Supplementary Information

Intragenic amplification of *PAX5*:
A novel subgroup in B-cell precursor acute lymphoblastic leukemia?

Supplementary Methods

Multiplex Ligation-dependent Probe Amplification (MLPA)

Copy number alterations (CNAs) of *PAX5*, *IKZF1*, C*DKN2A/B*, *EBF1*, *ETV6*, *BTG1*, *RB1*, and the *PAR1* region (*CRLF2, CSF2RA, IL3RA*), resulting in the expression of the *P2RY8-CRLF2* fusion, were determined in the individual centers by MLPA using various versions of the SALSA MLPA kit P335 *IKZF1* (MRC Holland, The Netherlands). Data analysis was conducted using either the Coffalyser (MRC Holland) or the GeneMarker V1.85 (Softgenetics, USA) software. Relative copy numbers were obtained after normalization of peaks against internal MLPA kit inherent and external (normal genomic DNA) controls. As previously determined in a validation study,[1] MLPA probe ratio values between 0.75 and 1.3 are within the normal range, equivalent to the normal copy number of 2. Values below 0.75 or above 1.3 indicate a loss or a gain, respectively, and a value below 0.25 indicates a biallelic loss. Accordingly, these values correspond to copy numbers of 1, 3-4, and 0. Previous data obtained in high hyperdiploid ALL[2] showed that a value ≥2.0 corresponds to a copy number of ≥4 and herein was interpreted as amplification of individual *PAX5* exons.

SNP array analysis

Eight and five *PAX5*^{AMP} samples were processed on the SNP6.0 array (Affymetrix, USA) by AROS Applied Biotechnology A/S, Aarhus, Denmark and on CytoScan HD Arrays *(*Affymetrix) and analyzed using the Genotyping Console (Affymetrix) and Chromosome Analysis Suite version 3.1 (ChAS; Affymetrix) software packages, respectively. All aberrations were mapped to the Genome Reference Consortium GRCh37, UCSC genome assembly hg19 reference genome. Copy number variants (CNVs) arising from B-cell and T-cell antigen receptor gene rearrangements as well as all known common benign CNVs were excluded. A complete list of all aberrations identified by SNP array analysis is provided in Supplementary Table 4.

Supplementary Tables

Supplementary Table 1. Demographic and clinical characteristics of BCP-ALL patients with *PAX5*^{AMP}

		Patients n (%)
Total		**77***
Age	**Mean (years)**	6.49
	Range	1 - 19
	< 1 year	0
	1-9 years	58 (75)
	≥ 10 years	19 (25)
Sex	**Male**	51 (66)
	Female	26 (34)
White Blood Cell Count (WBC) (x10^9/L)**	**Mean**	63.36
	Range	0.9 – 356.8
	< 50	46 (62)
	≥ 50	28 (38)
NCI Risk‡	**High**	43 (56)
	Standard	34 (44)
MRD post-induction††	**Positive**	18 (40)
	Negative	27 (60)
Complete Remission**	**Yes**	73 (99)
	No	1 (1)
Relapse	**Yes**	29 (40)
	No	44 (60)
	Median time to relapse	2.1 years
Follow-up (years)	**Median**	5.5
	Range	< 1 – 14.1
Survival % (95% CI)	**EFS @5 years**	49% (36-61)
	OS @5 years	67% (54-77)

* number of sample available from diagnosis, ** data available for 74 patients (95%),
‡ data available for 77 patients (99%), †† data available for 45 patients (24%)

Supplementary Table 2: Incidences of *PAX5*^{AMP} from population-based cohorts

Trial	Country	No. of *PAX5*^{AMP}	B-other ‡ screened	% B-other	Total screened	% BCP-ALL
UKALL2003	UK	9	197	4.6	787	1.1
ALL-BFM 2000	Austria	6	124	4.8	453	1.3
ALL-BFM 2000	Germany	7	ND	ND	1070	0.7
DCOG	Netherlands	7	171	4.1	522	1.3
EORTC 58951*	France	7	299†	2.3	1050	0.7
ALLIC 2002	Czech Republic	2	ND	ND	184	1.1
NOPHO	Sweden	5	37	13.5	116	4.3
TCCSG ALL L0416 & L0616	Japan	1	184	0.5	264	0.4
JACLS ALL02		2	176††	1.1	278	0.7
CCLSG ALL2004		1	83††	1.2	179	0.6
ANZCHOG ALL8	Australia	4	ND	ND	507	0.8
ALL-BFM 2009		1	ND	ND	153	0.7
Total B-other		**33**	**1271**	**3.0**		
Total BCP-ALL		**52**			**5535**	**0.9**

‡ B-other ALL was defined as negative for *ETV6-RUNX1, BCR-ABL1*, and *TCF3-PBX1* fusions, high hyperdiploidy (51-65 chromosomes), near-haploidy (<30 chromosomes), low hypodiploidy (30-39 chromosomes), *MLL* rearrangements, or intrachromosomal amplification of chromosome 21 (iAMP21)

* including Down syndrome patients, which were excluded from the other cohorts

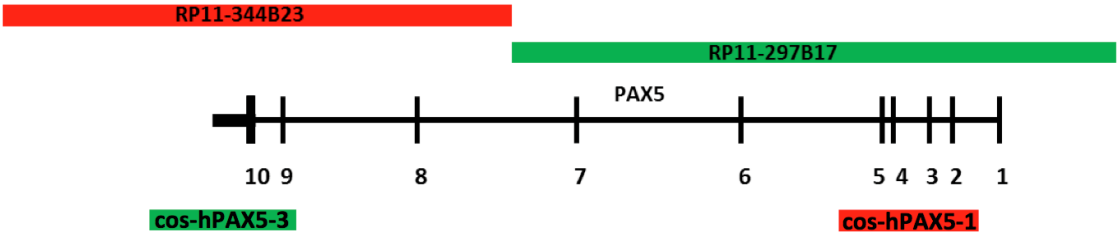
† excluding cases with *ERG* deletions

†† including iAMP21 cases

BCP-ALL, B-cell precursor acute lymphoblastic leukemia; ND, not determined

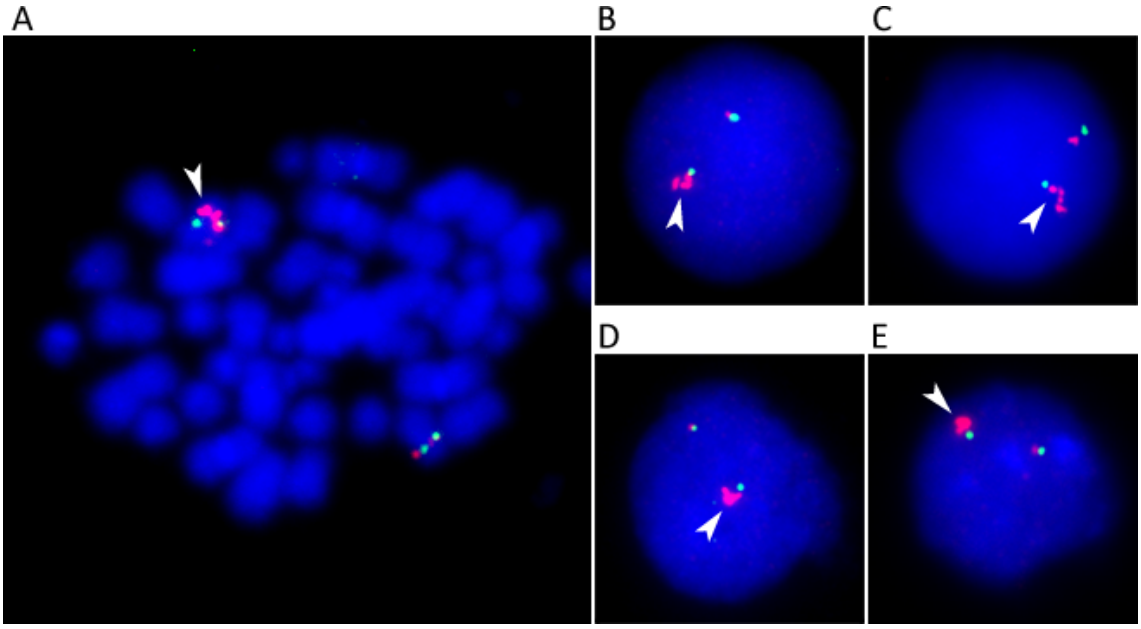
Supplementary Figures

Supplementary Figure 1. *PAX5* locus-specific FISH probes.



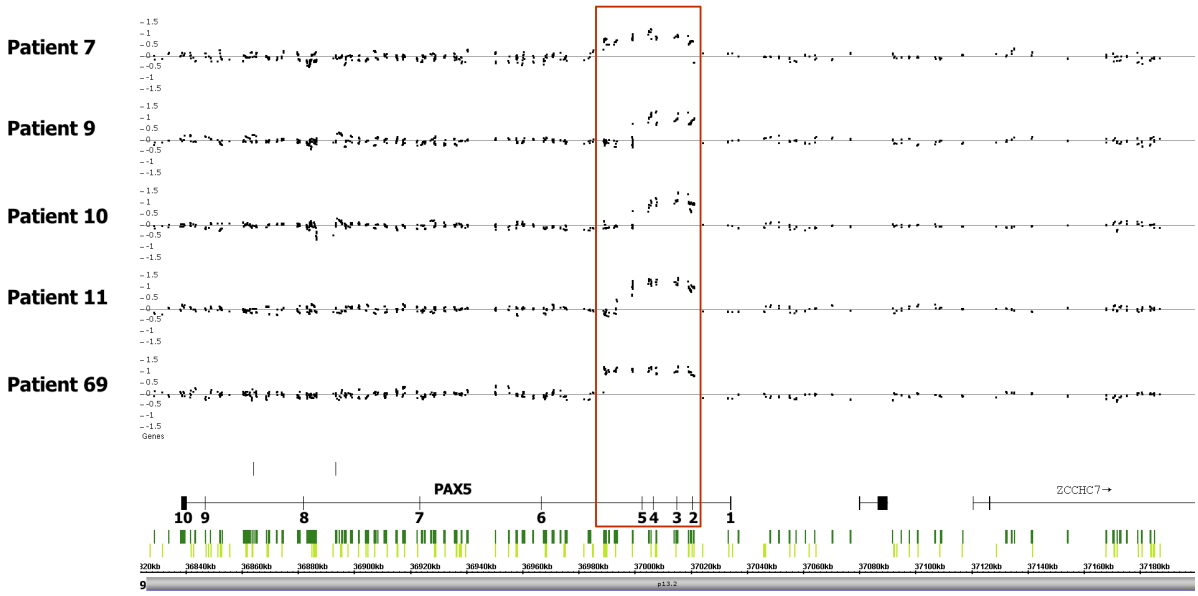
Schematic representation of the localization of the bacterial artificial chromosome (BAC) and cosmid clones[3,4] used for the detection of *PAX5* rearrangements and amplifications in 16 and 10 *PAX5*^{AMP} cases, respectively. Numbers denote *PAX5* exons.

Supplementary Figure 2. FISH analysis for *PAX5* in *PAX5*^{AMP} cases.



FISH analysis was performed using *PAX5* exon-specific cosmids cos-hPAX5-1 (exons 2-5; red signals) and cos-hPAX5-3 (exons 9-10; green signals). (A) Metaphase and (B-C) interphase nuclei of patient 7; (D) and (E) interphase nuclei of patients 73 and 74, respectively, showing that the *PAX5* amplification is located to 9p (A), and the tight clustering of the signals for the *PAX5* exons 2-5 specific probe in interphase cells (B-E). Arrowheads indicate the amplified allele.

Supplementary Figure 3. CytoScan HD profiles of patients with *PAX5*^{AMP}.



The copy number profiles (weighted log2 ratios) of five *PAX5*^{AMP} patients with amplifications of exons 2 and 5 detected by MLPA are depicted, showing that the amplification affects exons 2-5. Genes are indicated by black vertical bars; SNP markers, light green vertical bars; oligo markers, dark green vertical bars are indicated in the lower part of the figure.

Supplementary References

1. Schwab CJ, Chilton L, Morrison H, et al. Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features. *Haematologica*. 2013;98(7):1081-1088.

2. Schwab CJ, Jones LR, Morrison H, et al. Evaluation of multiplex ligation-dependent probe amplification as a method for the detection of copy number abnormalities in B-cell precursor acute lymphoblastic leukemia. *Genes Chromosomes Cancer*. 2010;49(12):1104-1113.

3. Busslinger M, Klix N, Pfeffer P, Graninger PG, Kozmik Z. Deregulation of PAX-5 by translocation of the Emu enhancer of the IgH locus adjacent to two alternative PAX-5 promoters in a diffuse large-cell lymphoma. *Proc Natl Acad Sci USA*. 1996;93(12):6129–6134.

4. Nebral K, Denk D, Attarbaschi A, et al. Incidence and diversity of PAX5 fusion genes in childhood acute lymphoblastic leukemia. *Leukemia*. 2009;23(1):134-143.