where a pairwise log-rank test indicated significant differences (*$p < 0.01$): (Bi) DME variable in ARC/ADM cohort with PFS outcome. (Bii) i-CLL patients in ARC/ADM ($n = 61$) stratified by TL groups. (C) Forest plots of variables significant in final multivariable models after stepwise backwards elimination for CLL4 and ARC/ADM. Non-significant factors within categorical variables are indicated (~). bi*ATM*, Biallelic *ATM* inactivation; Chl, Chlorambucil; FC, Fludarabine+Cyclophosphamide; n- / i-CLL, naïve- / intermediate-CLL; TL-S / -I, Telomere length-Short / -Intermediate; *TP53*ab, *TP53* deletion and/or mutations; U-CLL, Unmutated-CLL.

study aimed to determine whether TL or DME is more clinically useful for managing CLL patients. Both biomarkers provided valuable information on adverse clinical events, including progression and death, suggesting they capture different biological aspects driving an aggressive phenotype. Future studies with larger cohorts and inclusion of patients in targeted agent trials are needed. Our findings highlight the additional prognostic value of TL and DME compared to established genomic lesions in predicting PFS and OS post-(immuno)-chemotherapy, suggesting they may help identify IGHV-mutated patients who might benefit more from targeted agents.

AUTHOR CONTRIBUTIONS

LC, KN, HP, ANT, HA, DMB and CCO performed the experimental work; LC, LK, ME, DB and JG conducted the statistical and bioinformatics analyses; ME, AP, PH, AS, RW and DGO contributed patient samples and data; LK, JG, JCS and CP initiated and designed the study; LK, HP, JG, CP and JCS wrote the paper; and all authors critically reviewed the final paper.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge all patients who contributed to this study. The authors are indebted to Professor Daniel Catovsky for the generation and curation of the CLL4 trial. This work was funded by Bloodwise (11052, 12036), the Kay Kendall Leukaemia Fund (873), Cancer Research UK (ECRIN-M3 accelerator award C42023/A29370, Southampton Experimental Cancer Medicine Centre grant C24563/A15581, Cancer Research UK Southampton Centre grant C34999/A18087 and programme C2750/A23669) and the Bournemouth Leukaemia Fund. The LRF CLL4 trial was funded by a core grant from Leukaemia and Lymphoma Research. ME acknowledges the support by The Arbib Charitable Fund. The views expressed in this paper are those of the authors and not necessarily those of the funding agencies. This work was presented at the 65th American Society of Hematology Annual Meeting in San Diego.

FUNDING INFORMATION

The authors have no sources of funding to disclose.

CONFLICT OF INTEREST STATEMENT

DMB and CP have received funding from TeloNostiX.

DATA AVAILIBILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Approval was provided by Somerset Research Ethics committee (REC number 06/Q2202/30).

PATIENT CONSENT STATEMENT

All patients signed an informed consent form.

CLINICAL TRIAL REGISTRATION (INCLUDING TRIAL NUMBER)

These data are from three prospectively registered studies; UK LRF CLL4 (NCT00004218), UKCRN ARCTIC ('ARC', ID7136) and UKCRN ADMIRE ('ADM', ID6897).

Louise Carr[1]
Kevin Norris[2]
Helen Parker[1]
Anna Nilsson-Takeuchi[1]
Dean Bryant[1]
Harindra Amarasinghe[1]
Latha Kadalayil[3]
Monica Else[4]
Andrew Pettitt[5]
Peter Hillmen[6]
Anna Schuh[7]
Renata Walewska[8]
Duncan M. Baird[2]
David G. Oscier[8]
Christopher C. Oakes[9]
Jane Gibson[1]
Chris Pepper[10]
Jonathan C. Strefford[1]

[1]*Cancer Genomics, School of Cancer Sciences, University of Southampton, Southampton General Hospital, Southampton, UK*
[2]*Division of Cancer and Genetics, School of Medicine, Cardiff University, Cardiff, UK*
[3]*Faculty of Engineering and Physical Sciences, School of Chemistry, University of Southampton, Southampton, UK*
[4]*Division of Molecular Pathology, The Institute of Cancer Research, London, UK*
[5]*Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, UK*
[6]*Section of Experimental Haematology, Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, UK*
[7]*Oxford National Institute for Health Research Biomedical Research Centre/Molecular Diagnostic Centre, University of Oxford, Oxford, UK*
[8]*Division of Haematology, University Hospitals Dorset, Bournemouth, UK*
[9]*Division of Haematology, Department of Internal Medicine, The Ohio State University, Columbus, Ohio, USA*
[10]*Brighton and Sussex Medical School, University of Sussex, Brighton, UK*

Correspondence
Jonathan C. Strefford, Faculty of Medicine, University of Southampton, Cancer Genomics Group, MP824 Somers Building, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK.
Email: jcs@soton.ac.uk

ORCID

Andrew Pettitt https://orcid.org/0000-0002-0907-8950
Peter Hillmen https://orcid.org/0000-0001-5617-4403
Renata Walewska https://orcid.org/0009-0007-5693-2789
Duncan M. Baird https://orcid.org/0000-0001-8408-5467
Jonathan C. Strefford https://orcid.org/0000-0002-0972-2881

TWITTER

Jonathan C. Strefford jonstrefford

REFERENCES

1. Cohen JA, Bomben R, Pozzo F, Tissino E, Härzschel A, Hartmann TN, et al. An updated perspective on current prognostic and predictive biomarkers in chronic lymphocytic leukemia in the context of chemoimmunotherapy and novel targeted therapy. Cancers (Basel). 2020;12(4):894–915.
2. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018;131(25):2745–60.
3. Walewska R, Parry-Jones N, Eyre TA, Follows G, Martinez-Calle N, McCarthy H, et al. Guideline for the treatment of chronic lymphocytic leukaemia. Br J Haematol. 2022;197(5):544–57.
4. Kulis M, Heath S, Bibikova M, Queirós AC, Navarro A, Clot G, et al. Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia. Nat Genet. 2012;44(11):1236–42.
5. Queirós AC, Villamor N, Clot G, Martinez-Trillos A, Kulis M, Navarro A, et al. A B-cell epigenetic signature defines three biologic subgroups of chronic lymphocytic leukemia with clinical impact. Leukemia. 2014;29(3):598–605.
6. Fang T, Zhang Z, Ren K, Zou L. Genetically determined telomere length as a risk factor for hematological malignancies: evidence from Mendelian randomization analysis. Aging. 2024;16(5):4684–98.
7. Rampazzo E, Bonaldi L, Trentin L, Visco C, Keppel S, Giunco S, et al. Telomere length and telomerase levels delineate subgroups of B-cell chronic lymphocytic leukemia with different biological characteristics and clinical outcomes. Haematologica. 2012;97(1):56–63.
8. Norris K, Oakes CC, Giacopelli B, Shanafelt TD, Kay NE, Chaffee KG, et al. Telomere length is associated with epigenetic programming in CLL and is a superior predictor of clinical outcome with the ability to bifurcate patients with the same CLL-IPI score. Blood. 2018;132(Suppl 1):1833.
9. Wojdacz TK, Amarasinghe HE, Kadalayil L, Beattie A, Forster J, Blakemore SJ, et al. Clinical significance of DNA methylation in chronic lymphocytic leukemia patients: results from 3 UK clinical trials. Blood Adv. 2019;3(16):2474–81.
10. Lin TT, Norris K, Heppel NH, Pratt G, Allan JM, Allsup DJ, et al. Telomere dysfunction accurately predicts clinical outcome in chronic lymphocytic leukaemia, even in patients with early stage disease. Br J Haematol. 2014;167(2):214–23.
11. Strefford JC, Kadalayil L, Forster J, Rose-Zerilli MJJ, Parker A, Lin TT, et al. Telomere length predicts progression and overall survival in chronic lymphocytic leukemia: Data from the UK LRF CLL4 trial, Leukemia. Nature Publishing Group. 2015;29:2411–4.
12. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 2009;37(3):21.
13. Nadeu F, Royo R, Clot G, Duran-Ferrer M, Navarro A, Martín S, et al. IGLV3-21R110 identifies an aggressive biological subtype of chronic lymphocytic leukemia with intermediate epigenetics. Blood. 2021;137(21):2935–46.
14. Duran-Ferrer M, Mansouri L, Nadeu F, Clot G, Bhoi S, Ann Sutton L, et al. A comprehensive DNA methylome analysis of stereotyped and non-stereotyped CLL reveals an epigenetic signature with strong clinical impact encompassing IGHV status, stereotypes and IGLV3-21R110. Blood. 2022;140(Suppl 1):1800–2.

SUPPORTING INFORMATION

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