SUPPLEMENTARY APPENDIX

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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Supplementary Methods

Molecular diagnostic assays

FISH analysis was performed for the presence of established rearrangements using a range of commercially available probes (Abbott Diagnostics, Maidenhead, UK; DakoCytomation, Glostrup, Denmark). ZAP70 and CD38 expression was determined as previously described (30) where 10% and 30% positive cells were classed as positive, respectively. *IGHV* genes were sequenced as previously described (31) and a cut-off of ≥98% germ-line identity was taken to define the unmutated sub-set.

DNA extraction, SNP6 array hybridization, data extraction and analysis

The data was aligned (Build 36.3) and analyzed by two independent researchers using Partek Genomics Suite (Partek Inc, Missouri, USA). Copy number alterations (CNAs) were defined as a deviation of 50 consecutive probes from a normal value of 2 (±0.3), within a consecutive genomic window of 50 Kilobases. As we have previously shown that aberration identification are not compromised by the absence of germ-line DNA profiling (32), the 270 HapMap Reference baseline (Affymetrix) was used as a control and germline copy number variants were excluded based on the Database of Genomic Variants (http://projects.tcag.ca/variation/). The allele ratio was calculated for each sample using the HapMap Allele Reference baseline (Affymetrix) and in the absence of paired normal DNA; copy number neutral loss of heterozygosity (CNNLOH) was defined as a region greater than 20Mb, extending to a telomere.

Mutational analysis of *ATM* and *BIRC3 g*enes

Denaturing high-performance liquid chromatography (DHPLC) was applied to high-molecular weight genomic DNA to identify SNVs in the 62 coding exons and flanking intronic sequences of the *ATM* gene as previously reported (20). Sequence changes were confirmed by direct Sanger sequencing and SNVs were categorized as previously reported (20). For *BIRC3* analysis, whole genome amplified DNA (Illustra GenomiPhi V2 Amplification Kit®, GE Healthcare) was screened using high-resolution melt (HRM) analysis as previously reported (29). *BIRC3* exons 7 and 10 (Transcript NM_182962.2) were analyzed as these exons capture all previously reported CLL-specific somatic variation (26, 27). *BIRC3* HRM-PCR primer sequences and reaction conditions are described in **Supplementary table 2**. Products showing abnormal melt patterns were sequenced. All SNVs were sequence validated on genomic DNA (gDNA) from the archival sample. Furthermore, we sequenced the aforementioned regions of *BIRC3* in gDNA samples from 35 cases that exhibited normal HRM melt profiles on WGA material. In doing so, we found no additional SNVs present in

the gDNA. The strategy for assigning the somatic nature of each SNV is detailed in the Supplementary methods section.

Due to the historical nature of this cohort, matched germ-line DNA was not available on the majority of cases, so we ascertained the somatic nature of each SNV by ensuring the variant was not annotated as a polymorphism in dbSNP132 (www.ncbi.nlm.nih.gov/projects/SNP/). For *ATM*, SNVs were defined as pathogenic if they were; a) 'truncating' due to a sequence alteration (frame-shift or nonsense-STOP-codon or short in-frame deletion or splice site defect), that was predicted to cause premature termination of the protein, or b) 'non-truncating' if they were missense, either reported in AT patients or predicted to cause a non-synonymous amino acid substitution in the translated protein within the region encoding the functional domain of the ATM protein (20). For *BIRC3*, SNVs were defined as a) resulting in a protein sequence change (i.e. a nonsynonymous amino acid change) or b) were predicted to cause premature termination of the protein or a small in-frame deletion (26).

Statistical analysis

Overall response rate (ORR) was defined as complete (CR), nodular partial (nPR), partial response (PR) and non-response/ progressive disease (NR/PD) and was available on 131 of the 133 CLL4 cases. Overall survival (OS) was defined as time from randomization to death, or to the follow up date (August 2012) for survivors. Progression free survival (PFS) was defined as time from randomization to relapse needing further therapy, progression or death, or to the follow-up date (Oct 2010; final LRF CLL4 PFS update) for those with no progression/death.

Post-hoc sub-group power calculation for OS and PFS:

With clinical follow-up of 10 years, our CLL4 sub-group (11q23 deletions encompassing *BIRC3* and *ATM*, n=24) had 29% power with a significance level = 0.05 to detect a OS difference between cases with *ATM* and *BIRC3* deletion and those with *BIRC3* deletion and biallelic loss of *ATM* via mutation of the non-deleted allele, based on the median CLL4 OS times of 76 and 42 months, respectively. For, PFS we had 81% power with a significance level = 0.05 to detect a PFS difference between cases based on the median CLL4 OS times of 28 and 10 months, respectively.

Supplementary Table 1: Comparison of the CLL4 cases to the full LRF CLL4 trial

Variable	CLL4 Cases	%	CLL4 trial	%	P-Value
Total cases	133	-	777	-	
Male	98	74	573	74	ns
Female	35	26	204	26	
Age	63	-	64	-	ns
Binet Stage					
A	30	23	191	25	ns
B	65	49	352	45	
C	38	28	234	30	
IGHV unmutated	86	69	327	61	ns
mutated	38	31	206	39	
CD38 -ve	59	54	299	56	ns
+ve	50	46	236	44	
ZAP70 -ve	52	45	234	49	ns
+ve	63	55	244	51	
TP53 normal	102	89	532	92	ns
del/mut	13	11	48	8	
del(11q) -ve	84	67	462	80	0.002
+ve	42	33	116	20	
del(13q) -ve	50	40	361	62	<0.001
+ve	75	60	217	38	
del(17p) -ve	114	91	538	94	ns
+ve	11	9	33	6	
Tri12 -ve	110	87	487	84	ns
+ve	16	13	91	16	

TP53 abnormalities defined by deletion and/ or mutation (del/mut)

Supplementary Table 2: *BIRC3* primer sequences

Primer name	Primer sequence (5'- 3')	PCR conditions
***BIRC3* exon 7F**	TTCCATATAGTTATCCATTTTGAACCT	HRM-PCR: Ta=60°C
	TGCCTATACATTTTGTTGGTT	Seq-PCR: Ta=55°C
***BIRC3* exon 7 R**	ACATACTTGATTCTTTTTCCTCAGTTG	HRM-PCR: Ta=60°C
	AAAAACCTGACTGGATTGAG	Seq-PCR: Ta=55°C
***BIRC3* exon 10 F**	TGAAGAAGCAAACTGCCTTTTATT	HRM-PCR: Ta=60°C
	CCACAGAAGATGTTTCAGGT	Seq-PCR: Ta=55°C
***BIRC3* exon 10 R**	AAAGTTTAGACGATGTTTTGGTTCT	HRM-PCR: Ta=60°C
	GTGCTACCTCTTTTTCGTTC	Seq-PCR: Ta=55°C

Footnote: HRM-PCR = High-Resolution Melting PCR. Seq-PCR = Sanger Sequencing PCR

Supplementary Table 3: *ATM* mutations in our CLL patients

Regid	BIRC3 del	ATM del	del(11q) FISH	del(11q) FISH Clone Size (%)	ATM Mutation Nomenclature	c.DNA position	Amino Acid number	ATM mutation Type	Consequence
353	N	N	N	-	c.217_218del2:p.Gln73*fs*	217	73	T	Frameshift
37	Y	Y	Y	95	c.478_482del5:p.Ser160*fs*	478	160	T	Frameshift
349	N	N	N	-	c.1006_1020del15:p.Phe336_Ala340del5	1006	340	T	Frameshift
52	N	N	N	-	c.1048G>A:p.Ala350Thr	1048	350	NT	Nonsynonymous
48	N	N	N	-	c.1066-6T>G	1066	n/a	T	Splicing defect – termination (exon11)
346	N	Y	Y	-	c.1120C>T:pGlu374*	1120	374	T	STOP codon
181	Y	Y	Y	98	c.1402_1403del2:p.Lys468*fs*	1402	468	T	Frameshift
54	N	N	N	-	c.2193delC:p.Tyr731*fs*	2193	731	T	Frameshift
348	Y	Y	Y	30	c.2417T>G:p.Leu806Trp	2417	806	NT	Nonsynonymous
42	Y	Y	Y	93	c.2720_2723del4:p.Cys907*fs*	2720	907	T	Frameshift
39	N	Y	Y	87	c.3712_3716del5:p.Leu1238*fs*	3712	1238	T	Frameshift
72	Y	Y	Y	45	c.3720_3736del17:p.Asn1240*fs*	3720	1240	T	Frameshift
352	Y	Y	Y	89	3883_3885delCTT:p.Leu1295del	3883	1295	NT	Inframe deletion
46	Y	Y	Y	83	c.5006-2A>G	5006	n/a	T	Splicing defect - termination (exon 36)
55	N	N	N	-	c.5857A>G:p.Thr1953Ala	5857	1953	NT	Nonsynonymous
43	N	N	Y	11	c.6067G>A:p.Gly2023Arg	6067	2023	NT	Nonsynonymous
342	Y	Y	Y	86	c.6106T>A:p.Tyr2036Asn	6106	2036	NT	Nonsynonymous
51	Y	Y	Y	84	c.6375insT:p.Glu2126*	6375	2126	T	STOP codon
45	Y	Y	Y	75	c.6989_6995del7:p.Leu2330*fs*	6989	2330	T	Frameshift
49	Y	Y	Y	84	c.7327C>G:p.Arg2443Gly	7327	2443	NT	Nonsynonymous
174	N	N	N	-	c.7390T>C:p.Cys2464Arg	7390	2464	NT	Nonsynonymous
56	N	N	N	-	c.7438C>T:p.His2480Tyr	7438	2480	NT	Nonsynonymous
50	Y	Y	Y	97	c.7638_7646del9:p.Arg2547_Ser2549del3	7638	2547	T	Frameshift
354	Y	Y	Y	84	c.7883_7888del5	7883		T	Frameshift
341	Y	Y	Y	-	c.8056T>C:p.Phe2686Leu	8056	2686	NT	Nonsynonymous
57	N	N	N	-	c.8095C>T:p.Pro2699Ser	8095	2699	NT	Nonsynonymous
60	N	Y	Y	23	c.8161G>A:p.Asp2721Asn	8161	2721	NT	Nonsynonymous

36	Y	Y	Y	76	c.8249T>C:p.Leu2750Ser	8249	2750	NT	Nonsynonymous
61	N	Y	Y	69	c.8249T>C:p.Leu2750Ser	8249	2750	NT	Nonsynonymous
351	N	N	N	-	c.8428_8450del23:p.Lys2810*fs*	8428	2810	T	Frameshift
58	N	N	N	-	c.8663T>C:p.Ile2888Thr	8663	2888	NT	Nonsynonymous
47	Y	Y	Y	89	c.8672G>A:p.Gly2891Asp	8672	2891	NT	Nonsynonymous
357	Y	Y	Y	57	c.8787-1G>T	8787	n/a	NT	Splicing defect - termination (exon 62)
197	Y	Y	Y	88	c.9023G>A:p.Arg3008His	9023	3008	NT	Nonsynonymous
59	N	N	N	-	c.9032T>A:p.Met3011Lys	9032	3011	NT	Nonsynonymous
38	Y	Y	Y	66	c.9139C>T:p.Arg3047*	9139	3047	T	STOP codon

Footnote: In the BIRC3 del column underlined Y indicates an 11q23 deletion breakpoint in the BIRC3 gene body. T = Truncating mutation, NT = Non-Truncating mutation.

Supplementary Table 4: Gene body disruption by 11q23 deletion breakpoints which include *ATM* (excluding the *BIRC3* gene breakpoints)

Gene Symbol	Gene location	Sample ID with deletion breakpoint within a gene body	
		centromeric	telomeric
PHCA (ACER3)	76249601-76411617	72, *350*	
RSF1	77054922-77209528	42	
RAB30	82370126-82460532	354	
DLG2	82843701-84312113	*181*, *220*	
CNTN5	98397081-99732683	247, 264, 266	
GRIA4	104986010-105358029	44, 107	
CUL5	107384618-107483698	60, 61, 244	
DDX10	108041026-108316860		62, 346
CADM1	114549555-114880451		74, 180, 352
CEP164	116703781-116789192		37, 263, *350*
RNF214	116608614-116661614		50, 64, 76, 182, 348
DSCAML1	116803699-117173186		40, *181*
ASAM	122448230-122571217		49, *220*

Footnote: Reference genome is hg18 (Mar. 2006 NCBI36).