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Outcome of *ETV6/RUNX1*-positive childhood acute lymphoblastic leukaemia in the NOPHO-ALL-1992 protocol: frequent late relapses but good overall survival

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Summary

The prognostic impact of t(12;21)(p13;q22) [*ETV6/RUNX1* fusion] in paediatric acute lymphoblastic leukaemia (ALL) has been extensively debated, particularly with regard to the frequency of late relapses and appropriate treatment regimens. We have retrospectively collected 679 ALLs with known *ETV6/RUNX1* status, as ascertained by fluorescence *in situ* hybridization or reverse-transcription polymerase chain reaction, treated according to the Nordic Society of Paediatric Haematology and Oncology - ALL-1992 protocol. The assigned risk groups/treatment modalities for the 171 (25%) patients with t(12;21)-positive ALLs were 74 (43%) standard risk, 71 (42%) intermediate risk and 26 (15%) high risk. The 5- and 10-year event-free survival (EFS) of the 171 patients was 80% and 75% respectively, with no significant differences among the three risk groups. Most of the relapses occurred in boys and were late, with almost 50% of all relapses occurring ≥5 years after diagnosis. Of all relapses after 6 years, 80% occurred in the t(12;21)-positive group. The overall survival was 94% at 5 years and 88% at 10 years; thus, the treatment of patients in second or later remission is usually successful. As yet, there is no reliable plateau in the EFS curve, a fact that raises the question as to when the prognostic ramifications of ALLs harbouring *ETV6/RUNX1* should be evaluated.

Keywords: *ETV6/RUNX1*, t(12;21)(p13;q22), acute lymphoblastic leukaemia, childhood, prognosis, relapse.

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In the mid 1990s, the cytogenetically cryptic t(12;21)(p13;q22), resulting in an *ETV6/RUNX1* transcript, was found to be the most common translocation in childhood B-cell precursor acute lymphoblastic leukaemia (BCP ALL), occurring in approximately 25% of the cases (Romana *et al*, 1994, 1995a,b; Golub *et al*, 1995; Forestier *et al*, 2007). Although there are some chromosomal changes that strongly indicate the presence of t(12;21), for example del(12p) and trisomy 21 (Romana *et al*, 1994; Raynaud *et al*, 1999; Karrman *et al*, 2006; Forestier *et al*, 2007), the identification of this cytogenetic subgroup relies upon demonstrating the fusion of *ETV6* on 12p13 with *RUNX1* on 21q22 by the use of either fluorescence *in situ* hybridization (FISH) or reverse-transcription polymerase chain reaction (RT-PCR) analyses. The t(12;21)-positive cases constitute, together with high hyperdiploid (>50 chromosomes) ALLs, the majority of paediatric ALLs and also most of the incidence peak at 2–7 years (Forestier & Schmiegelow, 2006). Except for one T-ALL reported to harbour the *ETV6/RUNX1* chimaera (Ma *et al*, 2001), all published cases have had a BCP phenotype, often with a dim aberrant expression of the myeloid markers CD13 and CD33 (Baruchel *et al*, 1997; Borkhardt *et al*, 1997; Borowitz *et al*, 1998; De Zen *et al*, 2000; Rafi *et al*, 2000; Alessandri *et al*, 2002).

The prognostic implications of the *ETV6/RUNX1* fusion have been investigated and debated for more than a decade, with different groups coming to disparate conclusions with regard to outcome, frequency of late relapses, and proper treatment strategies. Shurtleff *et al* (1995) suggested that this ALL subgroup was characterized by an excellent prognosis, although the 5-year event-free survival (EFS) was not significantly better than for the t(12;21)-negative cases. Similar data indicating a favourable outcome for this patient cohort have since come from several retrospective studies (McLean *et al*, 1996; Borkhardt *et al*, 1997; Avigad *et al*, 1999; Maloney *et al*, 1999; Jamil *et al*, 2000; Uckun *et al*, 2001) as well as from a recent prospective study in which the survival rate was significantly better for BCP ALLs with *ETV6/RUNX1* than for those without this fusion (Loh *et al*, 2006). However, many investigators have not observed any statistically significant survival differences between t(12;21)-positive and -negative ALLs (Lanza *et al*, 1997; Takahashi *et al*, 1998; Kempski *et al*, 1999; Codrington *et al*, 2000; Hann *et al*, 2001; Hubeek *et al*, 2001; Pajor *et al*, 2001). Furthermore, frequent late relapses and similar incidences of the *ETV6/RUNX1* transcript in ALLs at diagnosis and relapse have been noted by some (Nakao *et al*, 1996; Harbott *et al*, 1997; Seeger *et al*, 1998, 1999; Tsang *et al*, 2001) but not by other authors (Loh *et al*, 1998; Rubnitz *et al*, 1999; Zuna *et al*, 1999). The fact that the *ETV6/RUNX1* fusion may persist for a long time after cessation of chemotherapy without heralding relapse and that the relapses occasionally are derived from an *ETV6/RUNX1*-positive clone other than the one present at diagnosis, as evidenced by different *ETV6* deletions and *IGH/TCR* rearrangements at diagnosis and relapse, strongly suggests that some late 'relapses' originate in lingering preleukaemic t(12;21)-positive clones, a finding which may explain why a sustained second remission often can be achieved and which should be taken into account in treatment decisions (Ford *et al*, 2001; Endo *et al*, 2003; Konrad *et al*, 2003; Peham *et al*, 2004; Zuna *et al*, 2004; Metzler *et al*, 2006).

There are probably several reasons for the above-mentioned discrepancies concerning outcome and risk of late relapses, including differences in patient accrual, number of cases investigated, follow-up time, and type of treatment given. As regards the latter, *in vitro* drug resistance analyses have revealed that t(12;21)-positive blasts are particularly sensitive to asparaginase, doxorubicin and etoposide but more resistant to vincristine and cytarabine (Ramakers-van Woerden *et al*, 2000; Frost *et al*, 2004). There are conflicting reports on the benefits of intensive *in vivo* therapy, with one study suggesting a crucial impact of upfront intensive treatment (Avigad *et al*, 1999) but another favouring low intensity antimetabolite-based therapy (Rubnitz *et al*, 1997). This notwithstanding, it is highly likely that EFS and overall survival (OS) are greatly influenced by the therapeutic approaches taken and that the risk of late relapses, whether they represent true relapses or second leukaemias originating in preleukaemic clones, is therapy-intensity dependent and/or associated with the duration of anti-metabolite maintenance therapy. To address these issues, we here report the outcome and frequency of relapses in t(12;21)-positive paediatric ALLs treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO)-ALL-1992 protocol.

Materials and methods

Patients and treatment protocol

Between 1 January, 1992 and 31 December, 2001, 1428 children without Down syndrome aged 1 to <15 years were diagnosed with BCP ALL in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) and treated according to the NOPHO-ALL-1992 protocol. This cohort includes all children with BCP ALL in that age group during the specified time period and is hence all-inclusive and truly population-based.

The NOPHO-ALL-1992 (described in detail by Gustafsson *et al*, 2000) treatment stratification for BCP ALL was mainly based on white blood cell (WBC) count, age, cytogenetic findings, extra-medullar leukaemia (EML) at diagnosis, and morphologically evaluated response to treatment on days 15 and 29. Three major risk groups were recognized, namely standard risk (SR), intermediate risk (IR) and high risk (HR). The criteria for SR were 2 to <10 years of age, WBC count $<10 \times 10^9$/l, no EML, and no cytogenetic HR features [t(4;11)(q21;q23) or t(9;22)(q34;q11)], whereas those for IR were 2 to <10 years of age and WBC count 10 to $<50 \times 10^9$/l or 1 to <2 years or ≥10–15 of age and WBC count $<50 \times 10^9$/l, no EML, and no cytogenetic HR features. The 1992 protocol distinguished three HR groups, which were

combined in the present analysis: HR-1: 1 to <5 years of age, WBC $>50 \times 10^9$/l, EML and/or cytogenetic HR features; HR-2: ≥5 years of age, WBC $>50 \times 10^9$/l, EML and/or cytogenetic HR features; and VHR (very HR): ≥5 years of age, WBC $>50 \times 10^9$/l, EML, lymphomatous leukaemia and/or cytogenetic HR features. HR also included cases with slow response (day 15 M3 (≥25% blasts) or day 29 M2 (5–24% blasts)/M3 bone marrow).

The main treatment features of the SR protocol was induction, consolidation and maintenance (total treatment time 2·5 years) and for the IR protocol induction, consolidation, delayed intensification and maintenance (treatment time 2 years). The treatment of the HR patients comprised induction, consolidation, delayed intensification, systemic central nervous system (CNS) therapy with alternating methotrexate and high-dose cytarabine (VHR patients also received prophylactic CNS radiation), and maintenance (LSA2L2 for VHR), with a total treatment time of 2 years.

Cytogenetic studies and inclusion criteria

Chromosome banding analyses were performed using standard methods in 15 cytogenetic laboratories in the Nordic countries. All abnormal karyotypes have been centrally reviewed annually since 1996 (Sweden)/2000 (all five Nordic countries). Screening for t(12;21), using FISH and/or RT-PCR, has been performed prospectively from 1996 in Sweden and from 2000 in the other Nordic countries; positive cases prior to this time period were identified in retrospective analyses.

For the purpose of this report, the following inclusion criteria were applied: (i) BCP immunophenotype, (ii) age ≥1 and <15 years, (iii) not Down syndrome and (iv) known *ETV6/RUNX1* status. A few ALLs not screened for the presence of t(12;21) were nevertheless included in the present study as negative for this translocation, namely those shown to be positive for *MLL* rearrangements, *BCR/ABL1* [t(9;22)(q34;q11)], or *TCF3/PBX1* [t(1;19)(q23;p13)]; these genetic changes are not present in *ETV6/RUNX1*-positive ALLs (reviewed in Forestier *et al*, 2007).

Statistical methods

The Statistical Package for the Social Sciences (SPSS) software 11.0 for Macintosh was used for the statistical analyses. The probability of EFS was calculated using the Kaplan–Meier method and the different subgroups were compared using the Log rank test. The significance limit for *P*-values was set to 0·05 in all tests. In the analysis of EFS, events comprised induction failures, death in remission, relapse and second malignancy. In the OS analysis, death was the endpoint. Patients in continuous first complete remission were followed between 49 and 173 months (median 91 months; 89 months for t(12;21)-negative and 93 months for t(12;21)-positive cases; last date of follow-up 19 April, 2007).

Results

Patients

Among the 1428 BCP ALLs, 679 (48%) had a known *ETV6/RUNX1* status (the vast majority of the other cases were in the retrospective cohort, i.e., were diagnosed prior to screening for the 12;21 translocation). There were no differences between these patients and the 749 (52%) that had not been tested for the presence of t(12;21) with regard to age, gender, WBC counts and frequency of EML (data not shown). Also the distributions of the three risk groups were similar in the two patient groups: 290 (38·7%) SR, 298 (39·8%) IR and 161 (21·5%) HR among the 749 cases not tested for t(12;21) and 259 (38·1%) SR, 282 (41·5%) IR and 138 (20·3%) HR for the 679 ALLs tested.

The number of patients in the different genetic subgroups as well as age, gender, WBC, EML, EFS and OS for the various groups in the 679 ALLs tested for the presence of t(12;21) are summarized in Table I.

Initial clinical features of the t(12;21)-positive cases

Among the 171 (25·2%) cases with t(12;21), the median age was 4·2 years, the median WBC count 9·2, and none had EML (Table I). Thus, the vast majority were stratified as SR or IR (Table II). Of the HR patients, all but two (see below) had high WBC counts as the reason for the risk stratification.

Early response

Morphological bone marrow examination at day 29 showed that 2 (1·3%) of the 160 t(12;21)-positive cases evaluated in this regard had M2/M3 marrows; these two patients were stratified into the HR group (see above). Among the t(12;21)-negative cases, 22/466 (4·7%) displayed M2/M3 marrows at day 29.

EFS of t(12;21)-positive cases

The EFS for the 171 patients with t(12;21)-positive ALL was 80% at 5 years and 75% at 10 years (Table I). Thus, the EFS continued to decrease also after 5 years in this patient cohort, in contrast to most other genetic subgroups, in which the EFS was similar at 5 and 10 years (Table I). In fact, no clear-cut plateau could be discerned in the t(12;21)-positive group during the first 11 years (Fig 1A). There was no significant difference in EFS at 10 years among the three risk groups: 0·77 [standard error (SE) 0·05] for SR, 0·74 (SE 0·06) for IR and 0·69 (SE 0·09) for HR ($P = 0·4$).

The EFS values at 5 and 10 years for the t(12;21)-positive cases did not differ significantly from that of all other ALLs combined: 0·80 vs. 0·76 ($P = 0·11$) and 0·75 vs. 0·74 ($P = 0·17$) respectively. A comparison was also made between t(12;21)-positive cases and BCP ALLs without abnormalities of

Table I. Clinical and genetic features of the 679 paediatric B-cell precursor acute lymphoblastic leukaemias tested for the presence of t(12;21).

Genetic subgroup	*n* (%)	Sex ratio (m/f)	Median age (range)	Median WBC (range)	EML (M/T/C)	5-year EFS (SE)	10-year EFS (SE)	10-year OS (SE)
t(12;21)(p13;q22)	171 (25·2)	1·3	4·2 (1·2–14)	9·2 (0·8–163)	0/0/0	0·80 (0·03)	0·75 (0·04)	0·88 (0·03)
51–61 chromosomes*	168 (24·7)	1·0	3·9 (1·1–15)	6·2 (0·5–169)	1/1/1	0·83 (0·03)	0·81 (0·03)	0·89 (0·03)
Normal	104 (15·3)	1·2	5·1 (1·2–15)	5·8 (0·5–167)	0/0/0	0·78 (0·04)	0·78 (0·04)	0·89 (0·03)
45–50 chromosomes*	89 (13·1)	0·9	5·5 (1·1–15)	14 (0·8–600)	0/2/3	0·65 (0·05)	0·65 (0·05)	0·78 (0·05)
No genetic result	61 (9·0)	1·8	4·0 (1·2–14)	7·8 (0·7–296)	0/0/3	0·85 (0·05)	0·82 (0·05)	0·89 (0·04)
t(9;22)(q34;q11)	32 (4·7)	1·1	7·0 (1·4–15)	44 (1·3–570)	0/0/2	0·44 (0·09)	0·40 (0·09)	0·53 (0·10)
t(1;19)(q23;p13)	21 (3·1)	0·7	6·2 (1·3–14)	15 (1·6–113)	0/0/0	0·81 (0·09)	0·81 (0·09)	0·88 (0·08)
>61 chromosomes*	19 (2·8)	1·1	4·3 (1·7–14)	5·7 (0·9–40)	0/0/0	0·84 (0·08)	0·78 (0·10)	0·84 (0·09)
der(11q23)/*MLL*	10 (1·5)	1·0	2·3 (1·1–3·9)	21 (2·2–149)	0/0/0	0·70 (0·14)	0·70 (0·14)	0·80 (0·13)
<45 chromosomes*	4 (0·6)	0 m, 4 f	5·8 (2·6–13)	19 (12–41)	0/0/0	0·50 (0·25)	NA	NA
Total	679 (100)	1·1	4·3 (1·1–15)	8·6 (0·5–600)	1/3/9	0·77 (0·02)	0·74 (0·02)	0·85 (0·02)

C, central nervous system involvement; EFS, event-free survival; EML, extra-medullar leukaemia; f, female; m, male; M, mediastinal lymph nodes (>3 cm); NA, not analysed (no case followed for more than 90 months); OS, overall survival; SE, standard error; T, testicular involvement; WBC, white blood cell count.

*Cases with t(1;19), t(9;22), der(11q23), or t(12;21) are not included in these groups.

Table II. Risk group stratification among the 171 paediatric t(12;21)-positive B-cell precursor acute lymphoblastic leukaemias.

Risk group	No. of cases (%)
Standard risk	74 (43·3)
Intermediate risk	71 (41·5)
High risk	26 (15·2)

well-known prognostic importance, i.e., those with a normal karyotype, no genetic results and cases without t(1;19), t(9;22), der(11)(q23), <45 chromosomes, and 51–61 chromosomes. In this analysis, the presence of t(12;21) was a good prognostic factor only in the HR group (EFS at 5 years was 0·69 and 0·47 respectively; $P = 0{\cdot}008$).

Relapses in t(12;21)-positive cases

Of the 137 relapses among the 679 cases tested for t(12;21), 35 (26%) occurred in the t(12;21)-positive group. As seen in Fig 2, approximately half of the relapses in this group occurred ≥5 years after diagnosis, when compared with <20% of the relapses in the t(12;21)-negative group. In fact, 43% of all relapses after 5 years and 80% of those after 6 years were t(12;21)-positive. Boys were over-represented among the relapse cases, with a male/female ratio of 1·9 in t(12;21)-positive cases and 1·5 in the negative cases ($P < 0{\cdot}01$). The relapse sites of the t(12;21)-positive and -negative cases are given in Table III.

OS for t(12;21)-positive cases

The OS for the 171 patients with t(12;21)-positive ALL was 0·94 (SE 0·02) at 5 years and 0·88 (SE 0·03) at 10 years (Table I and Fig 1B). Fifteen of the 171 children have died, 10 (66%) from leukaemia and five either during induction ($n = 1$) or in first ($n = 3$) or second ($n = 1$) complete remission. A significant difference ($P = 0{\cdot}04$) in OS at 5 years between t(12;21)-positive and -negative patients was only found between those allocated to HR treatment: 0·78 vs. 0·65.

Discussion

The most important findings in the present Nordic series of paediatric BCP ALLs treated according to the NOPHO-ALL-1992 protocol are that the presence of t(12;21) is strongly associated with favourable risk factors, that late relapses are common, yielding an EFS curve without a reliable plateau, that boys seem to be over-represented in the relapse group, and that the OS, although good, is not significantly better than for non-t(12;21) patients, except for those treated as HR.

The incidence (25%), male/female ratio (1·3), median age (4·2 years), median WBC count ($9{\cdot}2 \times 10^9$/l), and the lack of EML at diagnosis agree very well with previous larger series of t(12;21)-positive BCP ALLs (Borkhardt *et al*, 1997; Raynaud *et al*, 1999; Attarbaschi *et al*, 2004; Loh *et al*, 2006). Thus, the initial clinical characteristics of the patient cohort presented herein are typical for this genetic subtype, showing that there is no inclusion bias, as should be expected from a population-based series. Considering the favourable risk factors – the vast majority of the patients were treated either as SR or IR (Table II) – the high frequency of relapses and hence the steadily sloping EFS curve (Fig 1A), were disappointing, although not unexpected against the background of the findings found in previous studies of ALLs with t(12;21).

A relatively high frequency of late relapses, i.e., off therapy, has been emphasized by several groups (Nakao *et al*, 1996; Harbott *et al*, 1997; Seeger *et al*, 1998, 1999; Takahashi *et al*,

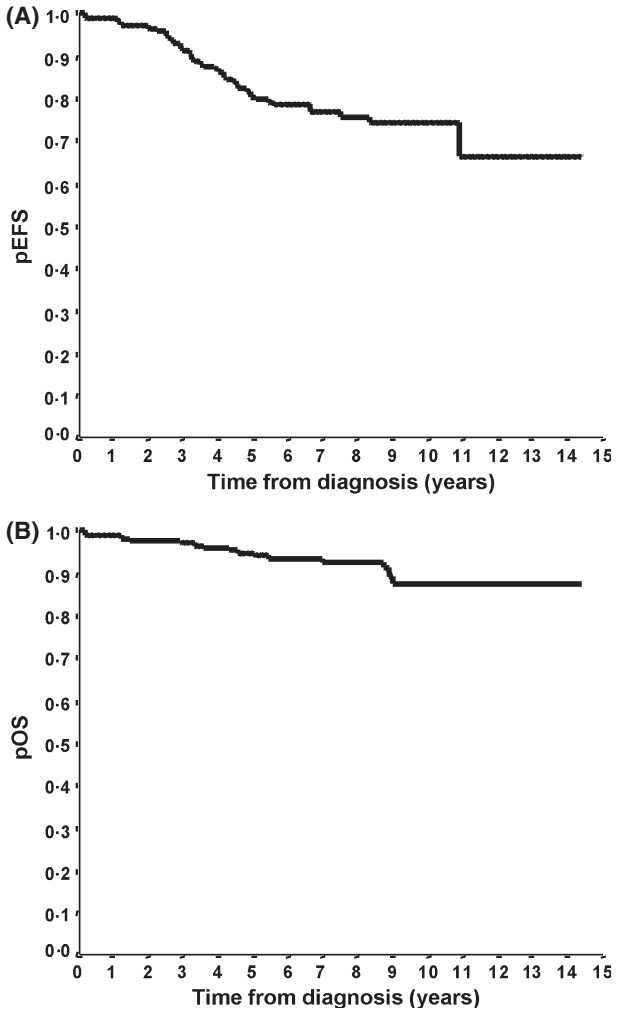

Fig 1. (A) Predicted event-free survival (pEFS) and (B) predicted overall survival (pOS) for 171 children with *ETV6/RUNX1*-positive B-cell precursor acute lymphoblastic leukaemia.

Table III. Relapse sites in t(12;21)-negative and -positive paediatric acute lymphoblastic leukaemias.

t(12;21) status	Site	No.	%	Cumulative (%)
Negative (n = 102)	BM	73	71·6	71·6
	CNS	11	10·8	82·4
	BM + CNS	9	8·8	91·2
	Testis	5	4·9	96·1
	BM + other site	4	3·9	100
Positive (n = 35)	BM	18	51·4	51·4
	BM + CNS	5	14·3	65·7
	BM + testis	5	14·3	80·0
	CNS	4	11·4	91·4
	Testis	2	5·7	97·1
	CNS + testis	1	2·9	100

BM, bone marrow; CNS, central nervous system.

1998; Avigad *et al*, 1999; Codrington *et al*, 2000; Tsang *et al*, 2001). In the present series, almost 50% of all relapses after 5 years and 80% of those after 6 years occurred in t(12;21)-positive patients. Interestingly, in several of these studies BFM/BFM-like treatment protocols were used, and the NOPHO-ALL-1992 is quite similar, with only a short period of asparaginase therapy. On the other hand, the protocols in the studies by Loh *et al* (1998, 2006) and Rubnitz *et al* (1999), who did not find frequent t(12;21)-positive relapses, are different in that they involve an extended use of this drug. Based on this, and also on *in vitro* studies demonstrating that blasts with *ETV6/RUNX1* are highly sensitive to asparaginase and doxorubicin (Ramakers-van Woerden *et al*, 2000; Frost *et al*, 2004), we would argue that treatment of this ALL subgroup should include long and effective asparagine depletion.

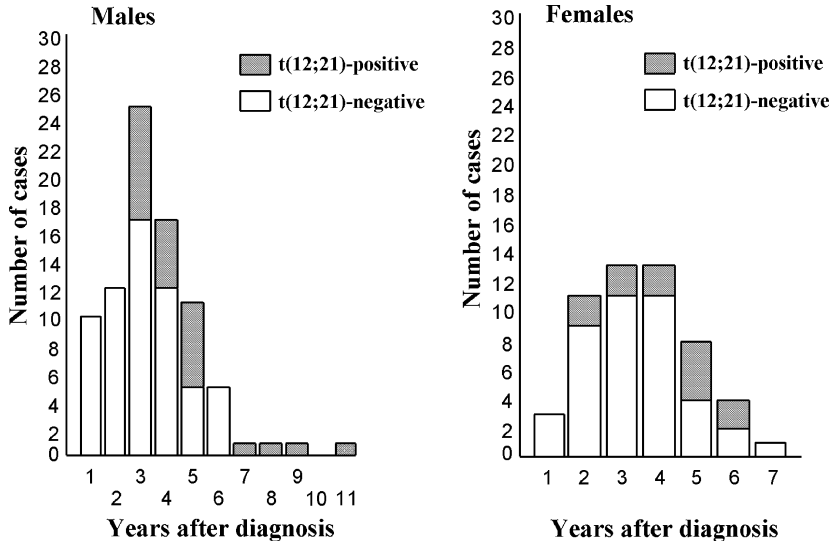

Fig 2. Number of relapses in t(12;21)-positive and negative paediatric B-cell precursor acute lymphoblastic leukaemias according to years postdiagnosis and gender.

The present finding of a male predominance among the relapses, particularly the late ones (Fig 2), has not been stressed previously, although a male/female ratio of >2 was reported by Seeger *et al* (1998). Most studies on the prognostic implications of t(12;21) have not reported individual data on gender and time in remission. However, the information that can be retrieved from studies providing pertinent data (Nakao *et al*, 1996; Harbott *et al*, 1997; Lanza *et al*, 1997; Satake *et al*, 1997; Loh *et al*, 1998; Rubnitz *et al*, 1999; Codrington *et al*, 2000; de Haas *et al*, 2000; Seeger *et al*, 2001; Tsang *et al*, 2001; Alvarez *et al*, 2005; Al-Sweedan *et al*, 2007) does not support that boys generally relapse later than girls; based on all studies referred to above, the median remission duration before relapse was 37 months (range 17–102) for boys and 32 months (range 11–109) for girls. Thus, our finding may well be fortuitous and should be interpreted with caution.

The fact that there is no plateau in the EFS curve during the first 11 years (Fig 1A), has two important clinical ramifications. First, it would seem prudent to follow patients with t(12;21)-positive ALLs for a longer time than those with other genetic ALL subtypes. Secondly, the lack of a safe plateau makes it difficult, maybe impossible; to decide when the prognostic impact *ETV6/RUNX1* in ALLs should be evaluated. In spite of the falling EFS curve, however, the OS is good, being 94% at 5 years and 88% at 10 years, similar to the 97% OS at 5 years reported in the prospective study by Loh *et al* (2006). Thus, the treatment of most of the patients in second or later remission was successful in the NOPHO-ALL-1992 protocol. This good response fits well with the hypothesis that many 'late relapses' in fact are new t(12;21)-positive ALLs originating in preleukaemic clones harbouring the *ETV6/RUNX1* fusions (Ford *et al*, 2001; Konrad *et al*, 2003; Peham *et al*, 2004; Zuna *et al*, 2004; Metzler *et al*, 2006).

In conclusion, t(12;21)-positive ALL paediatric patients in the Nordic countries, comprising 25% of all childhood BCP ALLs, has, despite frequent late relapses, a good overall survival. Whether increased use of asparaginase and/or doxorubicin in future treatment protocols will decrease the frequency of relapses, especially the late ones, and increase the OS further, remains to be seen.

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