Biallelic *BIRC3* inactivation in chronic lymphocytic leukaemia patients with 11q deletion identifies a subgroup with very aggressive disease

Chronic lymphocytic leukaemia (CLL) patients with the 11q22.3-q23.1 deletion (11q−) show a less favourable outcome (Döhner *et al*, 2000); however, a certain degree of prognostic heterogeneity within this subgroup has been recognised, with a reported time to first treatment (TFT) ranging from 13 to 31 months (Guarini *et al*, 2012; Jeromin *et al*, 2014; Baliakas *et al*, 2015). The minimal deleted region (MDR) on 11q always includes *ATM* and can also encompass *BIRC3*, located on the 11q22.2 band, 6 Mb centromeric to *ATM* (Rose-Zerilli *et al*, 2014). *BIRC3* inactivation by mutations and/or deletions has been associated with a chemorefractory phenotype in patients with wild-type (WT) *TP53* (Rossi *et al*, 2012).

We aimed to: (i) screen *BIRC3* disruption by copy number aberration (CNA) and mutation analysis; (ii) explore the clinical significance of *BIRC3* deletions and/or mutations among 11q- CLL patients.

The study included 134 untreated CLL patients with 11q− detected by fluorescence *in situ* hybridisation (FISH). *BIRC3* CNA analysis was performed by CytoScan HD array (Affymetrix, Santa Clara, CA) in a discovery cohort of 55 11q− CLL and by the QX200™ Droplet Digital™ polymerase chain reaction (ddPCR) system (Bio-Rad, Hercules, CA, USA) in a validation cohort of 79 11q− CLL (Table I). The two cohorts showed similar age, gender, white blood cell (WBC) count and a superimposable TFT (median: 14·4 vs. 18·3 months, $P = 0{\cdot}144$) and follow-up (median 49·4 vs. 43·8, $P = 0{\cdot}361$). Mutational analysis of *BIRC3*, *TP53*, *SF3B1* and *NOTCH1* was performed by Sanger sequencing in all cases and of *ATM* by next generation sequencing in the 10 cases harbouring the *BIRC3* biallelic lesion. TFT was assessed to evaluate the clinical impact of the *BIRC3* lesions, in order to avoid the confounding effects of different treatments (Supplementary Methods).

Table I. Clinical and biological characteristics of the 134 11q− CLL patients.

Gender (male/female)	103/31
Age, years (range)	63 (39–90)
White blood cell count $\times 10^9$/l (range)	36·4 (6–302·8)
Binet stage A/B/C/unknown	44/70/10/10
IGHV unmutated/mutated	103/28
% of 11q deletion by FISH (range)	72 (10–99)
11q deletion only (*n*)	54
11q deletion with 17p deletion (*n*)	3*
11q deletion with trisomy 12 (*n*)	6
11q deletion with 13q deletion (*n*)	71

*Cases with 17p deletion: 2 cases with *BIRC3* deleted/WT and 1 case with BIRC3 WT/WT.

In the discovery cohort ($n = 55$), the 11q− was detected by FISH in 80% of nuclei (range 25–99%). CytoScan HD array revealed a heterogeneous size of 11q− (0·36–65·14 Mbp), mostly large lesions (>5 Mbp in 51 patients, 92·7%). The MDR, located in the 11q22.3 region, encompassed 4 genes (*ACAT1*, *ATM*, *CUL5*, *NPAT*). In 45/55 cases (81·8%), the deleted region included the *BIRC3* gene. Fifty-one cases (92·7%) showed several additional CNAs (average 4·2, range 1–14/patient), mostly deletions, with gain 2p, del4(p15.2), del4(q22.1), gain 8q and del19(p13.3) recurring in ≥3 cases (Table SI, Figure S1 and S2).

In the validation cohort ($n = 79$), FISH detected the 11q− in 67% of nuclei (range 10–97%). ddPCR confirmed the *ATM* deletion in all cases and revealed the deletion of *BIRC3* in 60/79 cases (75·9%) (sensitivity: 10% of deleted cells) (Figure S3).

Overall, *BIRC3* deletion was identified in 105/134 11q− patients (78%), in line with the literature (Rossi *et al*, 2012; Rose-Zerilli *et al*, 2014). *BIRC3* mutations ($n = 15$) occurred in 10/134 cases (7·5%) (4 from the discovery, 6 from the validation cohort), all *BIRC3* deleted, resulting in a biallelic disruption of the gene.

TP53, *NOTCH1* and *SF3B1* mutations, all mutually exclusive, were identified in 1/98 (1%), 11/117 (9·4%) and 7/78 (8·9%) evaluated cases, respectively (Figure S4).

Among the 10 patients with *BIRC3* biallelic lesion, 1 harboured a *SF3B1* mutation, 1 a *NOTCH1* mutation and none harboured a 17p-/*TP53* mutation. *ATM* analysis revealed 3 subclonal mutations in 2 cases (Table SII). Clinical features were as follows: 6 were males, 4 females, median age 60 years (range 51–69), with an unmutated *IGHV* status in 7 cases; 3 were Binet stage C and 6 Binet B (one unknown), with a median WBC count of 100×10^9/l (range 11–$302{\cdot}8 \times 10^9$/l). 11q−/*BIRC3*del/*BIRC3*mut CLL patients had a significantly higher WBC count at diagnosis than 11q−/*BIRC3*del/*BIRC3*WT or 11q−/*BIRC3*WT/*BIRC3*WT cases ($P < 0{\cdot}0001$), with 8 cases showing WBC >100×10^9/l, including 3 with >200×10^9/l. Moreover, a higher proportion of these patients were stage C ($P = 0{\cdot}025$), although there was no

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difference in age, gender, *IGHV* mutations, additional FISH lesions, *NOTCH1*/*SF3B1*/*TP53* mutations or median time from diagnosis to sampling.

After a median follow-up of 47 months (range 1–236), 93/127 evaluable patients received chemotherapy/chemoimmunotherapy (median TFT 16 months, range 0·2–236). *BIRC3* deleted cases showed a TFT comparable with WT patients. Conversely, the *BIRC3* biallelic lesion was associated with a significantly shorter TFT than *BIRC3* deleted or WT (median TFT 5 vs. 16 months, $P < 0·004$) (Fig 1), also when the 2 *ATM* mutated cases were excluded from the analysis ($P = 0·036$) (Figure S5).

By multivariate analysis, stage ($P < 0·0001$), WBC count ($P = 0·02$) and biallelic *BIRC3* lesion ($P = 0·005$) were associated with a short TFT, whilst gender, age, CD38, ZAP70 and *IGHV* status were not.

Thus, CLL patients with biallelic *BIRC3* inactivation showed a more aggressive disease with significantly lower TFT compared to other 11q− cases. The absence of enrichment in mutations in the known CLL driver genes (i.e. *TP53*, *NOTCH1*, *SF3B1*) and the same ratio of unmutated/mutated IGHV genes compared to the other 11q− cases, reinforce the clinical impact of the *BIRC3* biallelic lesion, that holds true even after exclusion of the 2 patients with subclonal *ATM* mutations.

The effect of the *BIRC3* biallelic disruption on disease kinetics (i.e. the high WBC count and the short TFT) is in line with the function of the BIRC3 protein, because the inactivation of the *BIRC3* gene leads to the constitutive activation of the non-canonical NF-κB pathway (Rossi *et al*, 2012).

Clonal *ATM* mutations rather than *BIRC3* deletion and/or mutation have been associated with a worse outcome among 11q− CLL patients treated with chemotherapy (Rose-Zerilli *et al*, 2014). However, of 36 11q− CLL patients, 14 had *ATM* mutations, 32 *BIRC3* deletions and only 2 *BIRC3* mutation, thus, the prognostic impact of the *BIRC3* biallelic lesion could not be conclusively inferred.

We have previously reported the presence of *BIRC3* mutations in 27% of 11q− chemo-refractory CLL patients (Messina *et al*, 2014) and the clonal expansion in the peripheral blood at relapse of *BIRC3* deletion derived from the lymph node (Del Giudice *et al*, 2016). Thus, *BIRC3* lesions could have a role as a predictive marker in CLL treated with chemoimmunotherapy (Diop *et al*, 2017). Future evaluations will clarify whether *BIRC3* abnormalities are useful, not only to refine the prognostic stratification of 11q− CLL patients, but also to identify cases with a sub-optimal response to novel mechanism-based compounds.

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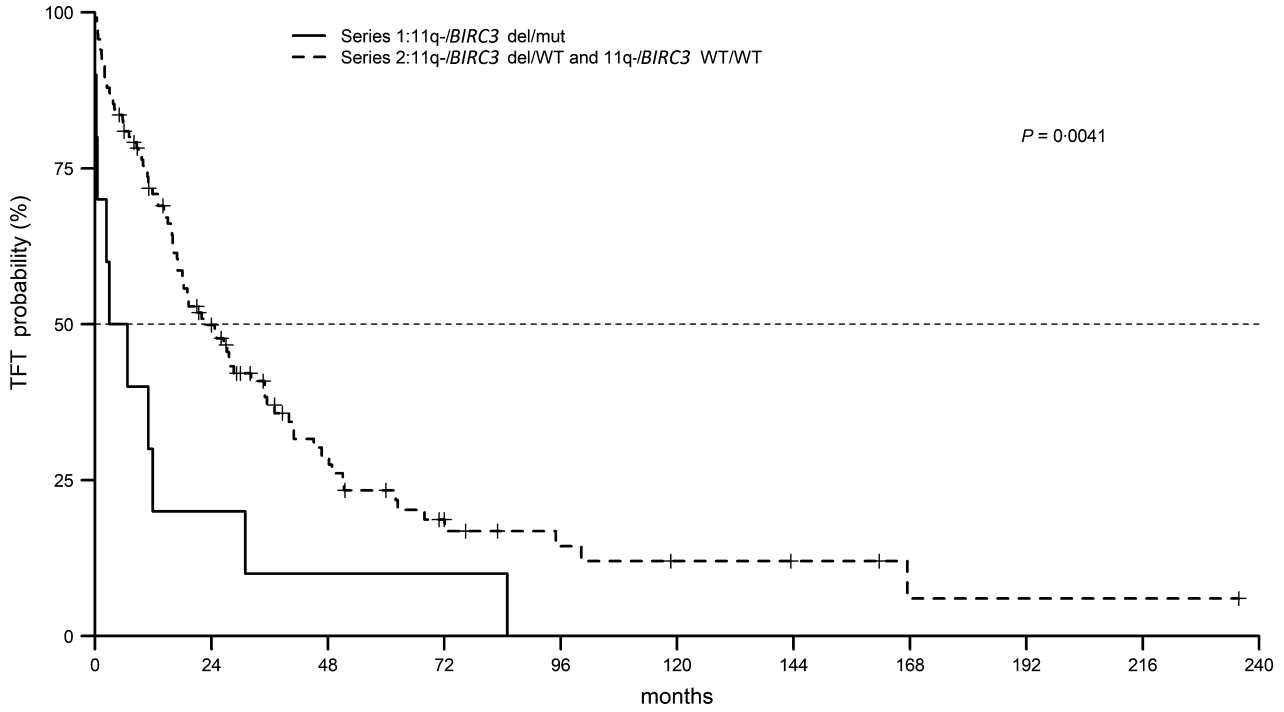

Fig 1. Time to first treatment of 127 11q− chronic lymphocytic leukaemia cases according to the presence of *BIRC3* deletion+mutation (*BIRC3* del/mut, $n = 10$), *BIRC3* deletion only (*BIRC3* del/WT, $n = 88$) or none (*BIRC3* WT/WT, $n = 29$). TFT probability was estimated using the Kaplan–Meier Product Limit estimator. Differences were evaluated by means of log-rank test. WT, wild type.

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Author contributions

SR and IDG designed the study, analysed the results and wrote the paper; CI, LC, MMarinelli and LVC performed and analysed Sanger sequencing and droplet digital PCR experiments; AP performed the statistical analysis; MMessina and SB performed and analysed the CNA study; FRM, GMR and GDP provided samples and clinical assistance; NP, PM, FR, RB, MDB provided samples and biological data; FD and CF performed and analysed NGS experiments; DR analysed and supervised NGS experiments; GG, AC, VG and AG analysed and discussed the results, and critically revised the manuscript; RF contributed to design the study, analysed and discussed the results and critically revised the manuscript.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Supporting Information

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

Table SI. Recurrent CNA in the 55 11q− CLL belonging to the discovery cohort.

Table SII. *BIRC3* and *ATM* mutations in 11q− CLL patients with *BIRC3* biallelic lesions.

Figure S1. CNAs per chromosome.

Figure S2. Heat-map of recurrent CNAs in 11q− CLL belonging to discovery cohort.

Figure S3. 2-D fluorescence amplitude plots.

Figure S4. Heat-map of 11q− CLL cases.

Figure S5. TFT of 125 11q− CLL cases according to the presence of *BIRC3* deletion+mutation (*BIRC3* del/mut, $n = 8$), *BIRC3* deletion only (*BIRC3* del/WT, $n = 88$) or none (*BIRC3* WT/WT, $n = 29$). The 2 *ATM* mutated cases were excluded from the analysis.

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