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

Claire Schwab,¹,* Karin Nebral,²,* Lucy Chilton,¹ Cristina Leschi,³,⁴ Esmé Waanders,⁵ Judith M. Boer,⁶ Markéta Žaliová,⁷,⁸ Rosemary Sutton,⁹ Ingegerd Ivanov Överholm,¹⁰ Kentaro Ohki,¹¹ Yuka Yamashita,¹² Stefanie Groenewold-Krentz,¹³ Eva Froňková,⁸ Marleen Bakus,¹⁴ Joelle Tchinda,¹⁵ Thayana da Conceição Barbosa,¹⁶ Grazia Fazio,¹⁷ Wojciech Miynarski,¹⁸ Agata Porębska,¹⁸ Giovanni Cazzaniga,¹⁷ Maria S. Pombo-de-Oliveira,¹⁶ Jan Trka,¹⁶ Renate Kirschner-Schwabe,¹³,¹⁹ Toshihiko Imamura,²⁰ Gisela Barbany,¹⁰ Martin Stanulla,²¹ Andishe Attarbaschi,²² Renate Panzer-Grümayer,² Roland P. Kuiper,⁵ Monique L. den Boer,⁶,²³ Hélène Cavé,³,⁴ Anthony V. Moorman,¹ Christine J. Harrison,¹,† and Sabine Strehl,²,† on behalf of the International Berlin-Frankfurt-Münster (I-BFM) Study Group

¹Leukaemia Research Cytogenetics Group, Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom; ²Children’s Cancer Research Institute, St Anna Kinderkrebsforschung, Vienna, Austria; ³INSERM Unité Mixte de Recherche 1131, Institut Universitaire d’Hématologie, Université Paris Diderot, Paris Sorbonne Cité, Paris, France; ⁴Département de Genétique, Hôpital Robert Debré, Assistance Publique–Hôpitaux de Paris, Paris, France; ⁵Department of Human Genetics, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands; ⁶Department of Pediatric Oncology, Erasmus MC, Rotterdam, The Netherlands; ⁷Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel, Germany; ⁸Pediatric Leukaemia Investigation Prague, Department of Pediatric Hematology/Oncology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic; ⁹Children’s Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, Australia; ¹⁰Clinical Genetics Section, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; ¹¹Department of Pediatric Hematology and Oncology, National Center for Child Health and Development, Tokyo, Japan; ¹²Clinical Research Center, Nagoya Medical Center, National Hospital Organization, Aichi, Japan; ¹³Division of Oncology and Hematology, Department of Pediatrics, Charité–Universitätsmedizin Berlin, Berlin, Germany; ¹⁴Department of Hematology, Universitair ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium; ¹⁵Pediatric Oncology, Children’s Research Centre, University Children’s Hospital Zürich, Zürich, Switzerland; ¹⁶Pediatric Hematology-Oncology Program, Research Center, Instituto Nacional de Cáncer, Rio de Janeiro, Brazil; ¹⁷Centro Ricerca Tettamanti, Clinica Pediatrica, Università di Milano-Bicocca, Monza, Italy; ¹⁸Department of Pediatrics, Oncology, Hematology and Diabetology, Medical University of Lodz, Lodz, Poland; ¹⁹German Cancer Consortium and German Cancer Research Center, Heidelberg, Germany; ²⁰Department of Pediatrics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan; ²¹Department of Pediatric Hematology and Oncology, Medical School Hannover, Hannover, Germany; ²²Department of Pediatric Hematology and Oncology, St Anna Children’s Hospital, Medical University of Vienna, Austria; and ²³Dutch Childhood Oncology Group, The Hague, The Netherlands

Key Points
- Intragenic PAX5 amplification defines a novel, relapse-prone subtype of B-cell precursor acute lymphoblastic leukemia with a poor outcome.

Introduction

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common childhood malignancy, characterized by a wide spectrum of genetic abnormalities, which are used in risk stratification for treatment.¹ PAX5 encodes a transcription factor, which plays a key role in B-cell commitment and maintenance² and is frequently (20% to 35%) deleted or mutated in BCP-ALL.³-⁵ Germline PAX5 mutations also occur in familial ALL.⁶,⁷ Furthermore, chromosomal rearrangements involving PAX5 result in the expression of potentially oncogenic PAX5 fusion genes.⁸-¹² Here we present a subset of patients with BCP-ALL lacking the major cytogenetic abnormalities (ETV6-RUNX1, BCR-ABL1, and TCF3-PBX1 fusions, high hyperdiploidy, near-haploidy, low hypodiploidy, MLL rearrangements, or intrachromosomal amplification of chromosome 21)¹ with intragenic amplifications of PAX5 (PAX5AMP).

Methods

Patients in this study originated from 15 international study groups. All participating centers obtained local ethical committee approval and written informed consent in accordance with the Declaration of Helsinki. Diagnosis of BCP-ALL was confirmed by immunophenotyping, according to standard criteria. Demographic and clinical details are summarized in supplemental Table 1.

The copy numbers of individual PAX5 exons were determined using the SALSA multiplex ligation-dependent probe amplification (MLPA) kit P335 IKZF1 (MRC Holland, Amsterdam, The Netherlands), as previously described (supplemental Methods).¹³-¹⁵ Thirteen PAX5AMP samples were processed on

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SNP6.0 or CytoScan HD arrays (Affymetrix, Santa Clara, CA; supplemental Methods). Fluorescence in situ hybridization (FISH), using PAX5 locus-specific probes, was carried out on 26 cases (supplemental Figures 1 and 2).

Survival analysis considered event-free survival, defined as time to relapse, and overall survival, defined as time to death, both censored at last contact. Very early relapse was defined as within 18 months of diagnosis, early relapse as >18 months and ≤6 months after the end of treatment, with late relapse defined as occurring >6 months posttreatment. Survival rates were calculated using the Kaplan-Meier method and compared using univariate Cox regression models. All analyses were performed using Intercooled Stata 14.0 (Stata, College Station, TX).

Results and discussion

PAX5<sup>AMP</sup> was identified in 79 patients with BCP-ALL, at diagnosis in 77 cases; only relapse material was available from 2 patients (Figures 1 and 2A). The amplified region encompassed exons 2 and 5.
Here we show that amplification of **PAX5** DNA-binding and octapeptide domains (Figure 1). The extent of **PAX5** copy-number changes was determined in 77 patients with intragenic amplification of **PAX5** identified at diagnosis. The copy numbers for each **PAX5** exon and the other genes targeted by the P335 **IKZF1** MLPA kit are shown (Figure 2B). Cytogenetic results were available for 57 patients, with abnormalities involving the short arm of chromosome 9, trisomy 5, and monosomy 7 being the most common recurrent chromosomal abnormalities. In patient 31, the probe ratio values for exons 2 and 5 were just below the cutoff of 2 for ≥4 copies; because the percentage of blast cells was low at 83.5%, this result was interpreted as amplification. In patient 69, MLPA showed that exon 2 had a ratio of 2.42 and exon 5 of 1.69; however on the single-nucleotide polymorphism array, exons 2 to 5 were amplified. **P2RY8-CRLF2** fusion assessed by MLPA, FISH, and/or reverse-transcriptase polymerase chain reaction. **PAX5** fusion, **PAX5** was mutually exclusive of other major risk-stratifying cytogenetic markers, including **IGH, PDGFRB/CSF1R, ABL1, ABL2, JAK2, and ZNF384** rearrangements, among 24 patient cases tested by FISH (data not shown).

Among the other genes assessed by MLPA, **CDKN2A/B** loss was the most common abnormality associated with **PAX5** (82%), higher than in other BCP-ALL subgroups. Gain of **EBF1** (26%), deletion of **IKZF1** (13%), and deletion of the PAR1 region resulting in **P2RY8-CRLF2** fusion (10%) were other common alterations, suggesting a collaborative role in **PAX5** leukemia development.

Consistent with the MLPA data, chromosomal abnormalities involving chromosome arm 9p (26%), trisomy 5 (23%), and monosomy 7 (12%) were observed among patients with successful karyotypes (n = 57; supplemental Table 3). Notably, trisomy 5 is a rare finding in BCP-ALL in the absence of high hyperdiploidy. Our previous study of trisomy 5 as the sole cytogenetic abnormality suggested an association with poor prognosis.

The main demographic and clinical features of the 77 patients with **PAX5** identified at diagnosis were male predominance (66%), age >10 years (25%), white blood cell count (WBC) ≥50 × 10^9 (39%), and National Cancer Institute high-risk status (55%). Minimal residual disease (MRD) data were available for 45 patients. Among ALL2003

### Table 1. Genetic features of patients with **PAX5**

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<tr>
<th>Exon</th>
<th><strong>PAX5</strong> Copy Number</th>
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**Figure 2. Genetic features of patients with **PAX5**.** (A) Data for the 77 patients with intragenic amplification of **PAX5** identified at diagnosis. The copy numbers for each **PAX5** exon and the other genes targeted by the P335 **IKZF1** MLPA kit are shown. Cytogenetic results were available for 57 patients, with abnormalities involving the short arm of chromosome 9, trisomy 5, and monosomy 7 being the most common recurrent chromosomal abnormalities. In patient 31, the probe ratio values for exons 2 and 5 were just below the cutoff of 2 for ≥4 copies; because the percentage of blast cells was low at 83.5%, this result was interpreted as amplification. In patient 69, MLPA showed that exon 2 had a ratio of 2.42 and exon 5 of 1.69; however on the single-nucleotide polymorphism array, exons 2 to 5 were amplified. (B) Data from 9 matched diagnosis-relapse pairs. *In patient 37, the difference in copy number of the amplified exons between diagnosis and relapse was due to reduced percentage of blasts at relapse. **P2RY8-CRLF2** fusion assessed by MLPA, FISH, and/or reverse-transcriptase polymerase chain reaction. ***Patient 16 presented with partial trisomy of chromosome 9 as a result of an unbalanced translocation involving chromosomes 1 and 5.

**Patients**
patients with evaluable MRD \((n = 8)\), 50% were positive at day 28 \((\approx 0.01\%\)) . Among patients treated in ALL-BFM 2000 with MRD data \((n = 14)\), 12 were classified as MRD intermediate risk and 1 each as high and low risk, whereas all European Organisation for Research and Treatment of Cancer patients \((n = 10)\) were intermediate risk, apart from 1 classified as high risk. From these limited data, we cannot assign an association between \(\text{PAX5}^{\text{AMP}}\) and MRD.

Among 74 patients with complete remission data available, 73 achieved complete remission by end of induction; 1 patient died before therapy. Relapse occurred in 40% \((29 \text{ of } 73)\) of these patients. The site of relapse, known for 22 patients, was isolated bone marrow \((n = 16)\), extramedullary \((n = 3)\), or combined relapse \((n = 3)\). The time to relapse \((median, 2.1 \text{ years})\) was known for 25 patients, with a ratio of very early to early to late relapse of 9:10:6, classifying 15 \((55\%)\) as high risk according to current criteria.\(^{20}\) Among patients experiencing relapse with sufficiently long follow-up, 17 \((59\%)\) died \((relapse, n = 9; \text{infection in remission, } n = 3; \text{unknown, } n = 5)\), and 10 remained alive \(>3\text{ years postrelapse}\).

The 5-year EFS and OS rates, evaluable for 74 patients, were 49\% \((95\% \text{ confidence interval [CI], 36\%–61}\%\)) and 67\% \((95\% \text{ CI, } 54\%–77\%)\), respectively. To identify risk factors, we examined the effects of age, WBC, National Cancer Institute status, year of diagnosis, and presence of additional genetic abnormalities, but only WBC was significant. Patients with a WBC \(>50 \times 10^9/\text{L}\) had a significantly increased risk of death \((\text{hazard ratio}, 3.48; 95\% \text{ CI, } 1.46–8.32; P = .005)\). In context, these low survival rates were generated from patients diagnosed over a 22-year period \((1993–2015)\), treated according to a wide range of trial protocols, highlighting the need for prospective studies.

The clinical, genetic, and outcome profiles of patients with \(\text{PAX5}^{\text{AMP}}\) were distinct from those harboring \(\text{PAX5}\) deletions, which occur at different incidences between BCP-ALL subgroups.\(^{3}\) Although present at an increased frequency in high-risk ALL, \(\text{PAX5}\) deletions are not associated with an inferior outcome.\(^{21,22}\) Because the number of patients with BCP-ALL with distinct \(\text{PAX5}\) fusions is limited, their prognostic relevance remains to be determined.

In conclusion, we have identified a rare subset of patients with BCP-ALL with \(\text{PAX5}^{\text{AMP}}\), who share a distinct spectrum of genetic abnormalities, including high frequencies of \(\text{CDKN2A/B}\) loss and trisomy 5. A majority of these patients lack established cytogenetic abnormalities, suggesting that \(\text{PAX5}^{\text{AMP}}\) may define a distinct subtype of BCP-ALL. Although several patients presented with \(\text{P2RY8-CRLF2}\) and 1 with \(\text{BCR-ABL1}\), both have been reported as secondary changes occurring alongside primary genetic abnormalities.\(^{3,23,24}\) Where matched diagnosis and relapse samples were available, the same amplification was present at both time points, indicating that \(\text{PAX5}^{\text{AMP}}\) may be an important driver of leukemogenesis. Because patients with \(\text{PAX5}^{\text{AMP}}\) showed a high incidence of relapse, we recommend testing for \(\text{PAX5}^{\text{AMP}}\) in future ALL trials to determine its true prognostic impact.

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Authorship

Contribution: C.S., K.N., S.S., and C.J.H. designed the study; C.S., L.C., K.N., S.S., C.J.H., and A.V.M. analyzed and interpreted data; the remaining authors provided genetic and clinical data; and all authors approved the final manuscript.

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The current affiliation for E.W. and R.P.K. is Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands.

A list of the members of the International Berlin-Frankfurt-Münster (I-BFM) Study Group appears on the I-BFM website \((https://bfminternational.wordpress.com/)\).

ORCID profiles: R.S., 0000-0002-0188-6005; K.O., 0000-0003-2838-4555; E.F., 0000-0002-6900-8145; J. Tschinda, 0000-0002-9450-2006; G.F., 0000-0001-7077-8422; G.C., 0000-0003-2955-4528; J. Trka, 0000-0002-9527-8608; H.C., 0000-0003-2840-1511; S.S., 0000-0002-0179-0628.

Correspondence: Sabine Strehl, Children’s Cancer Research Institute, St Anna Kinderkrebsforschung, Zimmermannplatz 10, 1090 Vienna, Austria; e-mail: sabine.strehl@ccri.at; and Christine J. Harrison, Leukaemia Research Cytogenetics Group, Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle University, Level 6, Herschel Building, Newcastle-upon-Tyne NE1 7RU, United Kingdom; e-mail: christine.harrison@newcastle.ac.uk.

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