The *PAX5* gene is frequently rearranged in *BCR-ABL1*-positive acute lymphoblastic leukemia but is not associated with outcome. A report on behalf of the GIMEMA Acute Leukemia Working Party

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Online Supplementary Table 1S. Primers used for *PAX5* analysis.

Primer	Description	Sequence (5'_3')
F1	*PAX5* RT-PCR, exon 1, S	ATCCGACTCCTCGGACCAGCA
R1	*PAX5* RT-PCR, exon 10, AS	CACCCTCAATAGGTGCCATCAGT
F2	GAPDH RT-PCR S	CCAGCCGAGCCACATCGCTC
R2	GAPDH RT-PCR AS	ATGAGCCCCAGCCTTCTCCAT
F3	*PAX5* genomic-PCR, exon 2, S	CATTTGTGATCAGAGGGGCT
R3	*PAX5* genomic-PCR, exon 2, AS	CGTGTGAAACAAAATGCCAC
F4	*PAX5* genomic-PCR, exon 3, S	CCCGTTATTTTGTTGCCAAT
R4	*PAX5* genomic-PCR, exon 3, AS	CCAGATCTTCAGGAAAGGCA
F5	*PAX5* genomic-PCR, exon 4, S	CCCAGCTTGGGTATGAGTTT
R5	*PAX5* genomic-PCR, exon 4, AS	CGTGTGCTGAAGTGTTTTATGC
F6	*PAX5* genomic-PCR, exon 5, S	CTGACCGCCCGTCTTTCT
R6	*PAX5* genomic-PCR, exon 5, AS	CTCCTCTGCAGGTAAGGGG
F7	*PAX5* genomic-PCR, exon 6 S	AACCTCCACTCACCCTTCCT
R7	*PAX5* genomic-PCR, exon 6, AS	CAGATGCCTCTGCCTTCAG
F8	*PAX5* genomic-PCR, exon 7, S	TGTGGAGTCTTGGTGCTGAG
R8	*PAX5* genomic-PCR, exon 7, AS	GCCAATCACATCCAAACACA
F9	*PAX5* genomic-PCR, exon 8, S	CCCTGTGATTTGTTGCTTTG
R9	*PAX5* genomic-PCR, exon 8, AS	AGAAGCGTAGAGGTCACCCA
F10	*PAX5* genomic-PCR, exon 9, S	GGGCCTTTTCTGAACTGGAT
R10	*PAX5* genomic-PCR, exon 9, AS	ACCCACCTCAGTGACCAGAC

Online Supplementary Table 2S. Distribution of *PAX5* and *IKZF1* deletions in 89 *BCR-ABL1*-positive ALL patients in whom SNP arrays (SNP-A) were performed. For the *PAX5* gene the type of copy number alteration, the start and end of the alteration (as cytoband) and its size are reported. In addition results of *PAX5* mutation analysis are reported in the cases for which material was available.

ID	Blast (%)	SNP-A type	*IKZF1* loss	*PAX5* loss	*PAX5* type of deletion	Size (kb)	Cytoband Start	Cytoband End	*PAX5* mutations
1	90	NspI 250K	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	595	p13.3	p13.2	-
2	93	NspI 250K	No	No	-	-	-	-	WT
3	90	NspI 250K	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	567	p13.2	p13.2	WT
4	55	NspI 250K	No	No	-	-	-	-	WT
5	90	NspI 250K	No	No	-	-	-	-	WT
6	91	NspI 250K	No	No	-	-	-	-	-
7	80	NspI 250K	No	No	-	-	-	-	-
8	90	NspI 250K	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	668	p13.2	p13.2	-
9	76	NspI 250K	No	No	-	-	-	-	WT
10	87	NspI 250K	Yes	No	-	-	-	-	
11	96	NspI 250K	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	2617	p13.3	p13.1	WT
12	97	SNP 6.0	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	18208	p21.3	p13.1	WT
13	90	NspI 250K	Yes	No	-	-	-	-	-
14	92	NspI 250K	Yes	No	-	-	-	-	-
15	49	NspI 250K	No	No	-	-	-	-	-
16	73	NspI 250K	No	No	-	-	-	-	-
17	70	SNP 6.0	Yes	No	-	-	-	-	WT
18	99	NspI 250K	No	No	-	-	-	-	-
19	90	NspI 250K	Yes	No	-	-	-	-	-
20	93	NspI 250K	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	295	p13.2	p13.2	-
21	65	NspI 250K	No	No	-	-	-	-	WT
22	90	NspI 250K	Yes	No	-	-	-	-	WT
23	80	NspI 250K	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	817	p13.3	p13.2	WT
24	98	NspI 250K	No	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	15122	p21.3	p13.1	-
25	92	NspI 250K	Yes	No	-	-	-	-	-
26	80	SNP 6.0	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	101	p13.2	p13.2	-
27	99	NspI 250K	Yes	No					
28	NA	SNP 6.0	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	16395	p21.3	p13.2	WT
29	94	NspI 250K	No	No	-	-	-	-	WT
30	90	NspI 250K	Yes	No	-	-	-	-	-
31	93	NspI 250K	No	Yes	Deletion of 9p	-	-	-	-
32	90	NspI 250K	Yes	No		-	-	-	-
33	90	NspI 250K	No	Yes	Deletion of 9p	-	-	-	-
34	96	NspI 250K	No	No	-	-	-	-	WT
35	83	NspI 250K	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	418	p13.2	p13.2	-
36	80	NspI 250K	No	No	-	-	-	-	
37	90	SNP 6.0	No	No	-	-	-	-	-
38	30	SNP 6.0	Yes	No	-	-	-	-	-
39	95	NspI 250K	No	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	18169	p21.3	p13.1	WT
40	96	NspI 250K	No	No	-	-	-	-	WT
41	60	NspI 250K	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	2281	p13.3	p13.2	-
42	90	NspI 250K	Yes	No	-	-	-	-	-
43	90	NspI 250K	Yes	Yes	Deletion of 9p	-	-	-	-
44	72	NspI 250K	No	No	-	-	-	-	WT
45	90	NspI 250K	No	No	-	-	-	-	-
46	90	NspI 250K	Yes	No	-	-	-	-	-
47	90	NspI 250K	Yes	No	-	-	-	-	-

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48	85	SNP 6.0	No	Yes	Deletion of 9p	-	-	-	-
49	90	NspI 250K	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	2281	p13.3	p13.2	-
50	85	NspI 250K	Yes	Yes	Deletions involving a portion of *PAX5* and flanking genes	p13.2 310	p13.2	-	
51	46	SNP 6.0	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	154	p13.2	p13.2	-
52	NA	NspI 250K	No	No	-	-	-	-	-
53	60	NspI 250K	Yes	No	-	-	-	-	WT
54	70	NspI 250K	Yes	No	-	-	-	-	-
55	87	NspI 250K	No	No	-	-	-	-	WT
56	96	NspI 250K	Yes	No	-	-	-	-	WT
57	NA	NspI 250K	Yes	No	-	-	-	-	WT
58	20	NspI 250K	Yes	No	-	-	-	-	-
59	NA	SNP 6.0	Yes	Yes	Deletion of 9p	-	-	-	WT
60	60	SNP 6.0	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	1718	p13.3	p13.2	WT
61	90	NspI 250K	No	Yes	Deletion of 9p	-	-	-	WT
62	37	NspI 250K	Yes	No	-	-	-	-	-
63	85	NspI 250K	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	154	p13.2	p13.2	-
64	87	NspI 250K	Yes	Yes	Deletion of 9p				-
65	96	NspI 250K	Yes	No	-	-	-	-	-
66	NA	SNP 6.0	Yes	Yes	Focal deletion	101	p13.2	p13.2	-
67	20	NspI 250K	No	No	-	-	-	-	WT
68	92	NspI 250K	Yes	No	-	-	-	-	-
69	86	NspI 250K	Yes	No	-	-	-	-	-
70	83	NspI 250K	Yes	No	-	-	-	-	WT
71	80	NspI 250K	No	No	-	-	-	-	WT
72	85	SNP 6.0	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	242	p13.2	p13.2	WT
73	96	NspI 250K	Yes	No	-	-	-	-	WT
74	92	NspI 250K	Yes	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	692	p13.3	p13.2	WT
75	98	NspI 250K	Yes	Yes	Deletion involving a portion of *PAX5* and flanking genes	833	p13.3	p13.2	-
76	96	NspI 250K	Yes	No	-	-	-	-	WT
77	98	NspI 250K	No	No	-	-	-	-	-
78	90	SNP 6.0	Yes	No	-	-	-	-	-
79	95	SNP 6.0	Yes	No	-	-	-	-	-
80	NA	SNP 6.0	Yes	No	-	-	-	-	-
81	99	SNP 6.0	No	No	-	-	-	-	-
82	18	NspI 250K	Yes	No	-	-	-	-	-
83	60	SNP 6.0	Yes	No	-	-	-	-	WT
84	80	SNP 6.0	No	No	-	-	-	-	-
85	93	SNP 6.0	Yes	No	-	-	-	-	-
86	80	SNP 6.0	No	No	-	-	-	-	-
87	80	NspI 250K	No	No	-	-	-	-	WT
88	82	NspI 250K	Yes	No	-	-	-	-	-
89	95	NspI 250K	Yes	No	-	-	-	-	WT

WT indicates wild-type.

Online Supplementary Table 3S. *PAX5* genomic real-time genomic quantitative PCR (Q-PCR). Q-PCR of *PAX5* exons 3, 6 and 8 was performed in order to confirm SNP results and to characterize the extension of deletions in 20 *BCR-ABL1*-positive ALL cases and three germline (Normal) samples.

ID	*PAX5* del	*PAX5* type of deletion	ex3 gqPCR	ex6 gqPCR	ex8 gqPCR
1	Yes	Deletion involving a portion of *PAX5* and flanking genes	0.85	0.9	0.6
3	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.56	0.6	0.57
8	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.57	0.54	0.57
11	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.71	0.75	0.75
12	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.66	0.68	0.66
20	Yes	Deletion involving a portion of *PAX5* and flanking genes	0.66	0.73	0.85
23	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.52	0.65	0.63
24	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.56	0.58	0.62
26	Yes	Deletion involving a portion of *PAX5* and flanking genes	0.96	0.58	0.51
28	Yes	Deletion involving a portion of *PAX5* and flanking genes	1.02	1.21	0.6
39	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.59	0.56	0.37
41	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.71	0.75	0.74
59	Yes	Deletion of 9p	0.59	0.57	0.55
60	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.63	0.67	0.71
61	Yes	Deletion of 9p	0.69	0.52	0.62
63	Yes	Deletion involving a portion of *PAX5* and flanking genes	0.61	0.67	0.96
64	Yes	Deletion of 9p	0.51	0.58	0.47
66	Yes	Focal deletion	0.67	0.79	0.83
74	Yes	Broader deletion involving *PAX5* and a variable number of flanking genes	0.74	0.76	0.75
75	Yes	Deletion involving a portion of *PAX5* and flanking genes	0.78	0.66	0.89
Normal	No	-	1.25	1.42	1.41
Normal	No	-	1.34	1.23	1.10
Normal	No	-	1.23	1.15	1.21

Genomic quantitative PCR (gqPCR) results are expressed as PAX5/RNAseP *gqPCR ratios as described by Mullighan* et al.[7] PAX5/RNAseP *gqPCR ratios of less than 0.80 indicate heterozygous deletion.*

Online Supplementary Table 4S. Association of *PAX5* deletion with protocols.

PAX5	Protocol type				
	LAL2000	LAL1205	LAL0201B	Institutional	Total
Wild-type	8	32	10	10	
Row %	13.33	53.33	16.67	16.67	60
Column %	53.33	74.42	62.50	66.67	
Deleted	7	11	6	5	
Row %	24.14	37.93	20.69	17.24	29
Column %	46.67	25.58	37.50	33.33	
Total	**15**	**43**	**16**	**15**	**89**

Online Supplementary Table 5S. Complete hematologic response related to the presence of *PAX5* deletion (univariate analysis).

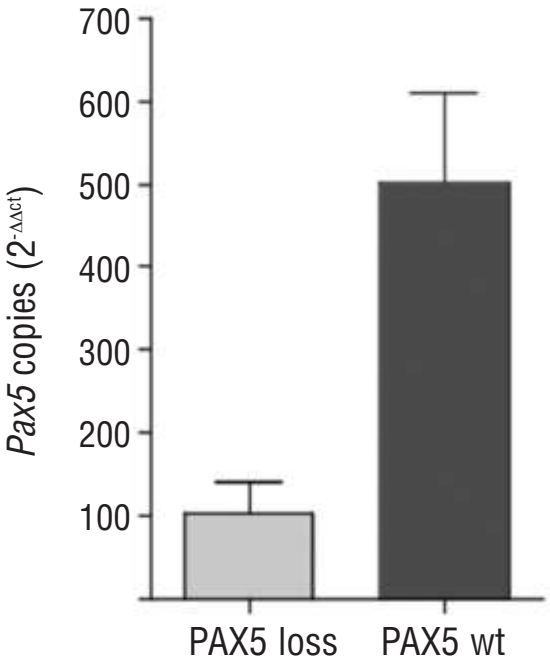
	PAX5 WT N. of pts (%)	*PAX5* Deleted N. of pts (%)	*P* value
Complete hematological response	12/18 (66.7 %)	11/11 (100%)	
			0.057
Resistance/refractory to induction therapy	6/18 (33.3 %)	0/11 (0)	

All patients started induction therapy and consequently were evaluable for complete hematologic response (by intention to-treat analysis). When data from the final evaluation were not available, the patients' data at the last visit available were analyzed (last observation carry forward or LOCF).

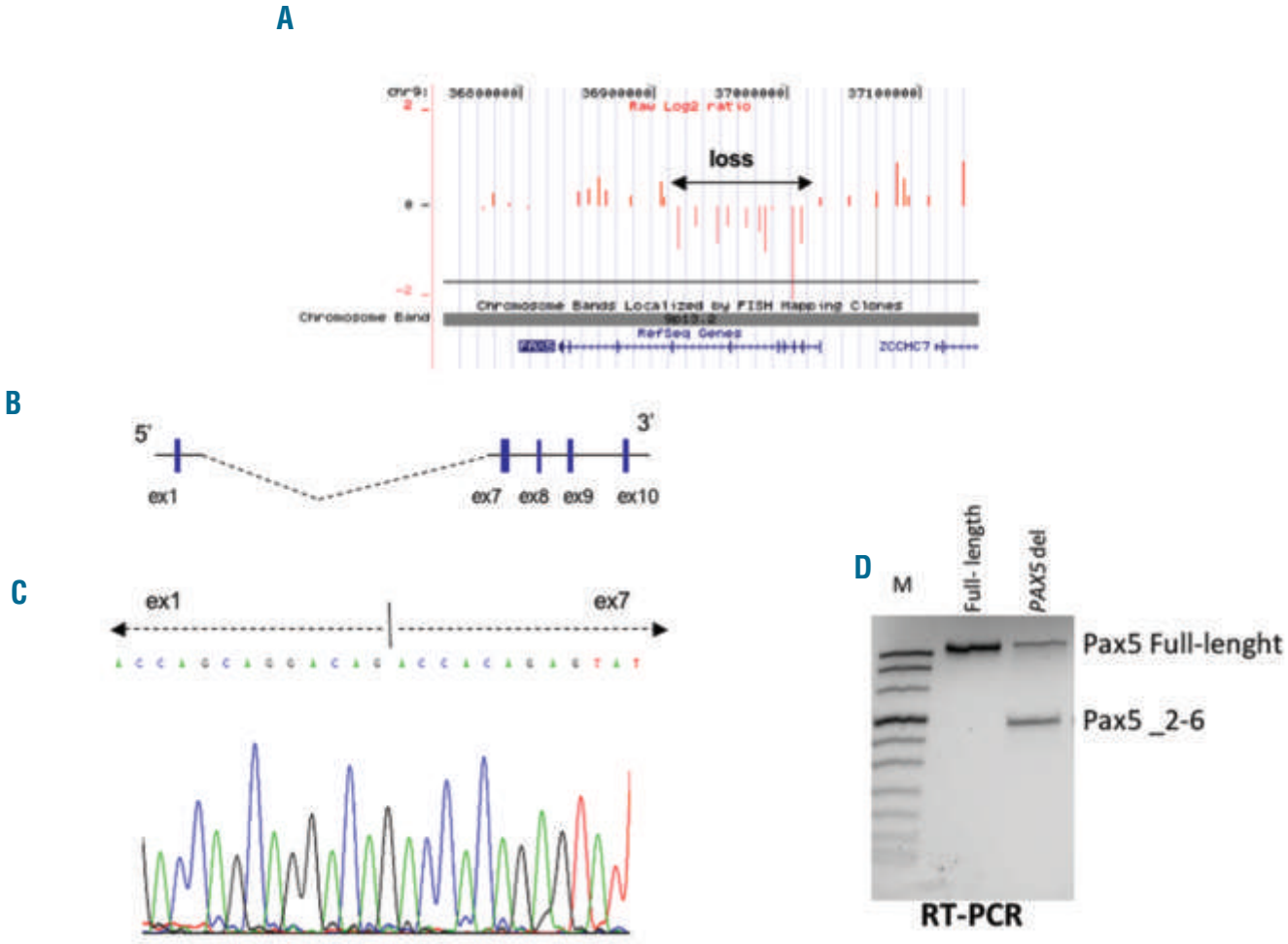
Online Supplementary Table 6S. Multivariate analysis of disease-free survival considering the combination of *PAX5* and *IKZF1*.

Variable	Hazard Ratio	95% CI		*P* value
Protocol treatment				
LAL1205 *versus* LAL2000	1.690	0.502	5.690	0.3969
LAL0201B *versus* LAL2000	5.232	1.451	18.86	0.0114
Other protocols *versus* LAL2000	1.808	0.465	7.035	0.3929
BCR type				
p190 *versus* p210	0.712	0.336	1.511	0.3767
p190/p210 *versus* p210	0.953	0.242	3.763	0.9457
PAX5 and *IKZF1* association				
PAX5 no and *IKZF1* yes *versus* *PAX5* no and *IKZF1* no	1.736	0.709	4.253	0.2277
PAX5 yes and *IKZF1* no *versus* *PAX5* no and *IKZF1* no	0.230	0.038	1.386	0.1088
PAX5 yes and *IKZF1* yes *versus* *PAX5* no and *IKZF1* no	1.610	0.641	4.043	0.3107
White blood cell at diagnosis (as continuous variable)	1.008	1.002	1.015	0.0146
Linear Hypotheses Testing Results (other significant comparisons)				
LAL1205 *versus* LAL0201B	0.323	0.134	0.777	0.0117
PAX5 no and *IKZF1* yes *versus* *PAX5* yes and *IKZF1* no	7.546	1.255	45.372	0.0272
PAX5 yes and *IKZF1* yes *versus* *PAX5* yes and *IKZF1* no	6.999	1.185	41.335	0.0318

PAX5/IKZF1 *yes indicates deletion;* PAX5/IKZF1 *no means wild-type.*



Online Supplementary Figure 1S. Real-time quantitative PCR on cDNA samples suggested that genomic alterations lead to a significant down-modulation of *PAX5* transcripts.



Online Supplementary Figure 2S. (A) Screen capture of SNP data from case #66 visualized in the University of California Santa Cruz (UCSC) Human Genome Browser showing the raw log-two ratio of SNP probes in the region containing *PAX5*. The position of each SNP probe set is represented by a vertical line. (B) Schematic diagram of the isoform generated following deletion in which exon 1 is juxtaposed to exon 7; (C) Pherograms of sequencing of transcript generated following *PAX5* focal deletion in case # 66 in whom exon 1 is directly juxtaposed to exon 7. (D) RT-PCR analysis showing the band of full-length *PAX5* in a case without deletion and the bands corresponding to the full-length isoform and the alternative isoform derived from deletion of exons 2-6 in the case with *PAX5* deletion. M: molecular size standard.

Online Supplementary Figure 3S. cDNA sequence of *PAX5* according to ENSG00000196092. The sequence of the isoform lacking exons 2-6 is underlined. The stop codons are highlighted in red while the start codon is shown in yellow.