Clinical, biological, and molecular genetic features of Richter syndrome and prognostic significance: a study of the French Innovative Leukemia Organization

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To the Editor:

Richter syndrome is the transformation of Chronic Lymphocytic Leukemia (CLL) or Small Lymphocytic Lymphoma into aggressive lymphoma. This study focuses on the diffuse large B-cell lymphoma (DLBCL) variant of Richter syndrome (RS). Richter syndrome prognosis is poor, with a median overall survival (OS) ranging from 4 months to 2 years.[1] In 80%-90% of cases, the RS clone arises from the CLL clone, whereas a minority of RS are clonally unrelated DLBCLs in a patient with CLL. The latter is associated with a median OS similar to de novo DLBCL. Two clinico-biological prognostic scoring systems are currently available.[2,3] Richter syndrome molecular profile shares only a few common features with de novo DLBCLs, with four highly prevalent genomic abnormalities: *TP53* abnormalities, *MYC* deregulations, *NOTCH1* mutations and *CDKN2A/B* deletions. Richter syndrome harbors an unmutated IGHV in 80% of cases and a high prevalence of stereotypic B-Cell Receptor (BCR).[1] To date, only a few RS molecular abnormalities were investigated for prognostic value.

Our objectives were to investigate the clinical and molecular genetic prognostic factors at RS diagnosis and to better characterize molecular abnormalities underlying RS and CLL-RS transformation in a multicenter cohort of 103 RS patients (Figure S1).

Clinical, biological and treatment data at CLL and RS phases are provided in Table S1. The FISH data were available for 27 CLL samples (Figure S2A). Sequencing data at RS stage were available for 59 samples, including 20/59 (34%) developing from a previously untreated CLL. The other CLLs were treated with one, two or at least three treatment lines in 15/59 (25%), 7/59 (12%) and 17/59 (29%) cases, respectively. Targeted therapies were used as subsequent lines of therapy in six patients at CLL stage and four patients at RS stage.

The IGHV mutational status at CLL stage was available in 34 cases, the majority of which (27/34; 79.4%) were IGHV unmutated. The CLL IGHV repertoire made exclusive use of V-families one, three and four. The V1-69 usage was the most frequent (7/34; 20.6%). Also, VH3-21 and VH4-39 IGHV were used by 3/34 (8.8%) and 2/34 (5.9%) CLL samples, respectively. The global repartition was similar between the whole CLL series and the subgroup of clonally related CLLs (Figure S2B). A stereotypic BCR was found in 6/34 (17.6%) CLLs. Subset six was the most frequent, both in the full CLL cohort and in the subgroup of clonally related CLLs (Figure S2C). The DNA samples at CLL phase were available in 32 cases (paired-CLLs), and analyzed with a 13-gene panel covering the most frequently mutated CLL genes (Figure 1A). We observed a high prevalence of *NOTCH1* (31%), *TP53* (19%), *EGR2* (16%), *XPO1* (9%) and *RPS15* (9%) pathogenic single nucleotide variants (SNV) or insertions/deletions (Indels). In contrast, *SF3B1* (16%), *POT1* (7%), *BIRC3* (6%), *BRAF* (3%), *MYD88* (3%) and *FBXW7* (3%) pathogenic variants rates were similar to those of unselected CLL.

Overall, 46/59 (78.0%) RS patients had unmutated IGHV on RS biopsy. The vast majority of IGHV rearrangements belonged to the VH families one, three and four, with a comparable distribution pattern in clonally related RS (Figure S3A). The BCR subsets were found in 14/59 (23%) RS, including 6/14 (42.9%) from subset six, 2/14 (14.3%) from subset eight and 1/14 (7.1%) from subset two (Figure S3B). The clonal relationship between CLL and RS was detected in 29/34 (85.3%) patients. Among these 29 clonally-related RS, 23 had received at least one treatment line for underlying CLL (Table S2). In our series, 34/57 (59.6%) RS patients showed a *TP53* abnormality at RS phase, including eight (14.0%) with isolated *TP53* pathogenic SNV or Indels, 11 (19.3%) with isolated 17p deletion and 15 (26.3%) with both. Sequencing data at RS stage were available for 59 samples. More than 38% of *TP53* pathogenic SNVs were identified in RS arising in untreated CLL. In contrast, prevalence of *NOTCH1*, *SF3B1*, *EGR2*, *XPO1* and *ATM* pathogenic variants was higher in RS diagnosed in previously treated CLL, and *BIRC3*, *POT1*, *FBXW7* and *RPS15* pathogenic variants exclusively occurred in that context (Figure 1B).

Paired sequential analysis of our 13-gene panel was performed for 32 cases with DNA available at both CLL and RS phase. Twenty-six out of these 32 RS were clonally related to the CLL component, according to IGHV comparison (Figure 1C, Table S3). A first group of pathogenic SNVs or Indels detected at RS setting were already present at CLL phase in most cases, including mutations of *NOTCH1* (already present at CLL phase in 10/15; 66.7% of cases), *EGR2* (5/6 cases; 83.3%), *XPO1* (3/4; 75.0%) and *RPS15* (3/3; 100%). A more balanced distribution between cases with pathogenic variants already present at CLL phase and cases with mutation acquired at RT was observed for *TP53* (4/11; 36.4% and 7/11; 63.6%, respectively) and *SF3B1* (4/7; 57% and 3/7; 43%, respectively). The *EGR2*, *XPO1* and *RPS15* pathogenic variants were only found in clonally related RS, and co-occurred with those of *NOTCH1* at RT in 5/6, 3/3 and 3/4 cases, respectively. Also, *TP53*, *NOTCH1* and *SF3B1* mutations were the most frequently acquired at RT (Figure S3C). In a majority of RS cases, all detected abnormalities identified at CLL phase were also

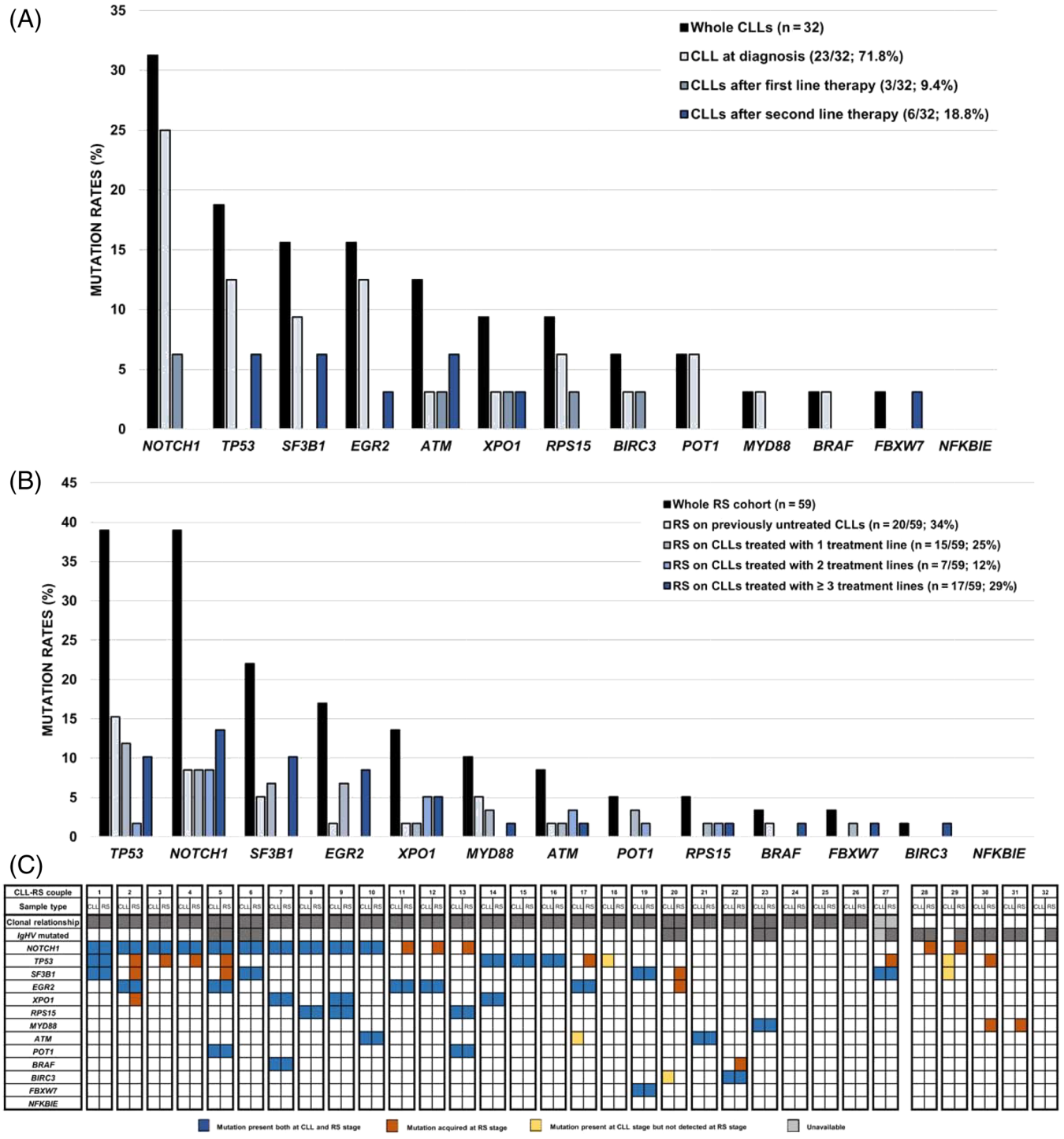

FIGURE 1 Cytogenetic and molecular features at CLL and RS phases. (A) Mutation rates for a panel of 13 frequent CLL-mutated genes (n = 32 paired-CLLs); (B) Mutation rates at RS stage according to the number of previous CLL treatment lines (n = 59); (C) Heatmap of gene mutations for CLL-Richter matched samples (n = 32), distinguishing mutations present at both CLL and RS stages, mutations acquired at Richter transformation and mutations detected at CLL phase but not at RS setting. Twenty-six out of these 32 RS were clonally related to the CLL component, according to IGHV comparison. An additional RS case was likely clonally related since we found the same *SF3B1* mutation both at CLL and RS phase

observed at RS stage, together with additional genomic lesions acquired at RT (Figure S4).

The median OS from RS diagnosis was 9.0 months (95%CI 7–13). In univariate analysis (Table S4), the clinical and biological factors significantly associated with shorter OS were ECOG PS score > 1 ($p < 0.001$), platelet count <100 × 10^9/L ($p = 0.001$), absence of complete metabolic response ($p < 0.001$), hemoglobin level ($p = 0.017$) and LDH > N ($p = 0.0002$). Factors tending to be associated with worse OS ($p < 0.2$), included Ann Arbor stage ($p = 0.153$), prior CLL treatment ($p = 0.053$), unmutated IGHV ($p = 0.072$), *TP53* abnormality ($p = 0.072$), *NOTCH1*

pathogenic SNVs/Indels ($p = 0.137$), absence of *MYD88* pathogenic variants ($p = 0.122$) and *EGR2* pathogenic variants ($p = 0.162$), all evaluated on RS biopsy. Because of significant correlation between some of these variables with $p < 0.2$ (Table S5), we selected ECOG PS, IGHV mutational status, and *TP53* abnormality for multivariable analysis. On multivariable analysis, poor survival was associated with ECOG PS score > 1 (HR 3.36; 95%CI 1.69–6.67; $p < 0.001$), unmutated IGHV status (HR 2.48; 95%CI 1.10–5.60; $p = 0.029$) and *TP53* abnormality (HR 2.31; 95% CI 1.21–4.41; $p = 0.011$) (Table S4; Figure S5). Prevalence of pathogenic variants were different between IGHV mutated and unmutated RS. IGHV unmutated RS were associated with lower prevalence of *MYD88* mutations ($p = 0.0014$) (Figure S6).

Disentangling the RS heterogeneity and its evolution is critical to improve management. *NOTCH1* and *TP53* pathogenic variants were the most frequent in patients with RS at CLL stage, supporting close monitoring of these patients. The *TP53* and *NOTCH1* mutations were also frequently acquired at RT, underlining the importance of these variants for transformation. The *SF3B1* mutations were the third most frequently acquired at RT, which is consistent with their role as an oncogenic driver of CLL evolution.[3] *NOTCH1*, *TP53*, *EGR2*, *XPO1* and *RPS15* pathogenic variants were frequent at RS setting, and already present at CLL phase in most cases, meaning that most RS samples inherit CLL mutations. Another important finding is the high prevalence of *EGR2* pathogenic variants in CLL projected to develop RS, in line with previous studies reporting *EGR2* mutations as an early event in CLL development, but virtually absent in de novo DLBCLs.[4]

The IGHV unmutated status on RS biopsy emerged as an independent adverse prognostic factor, with a median OS of 7 vs 52 months for patients with mutated IGHV, similar to de novo DLBCL and clonally unrelated RS.[1] This may be due to an overrepresentation of IGHV unmutated RS among clonally related RS ($p = 0.001$). However, CLL-RS clonal relationship was not a significant prognostic factor in our study, probably due to the lack of power. The IGHV unmutated status was a prognostic factor independent from *TP53* status. We confirmed that *TP53* abnormality specifically assessed at RS diagnosis was independently associated with shorter survival for RS patients. Predictably, we found a correlation between *TP53* abnormality and *TP53* pathogenic SNVs or Indels at RS, but only *TP53* abnormality had a significant effect on OS.

The vast majority of our patients benefited from R-CHOP-like regimens and positrons emission tomography for response assessment. Metabolic complete response was significantly associated with better OS ($p < 0.001$) in univariate analysis. Because only a few patients received novel agents, our study is relevant in the context of chemoimmunotherapy but has to be confirmed with novel agents.

Our study is limited to genes already described in CLL, and did not explore recently described genomic alterations recurrently observed in other B-cell malignancies.[5] However, most genomic aberrations associated with clonally related RS are commonly observed at CLL stage and our targeted NGS panel covered the most frequent CLL pathogenic variants.[6]

In conclusion, this study documents new data on RS biology and preceding CLL phase through molecular genetic profiling and refines biological factors associated with shorter OS, including *TP53* abnormalities, and IGHV unmutated status. These observations suggest that IGHV status especially at RS diagnosis should be widely explored together with *TP53* status and the clonal relationship for an adequate management for patients.

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CONFLICT OF INTEREST

The authors declare no relevant conflicts of interest.

AUTHOR CONTRIBUTIONS

Charline Moulin, Eugen Tausch, Francis Guillemin, Thomas Remen, Florence Cymbalista, Stephan Stilgenbauer, Pierre Feugier and Julien Broséus designed the work. Charline Moulin, Eugen Tausch, Sébastien Hergalant, Anne Quinquenel, Caroline Dartigeas, Grégory Lazarian, Odile Blanchet, Sandra Lomazzi, Elise Chapiro, Florence Nguyen-Khac, Christof Schneider, Frédéric Davi, Mathilde Hunault, Cécile Tomowiak, Damien Roos-Weil, Catherine Thieblemont, Florence Cymbalista, Kamel Laribi, Marie-Christine Béné, Pierre Feugier, Julien Broséus acquired data. Charline Moulin, Francis Guillemin, Thomas Remen, Sébastien Hergalant, Eugen Tausch, Reiner Siebert, Stephan Stilgenbauer, Florence Cymbalista, Pierre Feugier and Julien Broséus interpreted the results. Charline Moulin, Francis Guillemin, Eugen Tausch, Stephan Stilgenbauer, Florence Cymbalista, Pierre Feugier and Julien Broséus drafted the first version of the manuscript. All authors participated to the writing of the revised version of the manuscript. All authors approved the final version. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

PATIENT CONSENT

Since most patients were not alive at the time of the study start, the French National Ethics Committee exempted us from retrospectively requiring patient's consent form.

TRIAL REGISTRATION

ClinicalTrials.gov identifier: NCT03619512.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by local Institutional Review Board and the National Ethics Committee (CPP OUEST-IV).

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