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CORRESPONDENCE

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Circular RNA signature of aggressive CLL with t(14;19)(q32;q13). An ERIC study

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Abstract

In Chronic Lymphocytic Leukemia (CLL), t(14;19)(q32;q13), leading to the overexpression of BCL3, is found in ~1% of cases and is associated with an aggressive disease. In this study, leveraging a large CLL patient cohort collected thanks to an international collaboration, we investigate for the first time the circular transcriptome (circRNAome) associated with the rare t(14;19), in comparison with CLL without t(14;19) and B cells of age-matched healthy donors. We described the circRNAs commonly dysregulated in CLL, including circCSNK1G3 and circEXOC6B(3–5), which were depleted, and circZNF609 and circLPAR3, which were overexpressed in malignant cells. Of importance, we disclosed the circRNA signature of CLL with t(14;19), formed by circRNAs with expression significantly altered specifically in link with this lesion, ectopically expressed like circCDK14(3–4), circCORO1C, circCLEC2D, and circEMB, or downregulated like circCEP70(3–6). Several of these molecules were previously shown to be dysregulated or play a role in cancer, whereas most of the signature circRNAs deserve further investigation. CLL patients with high circCORO1C and circCLEC2D expression had significantly worse clinical outcomes, with shorter time to first treatment and overall survival. This study disclosed new molecular features of the aggressive CLL subtype with t(14;19).

Keywords CLL, T(14;19), Circular RNA, BCL3

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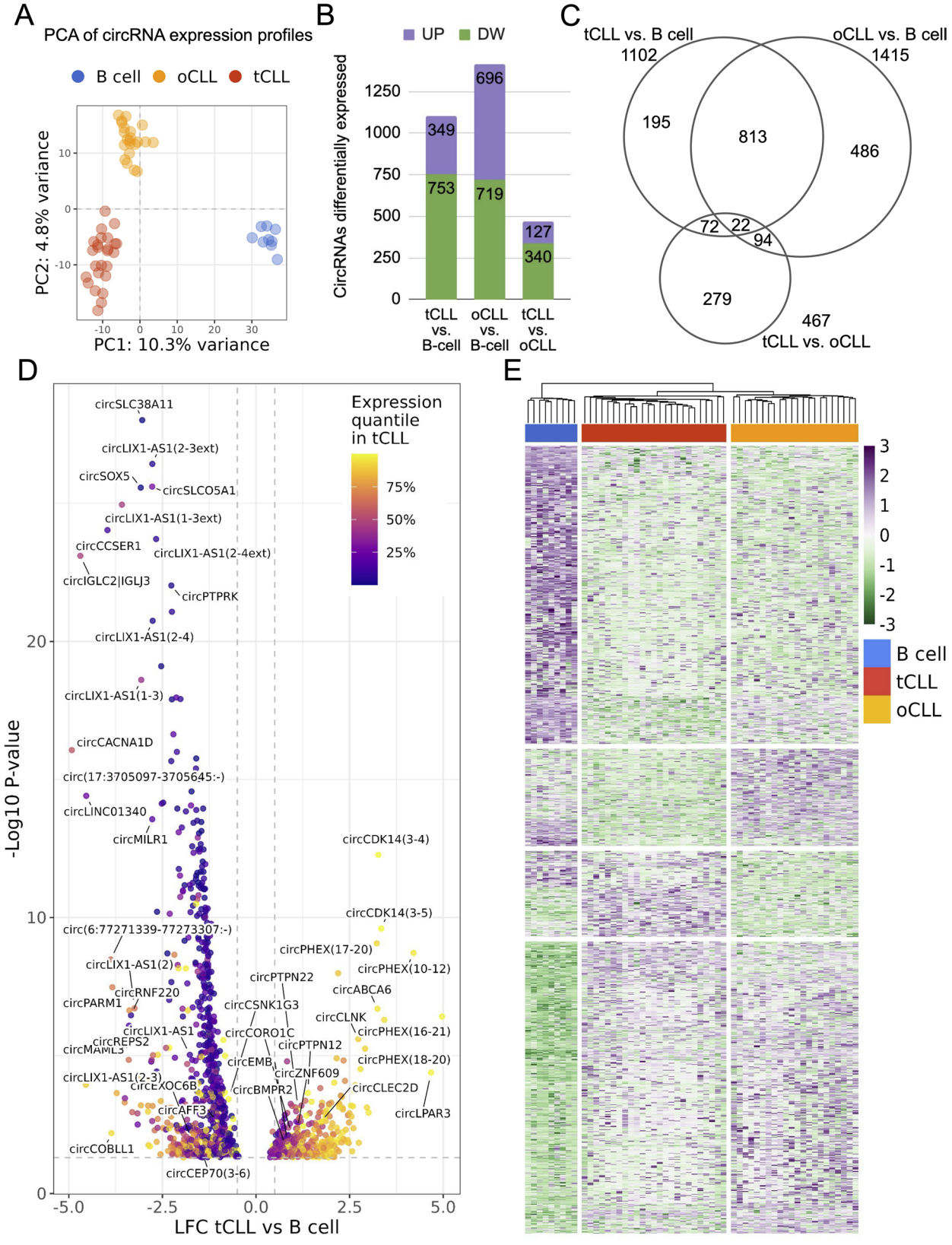

Fig. 1 (See legend on next page.)

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Fig. 1 CircRNA expression variation in tCLL, oCLL, and B cells, as a normal counterpart. **A**) Principal component analysis of circRNA expression profiles, normalized and adjusted to remove batch effects using surrogate variables methods; **B**) Number of circRNA differentially (Limma-Voom, Benjamini–Hochberg adjustment for multiple testing, adj. $p \leq 0.05$) expressed, overexpressed or less expressed, in pairwise comparisons of tCLL, oCLL and B cell samples (UP, overexpressed; DW, less expressed); **C**) Overlap of circRNAs differentially expressed in pairwise comparisons of tCLL, oCLL and B cell samples; **D**) Volcano plot of circRNAs significantly differentially expressed in tCLL compared to B cells (the x axis reports the Log of the Fold Change (LFC) in tCLL vs. B cell samples, the y axis the negative Log10 of the adj. P-value, the dot color indicates the expression level percentile in tCLL according to the color scale in the legend; **E**) Heatmap (Manhattan distance metric, Ward D2 clustering method) with sample clustering of the 1,961 circRNAs differentially expressed in at least one of the in pairwise comparisons of tCLL, oCLL and B cell samples

To the Editor,

We present the circular RNA (circRNA) signature of a rare chronic lymphocytic leukemia (CLL) harboring t(14;19)(q32.3;q13.2) (tCLL).

CLL, characterized by the clonal expansion of mature B-cells, is still an incurable disease with a heterogeneous clinical course. t(14;19), juxtaposing the transcription factor BCL3 with the immunoglobulin heavy chain locus, thus causing BCL3 overexpression and B cell transformation, is found in ∼1% of CLL cases and is associated with a more aggressive disease [1]. In CLL, most studies focused on gene expression or microRNAs, and only a few reported circRNA dysregulation [2–4]. CircRNAs are emerging as highly relevant molecules in leukemia biology for their contribution to disease mechanisms [5], where they can function as oncogenes [6] or tumor suppressors [7], and represent biomarkers, therapeutic targets, or agents as well.

In this study, we tackle the characterization of the molecular features of tCLL patients through the investigation of their circular transcriptome. We focused on 23,760 circRNAs detected and annotated with CircComPara2 [8] in leukemic B cells purified (> 97% purity) from peripheral blood of 25 tCLL, 22 CLL patients without t(14;19) (oCLL), and the normal counterpart, namely B cells isolated from healthy donors, profiled by RNA-seq allowed us to define the circRNA expression profile of this rare and aggressive CLL subtype. We found that tCLL more commonly presented an unmutated status of IGHV gene, mutation of TP53 and more than 3 chromosomal abnormalities (Supplementary Table S1) compared to oCLL. In addition, we observed distinct circRNA expression profiles among tCLL, oCLL, and normal mature B cells (Fig. 1A), indicating circRNA expression dysregulation in malignant cells, with statistical significance defined as adjusted P-value ≤ 0.05 (Supplementary Methods, Supplementary Results, Supplementary Table S1, Supplementary Fig. 1). Compared to B cells, most of the 1,102 circRNAs dysregulated in tCLL were less abundant in tCLL (753; 68%) (Fig. 1B-D and Supplementary Table S2), including circCEP70(3–6), and multiple circular isoforms with different backspliced exons from *CACNA1D* and *LIX1-AS1* genes. Several isoforms expressed from *CDK14* and *PHEX*, as well as circABCA6 and circLPAR3, were amongst the circRNAs most upregulated in tCLL. Moreover, 1,415 circRNAs were altered in oCLL. The dysregulation of highly expressed circRNAs, such as circBMPR2, circPTPN12, circPTPN22 and circPHEX upregulation, as well as the downregulation of circLIX1-AS1, circIQSEC1 and circRALGPS2, was consistent with the literature [2–4], whereas most of the identified alterations were novel findings. The direct comparison between CLL samples with and without the translocation pinpointed 467 circRNAs with different expression, 127 up- and 340 down-regulated in the tCLL group with the most aggressive disease (Fig. 1B-C and Supplementary Fig. 3). The 835 circRNAs altered in a similar way in both CLL patient groups compared to the mature B cells from healthy donors represent common features of CLL, independently of the presence of the BCL3 overexpressing translocation. For example, considering circRNAs that were previously associated with a functional role in cancer, the oncogenic circZNF609 [9] and the tumour suppressor circEXOC6B [10] were respectively more and less expressed in CLL as a whole, and also circAFF3, with extremely high expression in B-cells, was commonly depleted. We validated by RT-qPCR in samples form an extended cohort of cases (Supplementary Materials, Supplementary Table S3, Fig. 2A) the dramatic decrease in both CLL groups of circCSNK1G3, in line with its reported downregulation in acute myeloid leukemia [11], and a significant increase of circLPAR3, which has been described as oncogenic in carcinomas of the digestive system. Of note, according to sample clustering by the expression profiles of the 1,961 circRNAs with different expression (adjusted P-value ≤ 0.05) in at least one of the three above-mentioned comparisons, tCLL clusters separately from oCLL (Fig. 1E), further suggesting that t(14;19) and BCL3 overexpression are linked to a specific circRNAome. A circRNA signature of CLL with t(14;19) is defined by 80 circRNAs altered in tCLL and with different expression levels in tCLL vs. oCLL (Fig. 2B). These include 8 circRNAs dysregulated also in oCLL but significantly less than in tCLL, and 72 not altered in oCLL compared to B cells. We confirmed that circCDK14(3–4) is upregulated in both tCLL and oCLL compared to B cells, but significantly more in the former (Fig. 2C). Moreover, we validated a significant overexpression of circCORO1C, circCLEC2D, circEMB, and a lower expression of circCEP70(3–6) specific to tCLL compared to B-cells (Fig. 2C). Most (60; 75%) of the signature circRNAs are expressed from known BCL3 target genes.

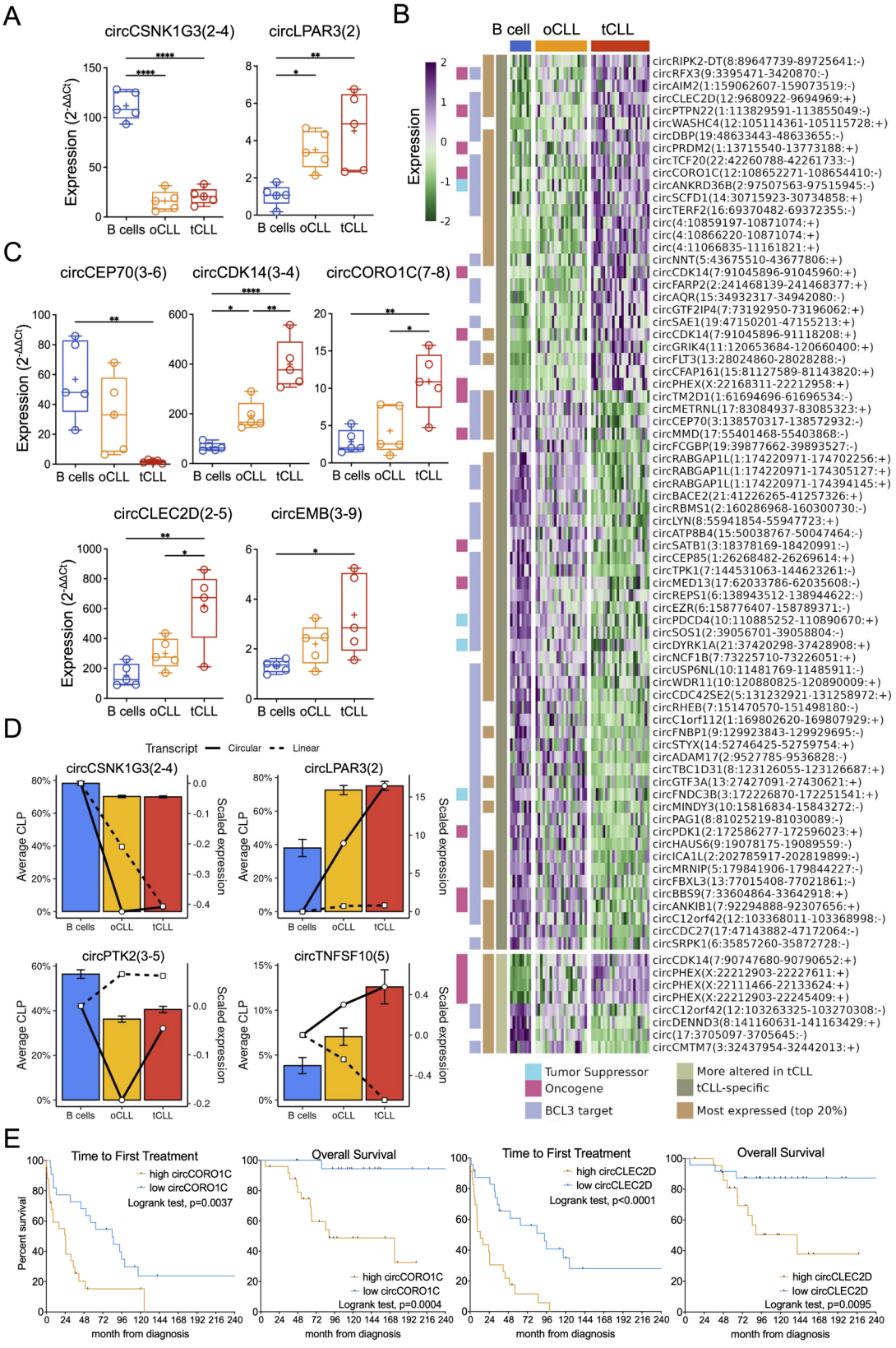

Fig. 2 (See legend on next page.)

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Fig. 2 Validation of circRNA commonly dysregulated in tCLL and oCLL and the circRNA signature of tCLL. **A**) RT-qPCR quantification of circLPAR3 and circCSNK1G3 in tCLL, oCLL and B cell samples confirmed a similar dysregulation in both CLL groups (relative amounts determined with the ΔΔCt method and normalized on GAPDH expression; Tukey's multiple comparisons test, *, ** and **** indicate adj. p.value ≤ 0.05, ≤ 0.01, and ≤ 0.0001; each circRNA has been quantified in 5 tCLL and 5 oCLL cases, from an extended cohort); **B**) Heatmap of 80 circRNAs differentially expressed in all the pairwise comparisons of tCLL vs. B cell samples, and in TCLL vs. oCLL, representing the circRNA signature of tCLL (Manhattan distance metric, Ward D2 clustering method; the color bar on the left indicates, according to the legend: i) the signature circRNAs also altered in oCLL compared to B cells but to a significant lesser extent than in tCLL, and those altered in oCLL, plus, according to the literature, ii) the circRNAs associated to tumor suppressor or oncogenic functions, iii) expressed by genes being known transcriptional targets of BCL3 and iv) highly expressed (average expression among the 20% highest in tCLL or B cell samples); **C**) RT-qPCR quantification of selected tCLL signature circRNAs (circCDK14(3–4), circCORO1C, circCLEC2D, circEMB, circCEP70(3–6)) validating RNA-seq based findings (relative amounts determined with the ΔΔCt method and normalized on GAPDH expression; Tukey's multiple comparisons test, *, ** and **** indicate adj. p.value ≤ 0.05, ≤ 0.01, and ≤ 0.0001; each circRNA has been quantified in 5 tCLL and 5 oCLL cases, from an extended cohort); **D**) Average circular-to-linear expression proportion (CLP; bars; vertical axis on the left) and scaled expression relative to the B cell expression mean (vertical axis on the right) of the circular (solid lines, round dots) and linear (dashed lines, square dots) transcript of the *CSNK1G3*, *LPAR3*, *PTK2*, and *TNFSF10* genes; error bars indicate the standard error from the average CLP; **E**) Kaplan-Meier plots of time to first treatment (TTFT) and overall survival (OS) of CLL patients stratified by circRNA expression (high, over the average; low, below the average; Log Rank test)

Considering the circular-to-linear expression proportion (CLP), 51 circRNAs showed preferential expression of the circular form (CLP > 0.5). CircRNA dysregulation can involve an imbalance between the expression of circRNAs and their linear counterparts. We identified hundreds of circRNAs with significantly varied CLP (Supplementary Table S2). Notably, circCDK14(3–4), circLIX1-AS1(2–3+), and circLPAR3 showed higher proportions in tCLL compared to B cells. Conversely, multiple circular isoforms of *ABCA6*, *CSNK1G3*, *PHEX*, and *PTPN22* displayed lower abundance. In particular, *CSNK1G3* and *LPAR3* exhibited exceptionally high proportions of circular isoform abundance in CLL (CLP ≥ 0.7). Moreover, the proportion of circCSNK1G3 was significantly higher in B cells, whereas that of circLPAR3 was lower (Fig. 2D). Aberrant circRNA expression can dramatically impact the function of its linear counterpart [12]. In genes crucial for CLL, we observed diverse expression relationship patterns between circular and linear isoforms. For instance, the tumor-suppressor, pro-apoptotic *TNFSF10* gene exhibited an increased CLP in tCLL due to elevated circTNFSF10(5) expression coupled with a marked decrease in its linear mRNA. Notably, the biogenesis of circTNFSF10(5) may compete with its linear counterpart, as the backsplicing involves a substantial portion of the mRNA's final exon. In contrast, the CLP variation in the *PTK2* gene stemmed from a different mechanism: the proportion of circPTK2(3–5) increased in tCLL compared to oCLL due to higher circRNA abundance. At the same time, the linear transcript levels remained constant (Fig. 2D). These examples illustrate the complex patterns of circRNA and linear mRNA expression variation interdependency, and how the study of circRNAs can inform additional aspects of expression dysregulation.

According to a literature survey (Fig. 2B), an oncogenic role has been described for 19 of the tCLL signature circRNAs, including circEMB, circCDK14(3–4), circCORO1C, and circPTPN22, four have been associated with a tumour suppressor role, and others were dysregulated in malignant cells, albeit lacking functional characterization. For instance, circFLT3 was upregulated in B-cell precursor acute leukemia and associated with an inferior outcome in acute myeloid leukemia. The hypothesis that circRNA specifically altered in tCLL can play a role in sustaining the disease phenotype needs to be explored by experimental investigation, but is supported by the observation that several of these molecules were previously shown to be dysregulated or play a role in cancer.

Considering clinical data, we observed that CLL patients with high circCORO1C and circCLEC2D expression have significantly shorter TTFT and OS (Fig. 2E). We performed survival analysis using the expression of all the validated circRNAs (circLPAR3, circCORO1C, circCLEC2D, circCEP70, circCSNK1G3, circEMB, circCDK14). In univariate analysis, TTFT was correlated with levels of circCORO1C, circCLEC2D, circCEP70, and circEMB, while OS was correlated with circCORO1C, circCLEC2D, and circCDK14 (P-value < 0.1). In multivariate analysis, including as covariate those statistically significant in univariate analysis and those differentially presented between tCLL and oCLL (Supplementary Table S1 and S4), circCORO1C, circCLEC2D retained prognostic significance (Supplementary Table S5).

In conclusion, we identified circRNAs with expression exclusively or significantly more markedly altered in CLL linked with t(14;19), defining the circRNA signature of this CLL subtype with aggressive behaviour. Owing to the emerging roles of circRNAs in leukemias and to the oncogenic functions described in other malignancies for several of these signature circRNAs, our findings provide new candidate markers of tCLL and pave the way for exploring new biological aspects of CLL heterogeneity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13045-025-01725-y.

Supplementary Material 1

Supplementary Material 2

Author contributions
ER, EG, and SB conceived the study, provided methods, analyzed transcriptomic data, and wrote the article. SB acquired funding.AV conceived the study, provided data and samples, analyzed data, wrote the article, and acquired funding. MF, KAR, PB, CC, CM, CH, KP, DO, ZD, FNK, GMR, AA, FBM, AVa, MJT, PA, BE, AP, AM, LB, FRM, LS, TC, ET, KAK, AK, FB, MD, PP, OK, VR, ACe, FA, CAD, AR, RF, SY, BE, JW, ACu, KSt, PG, and LT provided data and samples, gave intellectual input, revised the manuscript. SO, KSa, RM, ACa, and IG collaborated on the analysis of transcriptomic data, gave intellectual input, and revised the manuscript. GC and FF performed RT-PCR analysis and revised the manuscript.

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Data availability
RNA-Seq data were deposited into the ArrayExpress repository under accession number E-MTAB-15004.

Declarations

Ethics approval and consent to participate
The study was conducted according to the Declaration of Helsinki, approved by the ethics committee of the Padova University Hospital (protocol # 4430/AO/18), and informed consents were collected.

Competing interests
AV, LT, PG, LS, AC, and FRM attended scientific boards organized by Johnson & Johnson, AstraZeneca, BeiGene, and AbbVie. PG received honoraria from AbbVie, AstraZeneca, BeiGene, BMS, Galapagos, Johnson & Johnson, Lilly/Loxo, MSD, Roche, and research funding from AbbVie, AstraZeneca, BeiGene, BMS, Johnson & Johnson, Lilly/Loxo, MSD. LS received honoraria from AbbVie, AstraZeneca, BeiGene, Johnson & Johnson, Lilly, and MSD. The other authors declared no potential conflict of interest with this study.

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