bjh short report

In chronic lymphocytic leukaemia with complex karyotype, major structural abnormalities identify a subset of patients with inferior outcome and distinct biological characteristics

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Received 23 October 2017; accepted for publication 21 January 2018

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Summary

Complex karyotype (CK) is a negative prognostic factor in chronic lymphocytic leukaemia (CLL). However, CK is a heterogeneous cytogenetic category. Unbalanced rearrangements were present in 73·3% of 90 CLL patients with CK (i.e. ≥3 chromosome aberrations in the same clone), and were associated with a shorter overall survival ($P = 0·025$) and a shorter time to first treatment ($P = 0·043$) by multivariate analysis. Patients with unbalanced rearrangements presented a distinct mRNA expression profile. In conclusion, CLL patients with unbalanced rearrangements might represent a subset of very high-risk CLL patients with distinct clinical and biological characteristics.

Keywords: chronic lymphocytic leukaemia, complex karyotype, gene expression profile, overall survival, time to first treatment.

In chronic lymphocytic leukaemia (CLL), a complex karyotype (CK) represents a prognostic biomarker associated with an inferior outcome (Haferlach *et al*, 2007; Baliakas *et al*, 2014; Rigolin *et al*, 2015) and with a worse response to treatment, including novel agents (Thompson *et al*, 2015; Herling *et al*, 2016; Anderson *et al*, 2017). The negative prognostic impact of a CK has also been shown to be independent of the International Prognostic Index for patients

 doi: 10.1111/bjh.15174

with CLL (CLL-IPI) (Rigolin *et al*, 2017a) and of high-risk CLL features, including unmutated *IGHV* genes and/or 11q/17p deletions (Rigolin *et al*, 2017b).

CLL patients with CK may present a variety of cytogenetic aberrations, including numerical (i.e. monosomies and trisomies) and structural abnormalities (i.e. balanced and unbalanced translocations, derivative or marker chromosomes, isochromosomes, deletions, insertions and additions). It is unknown whether specific cytogenetic patterns within the CK may correlate with the clinical outcome.

We therefore investigated whether the presence of numerical or structural chromosomal abnormalities could be associated with distinct clinical or biological features in CLL patients with a CK.

Materials and methods

Patients

The study cohort included 90 patients with untreated CLL and a CK, diagnosed between 2000 and 2017. All patients were diagnosed and treated according to National Cancer Institute criteria (Hallek *et al*, 2008). The local ethics committee approved the study.

Cytogenetic analyses

Cytogenetic analysis was performed on PB samples and a karyotype was defined as complex in the presence of at least 3 chromosome aberrations in the same clone, as described by Rigolin *et al* (2017a). The following cytogenetic aberrations, each occurring at least in five patients, were considered: monosomies, trisomies, deletions, balanced translocations, unbalanced rearrangements (including chromosome additions, derivatives, insertions, duplications, ring, dicentric and marker chromosomes) and the presence of ≥5 abnormalities.

IGHV status, mutational analyses and gene expression profile (GEP) analysis

IGHV genes were amplified from genomic DNA and sequenced according to standard methods with the cut-off of 98% homology to the germline sequence to discriminate between mutated (<98%) and unmutated (≥98%) cases, as previously reported (Rigolin *et al*, 2015). Mutations of *NOTCH1*, *SF3B1*, *BIRC3* and *TP53* genes were analysed by next generation sequencing analysis using Ion Torrent PGM (Life Technologies, Foster City, CA), as described elsewhere (Rigolin *et al*, 2016). Details of GEP analysis are reported in Supplemental Methods (Appendix S1).

Statistical analysis

Fisher's exact test was applied for categorical variables. Time-to-first treatment (TTFT) was calculated as the interval between diagnosis and the start of first line treatment. Overall survival (OS) was calculated from the date of diagnosis until death due to any cause or until the last patient follow-up. Proportional hazards regression analysis was used to identify the significant independent prognostic variables on TTFT. The stability of the Cox model was internally validated using bootstrapping procedures (Rigolin *et al*, 2017b). Statistical analysis was performed using Stata 14·0 (Stata Corp., College Station, TX).

Results

Patients and outcome

Patients' characteristics are reported in Table I. The median age of this CLL cohort was 67·4 years (range 40–94) and the median period of follow-up was 51·3 months.

In univariate analysis (Table II), an inferior OS was associated with age >65 years ($P = 0{\cdot}007$), unbalanced rearrangements ($P = 0{\cdot}015$) and the presence of 5 or more abnormalities ($P = 0{\cdot}021$). Multivariate analysis (Table II) confirmed age >65 years ($P = 0{\cdot}038$) and unbalanced rearrangements ($P = 0{\cdot}025$; Figure S1A) to have a negative prognostic impact on OS.

A list of the karyotype abnormalities and of their distribution according to the presence of unbalanced rearrangements is presented in Table SI.

The principal clinical and biological characteristics of the patients with and without unbalanced rearrangements are reported in Table I. Patients with unbalanced rearrangements had a lower incidence of 11q deletion ($P = 0{\cdot}029$) and trisomies ($P = 0{\cdot}006$) and a higher incidence of *TP53* aberrations ($P = 0{\cdot}014$), monosomies ($P = 0{\cdot}004$) and a karyotype with more than 5 abnormalities ($P = 0{\cdot}003$).

When considering TTFT, in univariate analysis (Table II), an inferior outcome was associated with advanced Binet stage ($P = 0{\cdot}003$), unmutated *IGHV* ($P = 0{\cdot}009$) and unbalanced rearrangements ($P = 0{\cdot}018$; Figure S1B). In multivariate analysis (Table II), advanced stage ($P = 0{\cdot}001$), unmutated *IGHV* ($P = 0{\cdot}034$) and unbalanced rearrangements ($P = 0{\cdot}043$) retained their negative prognostic impact on TTFT.

Gene expression profiling (GEP)

The analysis of mRNA expression profiles of 23 patients with ($n = 11$) and without ($n = 12$) unbalanced rearrangements identified 160 differentially expressed genes ($P < 0{\cdot}1$, fold change cut-off >2·0). The cases investigated were representative of the entire cohort in terms of age, stage, TTFT and survival. A list of the most up- and down-regulated genes with the corresponding gene functions and deregulated pathways is reported in Table S2. Using this combined set of genes, a clear-cut separation of the analysed samples was evident (Figure S2). A heat-map representation of the average expression of the 30 most differentially expressed genes

Table I. Clinical and biological characteristics of the 90 patients with CK and according to the presence of unbalanced rearrangements.

Variable	Number of patients (%)	Unbalanced rearrangements		*P*
		Number of patients (%)		
		No	Yes	
Age ≤65/> 65 years	43/47 (47·8/52·2)	13/11 (54·2/45·8)	30/36 (45·5/54·5)	0·485
Gender male/female	58/32 (64·4/35·6)	16/8 (66·7/33·3)	42/24 (63·6/36·4)	1·000
Binet Stage A/B-C	63/27 (70·0/30·0)	19/5 (79·2/20·8)	44/22 (66·7/33·3)	0·306
CD38 negative/positive (cut-off 30%)	46/42 (52·3/47·7)	13/11 (54·2/45·8)	33/31 (51·6/48·4)	1·000
ZAP70 negative/positive (cut-off 30%)	36/24 (60·0/40·0)	11/3 (78·6/21·4)	25/21 (54·4/45·6)	0·130
IGHV mutated/unmutated	21/39 (35·0/65·0)	7/8 (46·7/53·3)	14/31 (31·1/68·9)	0·352
11q deletion yes/no	24/66 (26·7/73·3)	11/13 (54·2/45·8)	13/53 (80·3/19·7)	0·029
TP53 aberration yes/no	28/42 (40·0/60·0)	3/16 (15·8/84·2)	25/26 (49·0/51·0)	0·014
NOTCH1 mutated/wild type	5/41 (10·9/89·1)	2/12 (14·3/85·7)	3/29 (9·4/90·6)	0·633
SF3B1 mutated/wild type	6/31 (16·2/83·8)	2/11 (15·4/84·6)	4/20 (16·7/83·3)	1·000
BIRC3 mutated/wild type	2/35 (5·4/94·6)	1/12 (7·7/92·3)	1/23 (4·2/95·8)	1·000
Monosomies yes/no	42/48 (46·7/53·3)	5/19 (20·8/79·2)	37/29 (56·1/43·9)	0·004
Trisomies yes/no	34/56 (37·8/62·2)	15/9 (62·5/37·5)	19/47 (28·8/71·2)	0·006
Deletions yes/no	68/22 (75·6/24·4)	19/5 (79·2/20·8)	49/17 (74·2/25·8)	0·784
Balanced translocations yes/no	30/60 (33·3/66·7)	12/12 (50·0/50·0)	18/48 (27·3/72·7)	0·075
Unbalanced rearrangements yes/no	66/24 (73·3/26·7)	na	na	na
≥5 abnormalities yes/no	35/55 (38·9/61·1)	3/21 (12·5/87·5)	32/34 (48·5/51·5)	0·003

na, not applicable.

Table II. Univariate and multivariate analysis for overall survival and time to first treatment.

Overall survival	Univariate analysis		Multivariate analysis (n = 90)			
					Bootstrap	
Variable	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age >65 vs. ≤65 years	2·553 (1·291–5·049)	0·007	2·126 (1·043–4·331)	0·038	2·126 (1·133–3·987)	0·019
Binet Stage B-C vs. A	1·243 (0·900–1717)	0·187	–	–	–	–
IGHV unmutated vs. mutated	1·305 (0·530–3·215)	0·652	–	–	–	–
TP53 aberrations yes vs. no	1·134 (0·533–2·412)	0·744	–	–	–	–
CD38 positive vs. negative	1·847 (0·923–3·696)	0·083	–	–	–	–
ZAP70 positive vs. negative	1·388 (0·658–2·929)	0·390	–	–	–	–
Del11q yes vs. no	1·123 (0·564–2·237)	0·741	–	–	–	–
Unbalanced rearrangements yes vs. no	2·948 (1·237–7·029)	0·015	2·797 (1·135–6·897)	0·025	2·797 (1·159–6·753)	0·022
Deletions yes/no	0·811 (0·363–1·815)	0·612	–	–	–	–
Monosomies yes/no	1·420 (0·742–2·718)	0·290	–	–	–	–
≥5 abnormalities yes/no	2·131 (1·120–4·055)	0·021	0·716 (0·364–1·409)	0·334	0·716 (0·356–1·442)	0·350
Trisomies yes/no	0·717 (0·366–1·406)	0·334	–	–	—	–
Balanced translocations yes/no	0·881 (0·453–1·711)	0·708	–	–	–	–
Time to first treatment	Univariate analysis		Multivariate analysis (n = 60)			
					Bootstrap	
Variable	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age >65 vs. ≤65 years	0·950 (0·579–1·559)	0·841	–		–	
Binet Stage B-C vs. A	1·493 (1·148–1·942)	0·003	1·851 (1·305–2·627)	0·001	1·851 (1·305–2·627)	0·001
IGHV unmutated vs. mutated	2·624 (1·269–5·426)	0·009	2·238 (1·062–4·718)	0·034	1·911 (1·062–4·718)	0·034
TP53 aberrations yes vs. no	1·382 (0·800–2·388)	0·246	–	–	–	–
CD38 positive vs. negative	1·304 (0·789–2·155)	0·300	–	–	–	–
ZAP70 positive vs. negative	1·128 (0·603–2·113)	0·705	–	–	–	–
Del11q yes vs. no	1·156 (0·662–2·020)	0·609	–	–	–	–
Unbalanced rearrangements yes vs. no	2·007 (1·124–3·582)	0·018	2·375 (1·027–5·492)	0·043	2·375 (1·027–5·492)	0·043

HR, hazard ratio; 95% CI, 95% confidence interval.

between patients with and without unbalanced rearrangements is presented in Figure S3. Among the differentially expressed mRNAs, there were genes involved in the response to DNA damage and cell cycle regulation.

Discussion

A CK is found in 10–15% of CLL (Baliakas *et al*, 2014; Rigolin *et al*, 2015) and has been associated with an inferior outcome when present at diagnosis (Baliakas *et al*, 2014; Rigolin *et al*, 2017a), disease progression (Herling *et al*, 2016) or in relapsed/refractory patients treated with ibrutinib (Thompson *et al*, 2015) or venetoclax (Anderson *et al*, 2017). Interestingly, in high-risk CLL (Rigolin *et al*, 2017b), the prognostic impact of a CK was independent of *TP53* status, while a CK has been associated with a stronger prognostic significance than *TP53* aberrations in relapsed/refractory CLL treated with novel agents (Thompson *et al*, 2015; Anderson *et al*, 2017).

We found that, in CLL patients with CK, unbalanced rearrangements were independently associated with a worse OS and TTFT and that they identified a subset of patients with distinct features, including a lower incidence of 11q deletions and a higher incidence of *TP53* aberrations (17p deletions and/or *TP53* mutations). Moreover, the negative prognostic impact of unbalanced rearrangements was independent of the *TP53* status.

In CLL patients, conventional cytogenetics with novel mitogens represents a robust and valuable tool for a more precise refinement of prognosis. It enables the identification of, not only a CK and prognostically relevant abnormalities undetectable by fluorescence *in situ* hybridisation (Rigolin *et al*, 2015), but also structural and numerical lesions that may predict a worse clinical outcome (Haferlach *et al*, 2007; Rigolin *et al*, 2012; Baliakas *et al*, 2014).

We also found that the presence of unbalanced rearrangements was associated with a distinct mRNA expression profile with a deregulation of genes involved in cell cycle control and DNA damage response. Among these, *TRPM4*, *RASGRF1*, *CTTNBP2* and *SLAMF1* may be of interest as they may have possible prognostic and therapeutic implications. Transient receptor potential channel melastatin 4 (TRPM4) is an ion channel that plays a critical role in the control of the membrane potential, leading to the regulation of cell cycle, DNA replication, intracellular signal transduction and inflammatory responses. *TRPM4* has been found up-regulated in diffuse large B-cell lymphomas, where it was associated with a significantly worse outcome (Loo *et al*, 2017). RASGRF1 is a guanine exchange factor that plays a role in B-cell receptor (BCR) signalling. *RASGRF1* has been found overexpressed in CLL and its activation was blocked by Bruton Tyrosine Kinase inhibitors (Liao *et al*, 2014). Cortactin binding protein 2 (CTTNBP2) is an actin binding protein and Lyn substrate that is up-regulated in CLL patients and correlates to a poor prognosis, suggesting that this protein could be relevant in the pathogenesis and aggressiveness of the disease (Martini *et al*, 2017). Finally, reduced *SLAMF1* levels have been associated with clinical and molecular markers of unfavourable prognosis. SLAMF1 is involved in several pathways related to cell migration, cytoskeletal organization and intracellular vesicle formation. The loss of *SLAMF1* expression may affect responses to therapeutic agents, such as fludarabine and the BCL2 homology domain 3 mimetic, ABT-737 (Bologna *et al*, 2016).

In conclusion, we have shown that CLL patients with unbalanced rearrangements represent a subset of very high-risk CLL patients with distinct clinical and biological characteristics. These patients need to be identified at the time of treatment and should be considered upfront for alternative treatments, including combinations of novel agents.

Acknowledgements

This work was supported by the Fondo di Ateneo per la Ricerca 2013, 2014, 2016 of the University of Ferrara (G.M.R., A.C.), Programma Ricerca Regione Università 2007-2009, University of Ferrara (G.M.R., A.C.), Programmi di Ricerca di Rilevante Interesse Nazionale (PRIN) 2008 (A.C.), Ricerca Finalizzata Ministero della Sanità (A.C.; project RF-2011-02349712), Ministero dell'Istruzione, dell'Università e della Ricerca PRIN 2015 (A.C.; project 2015ZMRFEA), BEAT Leukemia Foundation Milan Italy, and AIL Ferrara. E.V. and E.S. are supported by AIL Ferrara.

Author contributions

G.M.R., M.C., L.T., G.S., R.F. and A.C., conceived and designed the study; G.M.R., M.C., F.M.Q., E.L., A.U., L.F., E.G., A.V. and F.R.M. acquired data and provided patient follow-up; M.A.B., E. T., C.I., L.C., E.V., A.M. and L.B. performed cytogenetic and molecular analyses; E.S., B.Z., C.B., L.L. and M.N. performed GEP studies; G.M.R., M.C., F.C., L.T., R.F. and A.C. analysed and interpreted data; and all of the authors contributed to the writing, approval and review of the manuscript.

Competing interests

The authors have no competing interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. OS (in A; $P = 0.015$) and TTFT (in B; $P = 0.018$) in patients with and without unbalanced rearrangements.

Fig S2. Cluster analysis of CLL patients with and without unbalanced rearrangements.

Fig S3. Heat-map representation of the average expression of the 30 most differentially expressed gene between patients with and without unbalanced rearrangements.

Table S1. List of CK according to the presence of unbalanced rearrangements.

Table S2. List of consistently down-modulated and up-modulated genes identified in CLL samples of patients with and without unbalanced rearrangements

Appendix S1. Materials and methods

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