ATM mutation rather than BIRC3 deletion and/or mutation predicts reduced survival in 11q-deleted chronic lymphocytic leukemia: data from the UK LRF CLL4 trial

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ABSTRACT

ATM mutation and *BIRC3* deletion and/or mutation have independently been shown to have prognostic significance in chronic lymphocytic leukemia. However, the relative clinical importance of these abnormalities in patients with a deletion of 11q encompassing the *ATM* gene has not been established. We screened a cohort of 166 patients enriched for 11q-deletions for *ATM* mutations and *BIRC3* deletion and mutation and determined the overall and progression-free survival among the 133 of these cases treated within the UK LRF CLL4 trial. SNP6.0 profiling demonstrated that *BIRC3* deletion occurred in 83% of 11q-deleted cases and always co-existed with *ATM* deletion. For the first time we have demonstrated that 40% of *BIRC3*-deleted cases have concomitant deletion and mutation of *ATM*. While *BIRC3* mutations were rare, they exclusively occurred with *BIRC3* deletion and a wild-type residual *ATM* allele. In 11q-deleted cases, we confirmed that *ATM* mutation was associated with a reduced overall and progression-free survival comparable to that seen with *TP53* abnormalities, whereas *BIRC3* deletion and/or mutation had no impact on overall and progression-free survival. In conclusion, in 11q-deleted patients treated with first-line chemotherapy, *ATM* mutation rather than *BIRC3* deletion and/or mutation identifies a subgroup with a poorer outcome.

Introduction

Deletion of chromosome 11q (termed del11q) was first recognized as a recurrent karyotypic abnormality acquired during the course of the disease in patients with progressive chronic lymphocytic leukemia (CLL).[1] Subsequent interphase fluorescence *in situ* hybridization (FISH) analysis identified 11q deletion in approximately 20% of patients with CLL, and associations with bulky lymphadenopathy and a poorer outcome for patients under the age of 55 years were noted.[2,3] Subsequent studies have documented associations with unmutated IGHV genes, del13q, genomic complexity, short telomeres, progressive disease and a poor outcome in response to alkylating agent or purine analog treatment, which was improved by their use in combination and ameliorated by the further addition of rituximab.[4–10]

Genomic profiling studies have refined previous karyotypic and FISH studies showing that 11q deletions are mono-allelic, frequently large and include a minimally deleted region (MDR), which encompasses the *ATM* gene.[11–14] Evidence that *ATM* is a key target of 11q deletions is derived from findings that: 1) mutation of the *ATM* gene is found in 30-40% of patients with an 11q deletion;[15,16] 2) the presence of an *ATM* mutation results in impaired DNA damage responses;[15,17–19] and 3) patients in the UK LRF CLL4 trial with biallelic ATM abnormalities (deletion and mutation) have a poorer outcome following the initial therapy with alkylating agent and/or purine analog therapy compared to those with mono-allelic *ATM* deletion or mutation.[20]

However, uncertainty remains as to whether the poorer outcome of patients with 11q deletion in the absence of an *ATM* mutation is simply a consequence of *ATM* haploinsufficiency. Alternative possibilities that have been considered include deletion, mutation or epigenetic silencing of other genes either within or outside the MDR or the associated genomic complexity.[8,21–23] Neither candidate gene sequencing nor whole exome sequencing studies have identified mutations within other genes located in the MDR.[24,25] However, recent data have revealed a high incidence of deletion or, more rarely, mutation of *BIRC3*, a negative regulator of non-canonical NFκB signaling located at 11q22. It has been reported that *BIRC3* deletion and/or mutation (previously termed "*BIRC3* disruption") occurs in a mutually exclusive manner with *TP53* abnormalities, is associated with fludarabine-resistance,[26] and when detected at diagnosis predicts for poor overall survival independent of 11q deletion.[27]

The above data strongly suggest that there are subsets of del11q patients that exhibit differing responses to standard

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treatment. However, the relative frequency and clinical significance of *ATM* and *BIRC3* abnormalities is unclear. This study addresses this issue in a large cohort of 11q-deleted patients detected by SNP6 profiling and screened for *ATM* and *BIRC3* mutations. As a consequence, in the context of a phase III clinical trial of chemotherapy, we show that *ATM* mutational status remains the most clinically informative genomic lesion in 11q-deleted CLL, identifying cases with outcome comparable to *TP53* deletion and/or mutation patients, and that the presence of *BIRC3* deletion and/or mutation is associated with outcome comparable to other 11q-deleted CLL cases.

Methods

Patients and molecular diagnostic assays

A total of 166 untreated CLL patients diagnosed according to standard morphological and immunophenotypic criteria were included in this study (Table 1 and *Online Supplementary Table S1*). This cohort principally included patients from the UK LRF CLL4 trial[9] (n=133) which allowed accurate clinical correlations to be made. An additional 33 11q-deleted patients were also included to allow more significant associations between the 11q deletion and other genomic variables to be made, such as with mutational status of *ATM* and *BIRC3*. The additional del11q patients were sampled subsequent to the development of progressive disease, with a median time from diagnosis of 3.2±5.2 years (±1 standard deviation (SD); range 1 month-27 years). For the entire cohort of 166 patients, mutational data were available for *TP53* (n=125), *SF3B1* (n=140) and *NOTCH1* (n=146).[28,29] Details on the molecular diagnostic assays[30,31] are available in the *Online Supplementary Methods*. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki and our local ethics committee gave their approval for the study.

DNA extraction, SNP6 array hybridization, data extraction and analysis

Genomic DNA was extracted from CLL B cells (n=166) and buccal swabs (n=32), prior to being purified, amplified, labeled and hybridized to the Affymetrix SNP6.0 platform (Affymetrix, Santa Clara, CA, USA) as previously described[32] (*Online Supplementary Methods*).

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Table 1. Cohort characteristics.

Characteristics	Sub-groups	All Cases (%)	CLL4 Cases n (%)[2]	
			non-del(11q)	del(11q)
Number of cases		166 (100)	97 (73)	36 (27)
Gender	Male	124 (75)	71 (73)	27 (75)
	Female	42 (25)	26 (27)	9 (25)
Age at diagnosis	Mean	63	71	62
	Range	39-89	56-86	44-80
Binet stage at diagnosis or randomization	A	48 (31)	23 (24)	7 (19)
	B	69 (44)	47 (49)	18 (50)
	C	40 (25)	27 (28)	11 (31)
Trisomy 12 by SNP6	Trisomy	13 (8)	9 (9)	3 (8)
	Normal	153 (92)	88 (91)	33 (92)
13q by SNP6	Deleted	94 (66)	51 (53)	22 (61)
	Normal	72 (44)	46 (47)	14 (39)
17p by SNP6	Deleted	12 (7)	9 (9)	1 (3)
	Normal	154 (93)	88 (91)	35 (97)
IGHV mutation status[a]	Unmutated	98 (68)	56 (64)	30 (83)[1]
	Mutated	47 (32)	32 (36)	6 (17)
CD38 positivity[b]	+	68 (50)	32 (42)	18 (55)
	–	69 (50)	44 (58)	15 (45)
ZAP70 positivity[c]	+	74 (54)	42 (51)	21 (64)
	–	63 (46)	40 (49)	12 (36)
ATM mutation status[d]	T-Mutated	17 (16)	5 (10)	10 (32)[3]
	NT-Mutated	19 (18)	8 (17)	4 (13)[4]
	Unmutated	69 (66)	35 (73)	17 (55)
TP53 mutation status[e]	Mutated	9 (7)	7 (8)	2 (6)
	Unmutated	116 (93)	76 (92)	30 (94)
BIRC3 deletion status	Deleted	57 (34)	0 (0)	32 (89)
	Normal	109 (66)	97 (100)	4 (11)
BIRC3 mutation status	Mutated	3 (2)	0 (0)	2 (6)
	Unmutated	159 (98)	95 (100)	34 (94)
NOTCH1 mutation status[f]	Mutated	14 (10)	10 (12)	2 (6)
	Unmutated	132 (90)	75 (88)	32 (94)
SF3B1 mutation status[g]	Mutated	28 (20)	19 (24)	3 (9)
	Unmutated	112 (80)	61 (76)	29 (91)

A proportion of cases were not screened for our panel of molecular and cytogenetic biomarkers; not screened= 21 a, 29 b, 29 c, 61 d, 41 e, 20 f, 26 g. [1]A significant positive association was identified between the presence of del11q and an unmutated IGHV sequences in the CLL4 cases (P=0.03, 2x2 χ^2 test). [2]The columns for del11q and non-del11q are based on the SNP6.0 profiling data. [3]A significant positive association was identified between the frequency of truncating ATM mutations (T-Mutated) and presence of del11q. (P=0.02, 2x2 χ^2 test). [4]The frequency of non-truncating ATM mutations (NT-Mutated) was similar in non-del11q and del11q cases. (P=0.76, 2x2 χ^2 test).

Mutational analysis of ATM *and* BIRC3 *genes*

The presence or absence of somatically-acquired single nucleotide variants (SNVs or mutations) in *ATM* and *BIRC3* were successfully ascertained in 105 (CLL4 cases: n=79 of 133) and 162 (CLL4 cases: n=131 of 133) patients, respectively. Denaturing high-performance liquid chromatography (DHPLC), high-resolution melt (HRM) polymerase chain reaction (PCR) analysis and Sanger sequencing were utilized.[20,26,27,29] More details of methods and the strategy adopted for assigning the somatic nature of each SNV are provided in the *Online Supplementary Methods* and *Online Supplementary Table S2*.

Statistical analysis

Statistical analysis was performed using SPSS (v.20). Associations with treatment response and clinical outcome were only performed on the LRF UK CLL4 cases due to the homogeneous and well-annotated nature of this cohort (*Online Supplementary Methods*). The LRF UK CLL4 cohort included in this current study did not differ significantly from the entire cohort for an extensive panel of variables, with the enrichment exception of del11q (*P*=0.002) and del13q (*P*<0.001) (*Online Supplementary Table S1*). Kaplan-Meier analysis with the log rank test or Cox regression was used for survival analyses on overall survival (OS) and progression-free survival (PFS). χ^2 test (Pearson or Fisher Exact test when necessary) were also employed for some comparisons and are described where relevant in the main text or table footnotes. *P*=0.05 was considered statistically significant. Previous reports have demonstrated that *BIRC3* disruption occurs in 50% of 11q deleted cases and is associated with survival comparable to *TP53* deleted CLL.[26,27] Therefore, with clinical follow up of ten years, our UK LRF CLL4 cohort (del11q, n=36) had 97% power with a significance level of 0.05 to detect a difference in OS between cases with and without *BIRC3* deletion and/or mutation based on the median CLL4 OS duration for cases with del11q (53 months) and del17p (14 months) detected by FISH.

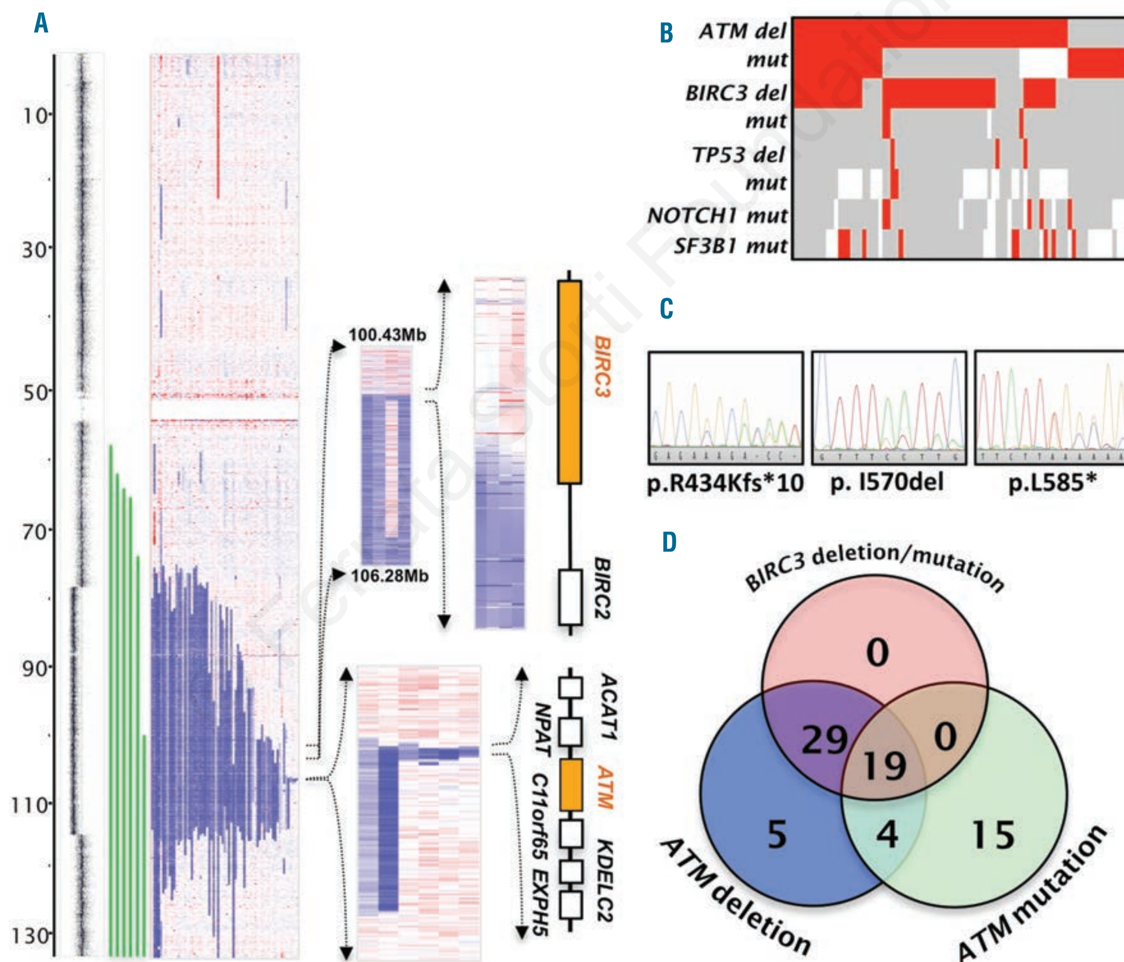

Figure 1. Chromosome 11 architecture, 11q23 minimally deleted region and *BIRC3* deletion and/or mutation in 11q-deleted CLL [In color] (A) Left to right: Genomic location in Mb from the telomere of 11p (top), through the centromere to the telomere of 11q (bottom); an example 11q deleted SNP6.0 probe profile showing an interstitial deletion between genomic locations 74 and 103Mb which includes the *ATM* and *BIRC3* loci; representative CNNLOH (green lines) observed in our cohort where the size and position of the line shows the genomic location and size of the CNNLOH event; heatmap of 69 11q deleted cases, where white, red and blue show regions of no copy number change, duplications and deletions, respectively. Focussed heatmap views of *ATM* and *BIRC3* genes demonstrating the *ATM* MDR and the 4 cases with a telomeric deletion breakpoint in the 3' *BIRC3* gene locus are also shown (the second case from the left has deletion of both *BIRC3* and *ATM*, which is the result of two deletion events in this patient). (B) Abnormality matrix of cases with *ATM* and *BIRC3* lesions. Each row is a genetic lesion present in our cohort, and each column is a patient where red, grey and white, shows the presence, absence or no data for the lesion in question, respectively. (C) Sanger sequencing traces of the three *BIRC3* gene mutations identified in our study. (D) Venn-diagram of the relationship between *BIRC3* deletion and/ or mutation and *ATM* deletion and mutation (n: number of observations).

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Results

Incidence of ATM *and* BIRC3 *deletions*

We analyzed all 166 patients for 11q deletions and copy number neutral-loss of heterozygosity (CNNLOH) events using the SNP 6.0 genome-wide copy number arrays. Sixty-nine patients had 11q deletions that all included a minimally deleted region (MDR) which was 416 Kb in size (chr11:107.498-107.914Mb) and contained the following genes: *ACAT1, NPAT, ATM, C11orf65, KDELC2, LOC1000127964, LOC1000128794* and *EXPH5* (Figure 1A). We observed that the majority of 11q-deleted cases (67 of 69) had deletions much larger than the MDR and 57 of 69 (83%) lost one copy of the *BIRC3* gene. In 56 of these patients, *ATM* and *BIRC3* genes were lost as part of a single deletion event while in one patient the genes were lost as a consequence of two separate deletions events. In 4 patients (6%), the centromeric deletion breakpoint was within the *BIRC3* gene body, resulting in loss of the 3' end of the gene that contains the C terminal RING domain important for the proteasomal degradation of *MAP3K14* (Figure 1A).

Incidence of ATM *and* BIRC3 *mutations*

BIRC3 mutation screening was successfully ascertained in 162 of 166 patients. A *BIRC3* gene mutation was detected in only 3 patients all of whom had a large mono-allelelic 11q-deletion encompassing both the *ATM* gene and the *BIRC3* gene located at 11q22. The *BIRC3* gene mutations are predicted to be deleterious for *BIRC3* protein function, the p.R434Kfs*10 (frameshift in/del) and p.L585* (STOP-codon) mutations both target highly conserved amino acid residues, resulting in truncation of BIRC3 protein with loss of the C terminal CARD-RING and RING domains, respectively. The p.I570del (3bp in-frame deletion) mutation targets another highly conserved amino acid, resulting in loss of the C-beta branched amino acid residue, isoleucine at the C terminal end of the beta-sheet structure in the RING domain (Figure 1C). *ATM* gene mutation screening was successfully completed in 105 patients and identified mutations in 36 patients. The mutations were classified as somatic and pathogenic based on our previously reported criteria (*Online Supplementary Methods*) and occurred in exons 2-63, between amino acids 37-3047, affecting the TAN, FAT, PI3K and FATc domains of the ATM protein (*Online Supplementary Table S3*). *ATM* mutations occurred in the absence of an 11q-deletion in 27% (13 of 48) of patients screened and in 40% (23 of 57) of 11q-deleted patients. Interestingly, we identified that a single case with an *ATM* mutation (c.8428-8450del23:p.Lys2810fs) also harbored CNNLOH of the region duplicating the variant. This case highlights that in

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Table 2. Survival models of *TP53*, *ATM* and *BIRC3* disruption in CLL4 cases.

Mutation/ Biomarkers	Overall survival (years) Events/ Total	Median[1]	95% CI[1]	HR[2]	95% CI[2]	*P*	Progression-free survival (years) Events/ Total	Median[1]	95% CI[1]	HR[2]	95% CI[2]	*P*
Model A												
Established prognostication model[a]												
mutated IGHV	18/38	118	96-139	–	–	–	23/38	61	24-99	–	–	–
un-mutated IGHV	75/86	56	49-64	3.19	1.89-5.39	**<0.001**	83/86	25	16-33	4.05	2.46-6.66	**<0.001**
trisomy 12	8/9	79	35-123	1.65	0.76-3.61	0.208	9/9	35	19-50	2.55	1.21-5.34	**0.013**
del(11q)	30/35	53	36-69	2.11	1.26-3.51	**0.004**	35/35	20	6-33	3.15	1.95-5.10	**<0.001**
del(17p)	10/10	8	0-16	44.28	16.11-121.67	**<0.001**	10/10	4	2-5	23.33	10.16-53.56	**<0.001**
Model B												
Inclusion of *BIRC3* deletion and /or mutation[b]												
Wild-type	48/72	78	55-102	–	–	–	55/72	43	39-48	–	–	–
del(11q)	3/3	42	0-86	3.00	0.93-9.73	0.066	3/3	38	0-81	18.0	0.56-5.79	0.323
BIRC3 deletion and/ or mutation (with/ without *ATM* mutation)	24/29	53	39-67	1.91	1.16-3.15	**0.011**	29/29	17	4-30	3.26	2.03-5.22	**<0.001**
TP53 deletion and/ or mutation	15/15	14	5-22	5.76	3.17-10.44	**<0.001**	15/15	5	2-7	5.20	2.89-9.38	**<0.001**
Model C												
Inclusion of *BIRC3* and *ATM* mutation[c]												
Wild-type del(11q)	16/28	91	48-134	–	–	–	19/28	46	5-87	–	–	–
(*ATM* deletion without *BIRC3* deletion and /or mutation)	2/2	15	–	3.16	0.71-13.76	0.132	2/2	11	–	2.31	0.53-10.01	0.265
BIRC3 deletion and/ or mutation (without *ATM* mutation)	7/11	76	40-111	1.41	0.57-3.45	0.456	11/11	28	16-40	2.80	1.29-6.04	**0.009**
Biallelic inactivation of *ATM* (deletion and mutation of *ATM*)	13/13	42	13-71	3.42	1.63-7.21	**0.001**	13/13	10	6-15	6.07	2.80-13.18	**<0.001**
TP53 deletion and/ or mutation	15/15	14	5-22	6.27	3.03-12.96	**<0.001**	15/15	5	2-7	6.13	3.03-12.38	**<0.001**

Survival analysis is based on [1]log rank testing and [2]univariate regression analysis. Significant P-values are shown in bold. [a]All 133 CLL4 cases were used, subdivided based on the presence of established aberrations identified by SNP6.0 and sub-grouped based on the established Döhner model (46). [b]119 CLL4 cases with the presence of del11q, BIRC3 and TP53 deletions and/or mutations determined by SNP6.0 profiling and mutational analysis. [c]69 CLL4 cases with del11q, BIRC3 and TP53 deletions, and mutations of ATM, BIRC3 and TP53 based on SNP6 profiling data and mutational analysis.

rare instances biallelic inactivation of *ATM* may occur independently of chromosome deletion yet current molecular diagnostic tests do not detect these events (N.B. this case was not included in our survival analyses). Importantly, we observed that within 11q-deleted patients, with loss of both *ATM* and *BIRC3* genes, and successfully screened for gene mutation, 40% (19 of 48) with a *BIRC3* deletion also had an *ATM* mutation (Figure 1D).

Relative importance of ATM *and* BIRC3 *abnormalities on outcome of patients with 11q deletions*

Within the CLL4 cohort (n=133), there were 36 patients with an 11q deletion of whom 32 had loss of both *ATM* and *BIRC3* genes, 2 had a *BIRC3* mutation, and 14 had an *ATM* mutation. Initially, we confirmed in univariate analysis that within this cohort, unmutated *IGHV* genes and deletions of 11q and *TP53* were associated with short PFS and OS, as found in larger studies of prognostic markers in this trial (Table 2 model A).[9,20,28,29,33] Specifically, 11q deleted patients exhibited a median OS and PFS of 53 months (95%CI:36-69; *P*=0.004) and 20 months (95%CI: 6-33; *P*<0.001), respectively.[9,20,28,29,33] Inclusion of *BIRC3* deletion and mutation into the model (Table 2 model B) showed that BIRC3 deleted/mutated cases had a reduced survival compared to non-11q deleted cases, with a median OS of 53 months (95%CI: 39-67) and PFS of 17 (95%CI: 4-30). As expected, there was no detectable difference (OS: *P*=0.52 and PFS: P=0.42) in outcome between all cases with del11q and those with *BIRC3* deletion/mutation, consistent with the high incidence of *BIRC3* loss in del 11q cases (Table 2 model B and Figure 2A and B).

Finally, we demonstrated that the most significant reduction in OS and PFS in 11q-deleted patients was observed in patients with mutations targeting the *ATM* gene. These patients exhibited a median OS and PFS of 42 (95%CI: 13-71) and 10 months (95%CI: 6-15), respectively. Importantly, cases with a *BIRC3* deletion/mutation that did not have an *ATM* mutation had significantly longer survival times for OS (76 *vs.* 42 months; *P*=0.05) and PFS (28 *vs.* 10 months; *P*=0.01) than cases with biallelic inactivation of ATM (Table 2 model C).

Discussion

This study extends recent observations on the incidence and clinical significance of *ATM* and *BIRC3* loss and/or mutation (SNV) in CLL patients with an 11q deletion. We had previously shown that the combination of an 11q deletion encompassing the *ATM* gene and mutation of the remaining *ATM* allele was associated with shorter progression-free and overall survival than a del11q with wild-type *ATM* in patients receiving first-line chemotherapy in the UK LRF CLL4 trial.[20] This observation is consistent with the importance of functional ATM protein in the response to DNA damage.[17,34-36] While several smaller studies have not identified an impact of *ATM* mutational status on patient survival, they did not differentiate *ATM* mutated individuals into those with and without deletion of 11q23.[37,38] Rossi and colleagues independently reported that *BIRC3* disruption resulting from complete or partial *BIRC3* loss with or without mutation of the remaining allele is common in patients who are refractory to, but not in those sensitive to, fludarabine-containing regimens.[26] Specifically, they noted that: 1) recurrent mutations target *BIRC3* in CLL, albeit at low frequencies; 2) *BIRC3* is recurrently deleted principally due to large genomic deletions on 11q; 3) in a single CLL case the *BIRC3* gene was deleted

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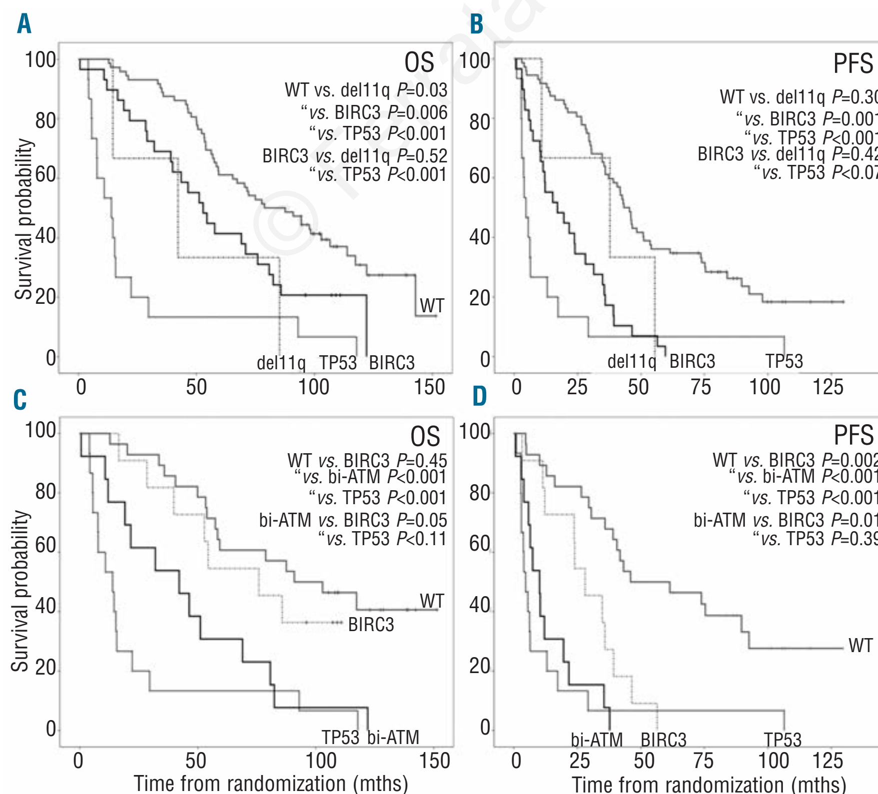

Figure 2. Overall and progression-free survival in CLL4 cases (A) and (B) show CLL4 overall and progression-free survival, respectively; for cases sub-grouped by *TP53* deletion/SNV (dashed black line), *BIRC3* deletion and/ or mutation (black line), 11q deletion without *BIRC3* (dashed gray line) or wild-type (gray line) for *TP53*, *BIRC3* genes (no deletion or mutation) and 11q-deletions. (C) and (D) show CLL4 overall and progression-free survival, respectively; for the cases sub-divided into *TP53* deletion/mutated (dashed black line), biallelic inactivation of *ATM* (black line), *BIRC3* deletion and/or mutation (dashed gray line) and wild-type (gray line) for *TP53*, *BIRC3*, *ATM* genes (no deletion or mutation) and 11q deletion. The survival curve for del11q (without an *ATM* mutation or *BIRC3* deletion and/or mutation) was precluded due to only two survival events.

without concomitant loss of *ATM*; and 4) the gene-body of *BIRC3* is targeted by rare proximal 11q-deletion breakpoints. These data are consistent with the tumor suppressor role of *BIRC3* as a negative regulator of the non-canonical NF-KB pathway and the known importance of NK-KB pathway activation in CLL by multiple mechanisms.

By studying a relatively large cohort of patients with del 11q screened for both *ATM* and *BIRC3* abnormalities, we confirm the high incidence of *BIRC3* deletion in cases with del 11q and the lack of *BIRC3* deletion in the absence of *ATM* deletion. In support of previously published work,[26] our data also show that *BIRC3* mutations are rare in a cohort of untreated CLL patients with progressive disease, suggesting limited clonal selection of these mutations prior to treatment. Our most important observation is the high frequency of missense or nonsense *ATM* mutations in *BIRC3* deleted cases (40%).

Investigating the clinical significance of these findings in patients entered into the UK LRF CLL4 trial confirmed that patients with bi-allelic *ATM* abnormalities had a shorter PFS and OS than cases with del 11q and wild-type *ATM*. Although 11q-deleted cases with *BIRC3* deletion and/or mutation did exhibit reduced OS and PFS compared to non-11q deleted cases, this was not significantly different from 11q-deleted cases without *BIRC3* deletion and/or mutation and much superior to cases with bi-allelic *ATM* abnormalities and *TP53* deletion/mutation. Furthermore, when we stratified the CLL4 cases that have large 11q23 deletions encompassing both *BIRC3* and *ATM* genes (n=24) by presence or absence of *ATM* mutation, we were able to identify that patients with an ATM mutation had a shorter PFS (HR=3.03, 95%CI: 1.22-7.49; *P*=0.017), but not OS, when compared to the patients with only *BIRC3* deletion/mutation (*data not shown*). Our negative finding for CLL4 overall survival in this sub-group analysis must be interpreted with caution due to the lack of power (29%) (*Online Supplementary Methods*).

Several limitations to this study are noted. 1) In view of the frequency with which *ATM* and *BIRC3* deletions co-exist, a much larger study would be required to identify differences in outcome between patients with *ATM* deletion compared to those with *ATM* and *BIRC3* deletion. 2) Given that the UK LRF CLL4 trial compared different chemotherapy regimens in previously untreated patients, it will be important to validate these observations in the context of chemo-immunotherapy trials, in studies of novel agents, such as B-cell receptor (BCR) signaling inhibitors, and in patients with relapsed or refractory disease. 3) Our study has focused on *ATM* and *BIRC3* abnormalities. However, deletion of 11q frequently results in the loss of many other genes involved in key regulatory pathways that could impact on CLL pathogenesis.[8] As an example, *MRE11A* and *H2AFX*, both of which are involved in DNA damage response, are also deleted in 58% (n=40) and 26% (n=18), respectively. Indeed, 53% (17 of 31) of our *BIRC3*-deleted cases also lost *H2AFX* and/or *MRE11A*. Furthermore, although we identified recurrent deletion breakpoints within the gene body of *BIRC3*, we also identified recurrent breakpoints in the bodies of other 11q genes (*Online Supplementary Table S4*), including *CEP164*, which encodes a mediator protein required for the maintenance of genomic instability.[39] Similarly we have not investigated the potential clinical consequences of the elevated genomic complexity associated with del 11q nor the incidence of epigenetic silencing of genes on the retained 11q allele. As some previous studies have noted an association between del 11q and *SF3B1* gene mutations we considered whether the incidence of *SF3B1* mutations, and the presence of CLL-specific *ATM* splice forms in *SF3B1* mutated cases.[40] might differ between cases with or without an *ATM* mutation but observed no significant enrichment of *SF3B1* mutations in either the 11q-deleted, *ATM* mutated or patients with biallelic *ATM* abnormalities suggesting that this mutation does not contribute to the poor prognosis associated with 11q abnormalities (*data not shown*).

In conclusion, we confirm that in patients treated with chemotherapy as part of the UK LRF CLL4 trial, the presence of an *ATM* mutation subdivided 11q-deleted CLL into a sub-group with significantly shorter overall and progression-free survival. *BIRC3* deletion/mutation did not identify an 11q-deleted patient subgroup with dismal outcome comparable to TP53 deletion/mutation. In fact, within 11q-deleted patients, *BIRC3* deletion/mutation was associated with significantly longer survival times than *ATM* mutation. We would caution against interpreting the clinical impact of *BIRC3* abnormalities without knowledge of *ATM* mutational status.

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Authorship and Disclosures

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