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Letter to the Editor

Additional aberrations of the *ETV6* and *RUNX1* genes have no prognostic impact in 229 t(12;21)(p13;q22)-positive B-cell precursor acute lymphoblastic leukaemias treated according to the NOPHO-ALL-2000 protocol

The *ETV6*/*RUNX1* fusion, which is the molecular consequence of the cytogenetically cryptic t(12;21)(p13;q22), is present in approximately 25% of paediatric B-cell precursor acute lymphoblastic leukaemia (BCP ALL) cases in the Nordic countries. *ETV6*/*RUNX1*-positive BCP ALL is generally considered to have an excellent prognosis; however, reports of frequent late relapses as well as similar incidences of t(12;21) in newly diagnosed and relapsed cases have cast doubts on its favourable prognostic impact [1–3]. Furthermore, the clinical consequences of additional aberrations involving the *ETV6* and *RUNX1* genes, e.g., deletions of wild-type *ETV6* and trisomy 21, harbouring either a non-rearranged *RUNX1* gene or an additional *ETV6*/*RUNX1* chimaera, have also been debated. For example, 12p deletions, involving the non-rearranged *ETV6* allele, have been suggested to influence adversely the outcome [4]. In addition, Stams et al. reported that the subgroups with either an additional *ETV6*/*RUNX1* fusion gene, located on an extra der(21)t(12;21), or without any secondary aberration involving the *ETV6* and *RUNX1* genes did worse in terms of disease-free survival [5]. Furthermore, the frequencies of additional aberrations of these genes, with the exception of *ETV6* deletions, have been shown to be higher in relapsed than in diagnostic samples [6]. On the other hand, in a large study comprising 245 t(12;21)-positive cases, the presence of secondary aberrations of 12p and 21q did not predict a higher risk of relapse [7], and, recently, cases with *ETV6* deletions were actually shown to have a more favourable prognosis than cases without deletions [8]. In order to address this potentially clinically important issue we have ascertained and reviewed all *ETV6*/*RUNX1*-positive cases treated according to the NOPHO-ALL-2000 protocol.

All fluorescence *in situ* hybridisation (FISH)-verified *ETV6*/*RUNX1*-positive BCP ALL cases in children aged 1–15 years diagnosed in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) between January 2000 and December 2008 and treated according to the NOPHO-ALL-2000 protocol were included in the present study. Among the 1001 BCP ALL cases diagnosed during this time period and analysed as regards the presence of t(12;21) by FISH and/or reverse transcription-polymerase chain reaction analyses, the *ETV6*/*RUNX1* fusion was detected in 242 (24%) cases, of which 229 (95%) had been analysed by FISH. Only the latter are included in the present study because they are the ones informative regarding additional *ETV6* and/or *RUNX1* abnormalities. The FISH patterns ascertained were: (i) t(12;21) alone; (ii) loss of wild-type *ETV6* ("del(ETV6)"), (iii) additional *ETV6*/*RUNX1* fusion ("+der(21)t(12;21)"), (iv), gain of *RUNX1* ("+21"), and (v) combinations of del(ETV6), +21, and +der(21)t(12;21). In total, additional changes involving the *ETV6* and/or *RUNX1* genes were detected in 131 (57%) of the 229 cases, with deletion of *ETV6* being most common (38%). The majority (86%) harboured only one additional aberration, whereas 14% had two or more secondary changes. The incidence of secondary aberrations in the present series is lower than the approximately 75–80% reported in some previous studies [4,5,7]. Whether this difference is fortuitous or reflects technical and/or geographic differences is presently unknown.

The clinical characteristics at presentation, risk stratification, and probabilities of event-free and overall survival (pEFS and pOS) for the entire patient cohort ($n = 229$) as well as for the five different genetic subgroups are listed in Table 1. There were no significant differences among the subgroups as regards white blood cell counts, age, and risk stratification. A total of 29 events (26 relapses, two second malignant neoplasms, and one induction failure) occurred during the observation period, with the median time to relapse being 36 months (range 20–86 months). Among the cases that relapsed, 14 (54%) had additional *ETV6* and/or *RUNX1* aberrations at diagnosis, a proportion similar to the one for the whole cohort (57%) indicating that cases with such secondary aberrations are not more likely to relapse. Patients with trisomy 21 had the highest frequency (17%) of relapses; however, the distributions of genetic subgroups among the children that relapsed and the whole cohort did not differ significantly. Neither pEFS nor pOS varied significantly among the five genetic subgroups (Fig. 1 and Table 1). Thus, in agreement with the findings in another large series of t(12;21)-positive cases [7] we found no evidence for any

Table 1
Clinical features and risk groups of the 229 t(12;21)-positive BCP ALL cases.

Genetic subgroup	Median WBC × 10^9/l (range)	Median age (range)	Risk group (SR/IR/HR in %)	Relapses (%)	pEFS (SE)	pOS (SE)
t(12;21) only ($n = 98$; 43%)	8.0 (0.8–164)	4.8 (1.4–16)	38/39/23	12 (12)	0.80 (0.06)	0.89 (0.05)
del(ETV6) ($n = 69$; 30%)	5.0 (0.2–160)	3.6 (1.4–15)	42/42/16	6 (8.7)	0.89 (0.04)	0.97 (0.02)
+der(21)t(12;21) ($n = 26$; 11%)	5.2 (1.2–194)	5.0 (1.7–11)	54/27/19	3 (12)	0.86 (0.08)	1.0 (0.00)
+21 ($n = 18$; 7.9%)	4.9 (1.9–225)	3.9 (2.5–10)	50/34/16	3 (17)	0.81 (0.10)	0.88 (0.12)
Combinations ($n = 18$; 7.9%)	3.6 (1.1–100)	4.3 (1.8–11)	45/33/22	2 (11)	0.74 (0.15)	0.94 (0.05)
Whole cohort ($n = 229$; 100%)	5.5 (0.2–225)	4.7 (1.4–16)	42/38/20	26 (11)	0.82 (0.04)	0.93 (0.03)

BCP ALL, B-cell precursor acute lymphoblastic leukaemia; HR, high risk; IR, intermediate risk; pEFS, probability of event-free survival; pOS, probability of overall survival; SE, standard error; SR, standard risk; WBC, white blood cell count.

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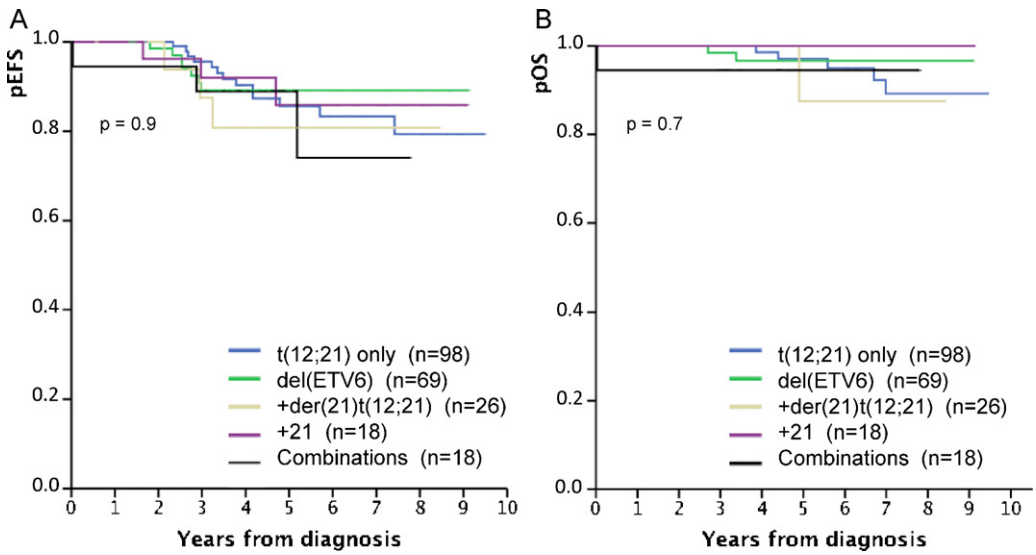

Fig. 1. (A) pEFS and (B) pOS at 9.5 years after diagnosis for the five different genetic subgroups (see Table 1) treated according to the NOPHO-ALL-2000 protocol. The pEFS and pOS were calculated using the Kaplan–Meier method, and the different genetic subgroups were compared using the log rank test. In the analysis of pEFS, events consisted of induction failure, relapse, death in complete remission 1 (CR1), and second malignant neoplasms, whereas in the analysis of pOS, death of any cause was the end point. The median observation times for patients in continuous CR1 and for surviving patients were 59 months (range 7–114 months) and 60 months (7–114), respectively. The date of last follow-up was January 31st, 2011. The SPSS software 19.0 for Windows (SPSS Inc., Chicago, IL) was used for all calculations.

prognostic impact of additional *ETV6* and/or *RUNX1* abnormalities in this cytogenetic BCP ALL subtype.

Conflicts of interest

The authors declare no potential conflicts of interest.

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Gisela Barbany*
Department of Molecular Medicine and Surgery, Karolinska Institutet, Sweden

Mette K. Andersen
Department of Clinical Genetics, Rigshospitalet, Denmark

Kirsti Autio
Georg Borgström
Department of Pathology and Clinical Genetics, Haartman Institute, University of Helsinki, Finland

Lucia Cavalier Franco
Department of Immunology, Genetics and Pathology, Clinical Genetics, Uppsala University, Sweden

Irina Golovleva
Department of Medical Biosciences, Medical and Clinical Genetics, University Hospital of Umeå, Sweden

Sverre Heim
Section for Cancer Cytogenetics, Institute for Medical Informatics, Radiumhospitalet, Oslo University Hospital, Oslo, Norway
University of Oslo, Oslo, Norway

Kristina Heinonen
Chromosome and DNA Laboratory, Kuopio University Hospital, Finland

Randi Hovland
Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Norway

Bertil Johansson
Department of Clinical Genetics, University and Regional Laboratories, Skåne University Hospital, Lund University, Lund, Sweden

Johann H. Johannsson
Department of Clinical Genetics and Cytogenetics, University Hospital, Reykjavik, Iceland

Eigil Kjeldsen
The Cancer Cytogenetic Laboratory, Department of Hematology, Århus University Hospital, Denmark

Ann Nordgren
Department of Molecular Medicine and Surgery, Karolinska Institutet, Sweden

Lars Palmqvist
Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Gothemburg, Sweden

Erik Forestier, on behalf of the Nordic Society of Paediatric Haematology and Oncology (NOPHO), the Swedish Cytogenetic Leukaemia Study Group (SCLSG) and the NOPHO Leukaemia Cytogenetic Study Group (NLCSG)
Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden

* Corresponding author at: Department of Molecular Medicine and Surgery, CMM, Karolinska Institutet, 17176 Stockholm, Sweden. Tel.: +46 8 51773788; fax: +46 8 327734.
E-mail address: gisela.barbany@ki.se (G. Barbany)

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