Prospective analysis of *TEL/AML1*-positive patients treated on Dana-Farber Cancer Institute Consortium Protocol 95-01

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In a retrospective analysis, we previously reported that children whose leukemia cells harbored the *TEL/AML1* gene rearrangement have excellent outcomes. From 1996 to 2000, we conducted a prospective study to determine the incidence and outcomes of children with *TEL/AML1*-positive acute lymphoblastic leukemia (ALL). Children with newly diagnosed ALL were treated on DFCI ALL Consortium Protocol 95-01. Patients were risk stratified primarily by current National Cancer Institute (NCI)–Rome risk criteria. With a median follow-up of 5.2 years, the 5-year event-free survival for *TEL/AML1*-positive patients was 89% compared with 80% for *TEL/AML1*-negative B-precursor patients ($P = .05$). The 5-year overall survival rate was 97% among *TEL/AML*-positive patients compared with 89% among *TEL/AML1*-negative patients ($P = .03$). However, in a multivariable analysis, risk group (age and leukocyte count at diagnosis) and asparaginase treatment group, but not *TEL/AML1* status, were found to be independent predictors of outcome. We conclude that *TEL/AML1*-positive patients have excellent outcomes, confirming our previous findings. However, factors such as age at diagnosis and presenting leukocyte count should be taken into consideration when treating this group of patients. (Blood. 2006;107:4508-4513)

Introduction

The *TEL/AML1* gene rearrangement results from a balanced, reciprocal, t(12;21)(p12;q22) and occurs in 25% of children with B-lineage acute lymphoblastic leukemia (ALL).[1,2] Current research focuses on determining the biologic function of the chimeric fusion protein, the cooperating events needed for leukemia development, the prognostic significance of this fusion gene, and the optimal therapy for these patients.

The *TEL* gene (also known as *ETV6*), first cloned in 1994 as a novel fusion partner to the *PDGFRβ* receptor in a patient with chronic myelomonocytic leukemia,[3] is a member of the ETS family of transcription factors whose protein product is a nuclear phosphoprotein.[4] Tel deficiency in mice results in early embryonic lethality in part caused by defective yolk sac angiogenesis and an inability to establish bone marrow hematopoeisis.[5] The *TEL* gene was subsequently described to partner with *RUNX1* (also known as *CBFA2* or *AML1*) in pediatric B-precursor cell ALL (Figure 1).[6,7]

One of the most important predictors of failure in current risk models of ALL is the therapy received.[8] Identifying cohorts of patients who can be treated with specific combinations of therapy has been one of the key goals of current clinical trials. The detection of recurrent somatic cytogenetic abnormalities has allowed investigators to more accurately predict the outcome of patients sharing these events. For instance, it has been well established that ALL patients with the *BCR/ABL* gene rearrangement have a poor prognosis with standard ALL therapy and often require allogeneic transplantation for optimal treatment.[9]

Several retrospective studies have divergent results with respect to the prognostic significance of the *TEL/AML1* fusion gene.[10] We previously reported that a subset of 22 *TEL/AML1*-positive patients treated on DFCI ALL Consortium protocols were 100% free of relapse at a median length of follow-up of 8.3 years.[11] Investigators from St Jude Children's Research Hospital (St Jude) also reported an exceptionally favorable outcome for pediatric patients with *TEL/AML1*.[1]

However, subsequent reports noted that as many as 25% of children on Berlin-Frankfurt-Münster (BFM) protocols who had relapses were *TEL/AML1*-positive.[12,13] In addition, another cooperative group could not confirm that the *TEL/AML1* fusion gene conferred a favorable prognosis.[14] Such differences in reported outcome may be explained by risk stratification and subsequent

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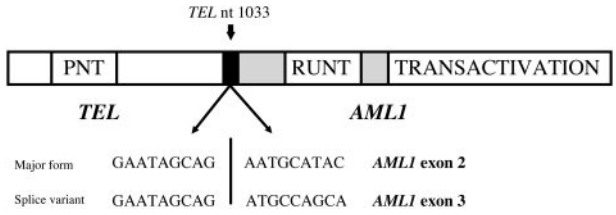

Figure 1. Schematic representation of the DNA sequence of the *TEL/AML1* fusion genes detected in this assay. The most common form fuses exon 5 of *TEL (ETV6)* with exon 1 of *AML1* (*RUNX1).* A frequent splice variant fuses exon 5 of *TEL (ETV6)* with exon 2 of *AML1* (*RUNX1),* and both were detected in this assay.

therapy received, and they indicated that a prospective analysis of outcome was warranted.

To address potential selection biases from previous retrospective reports, we conducted a prospective study on the prognostic significance of the *TEL/AML1* fusion gene in children enrolled on Dana-Farber Cancer Institute ALL Consortium Protocol 95-01, a phase 3 clinical trial that accrued patients with newly diagnosed ALL. Protocol 95-01 stratified patients according to age and presenting white blood cell count using current National Cancer Institute (NCI)–Rome criteria, thereby eliminating risk stratification differences with many other cooperative groups.

Patients, materials, and methods

Patients and leukemia samples

Diagnostic bone marrow and peripheral blood samples were collected prospectively from patients eligible to participate on DFCI ALL Consortium Protocol 95-01 between January 1996 and September 2000. Four hundred ninety-one patients enrolled on DFCI 95-01. For the purposes of this analysis, any bone marrow or peripheral blood sample falling within 5 days of the date of registration on study was considered diagnostic. Bone marrow or peripheral blood samples at diagnosis were obtained from 396 (81%) patients. Of the 396 patients, 352 had B-precursor ALL and 44 had T-cell ALL. Informed consent was obtained according to the Declaration of Helsinki. Approval for laboratory studies performed on Protocol 95-01 was obtained from the Committees on Human Research at the Dana-Farber Cancer Institute and the University of California, San Francisco.

The 9 consortium institutions were Children's Hospital, Boston and the Dana-Farber Cancer Institute (Boston, MA), Le Centre Hospitalier de l'Universite Laval (Quebec, Quebec), McMaster University (Hamilton, Ontario, Canada), Maine Children's Cancer Center, (Portland, ME), Mt Sinai Hospital (New York, NY), Ochsner Health Clinic (New Orleans, LA), Ste Justine Hospital (Montreal, Quebec), San Jorge Children's Hospital (San Juan, Puerto Rico), and University of Rochester (Rochester, NY). The institutional review boards of each participating institution approved the protocol before patient enrollment.

Therapy

Risk group stratification. Children at standard risk (SR) ranged in age from 1 to younger than 10 years of age at the time of diagnosis and had presenting white blood cell (WBC) counts of less than 50×10^9/L and no CNS 2 (less than 5/μL WBCs but cytospin-positive for blasts) or CNS 3 more than 5/μL WBCs and cytospin-positive for blasts or any cranial nerve palsy disease. In addition, SR patients could not have a T-cell immunophenotype, anterior mediastinal mass, or presence of the Philadelphia chromosome. Patients with mature B-cell leukemia were excluded from this trial. All other patients were deemed at high risk (HR). Infants (younger than 12 months) were considered at HR but were treated with an additional cycle of postremission consolidation, including high-dose methotrexate and high-dose cytarabine.

Systemic therapy. Types of therapy are summarized in Table 1. Chemotherapy was continued for a total of 24 months from the date of complete clinical remission. Patients could participate in 2 randomizations related to systemic therapy: (1) a comparison of *Escherichia coli* and *Erwinia* asparaginase during induction and postremission intensification (all patients) and (2) doxorubicin delivered with or without dexrazoxane (HR patients only). The asparaginase randomization closed on December 12, 1998, because accrual goals were met. All subsequent patients received *E coli* asparaginase.

Laboratory methods

After bone marrow or peripheral blood was collected, mononuclear cells were collected after density gradient centrifugation using Hypaque-Ficoll according to standard methods. A minimum of 5 million cells was placed directly into RNA-Stat 60 (January 1996 to November 1997) or into RNeasy (Qiagen, Valencia, CA) or RNAqueous buffer (November 1997 to 2001) (Ambion, Austin, TX). Total RNA was extracted according to the specific protocol and subjected to DNAse with DNAse I (Ambion) followed by inactivation by DNAse Inactivator (Ambion). Total RNA was reverse transcribed into cDNA using standard methods. An aliquot of cDNA was then used in duplicate control polymerase chain reaction (PCR) to verify the integrity of the RNA sample using *TEL*-specific primers 458 and 750R and were visualized on a 2.5% agarose gels, as previously described.[11,15] Only duplicate-positive samples for *TEL* were analyzed for the presence of the *TEL/AML1* fusion gene.

Amplification of the *TEL/AML1* fusion gene was performed using *TEL* forward primer 958 5′CTGGCTTACATGAACCACATCA3′ and *TEL/AML1* reverse primer 1058R 5′CGGCTCGTGCTGGCA3′. The 25-μL PCR reaction mixture included 2 μL cDNA, 10 × buffer (Applied Biosystems, Foster City, CA), 2.5 mM MgCl, 250 nM dNTPs (Boehringer Mannheim), 1000 nM primers, 0.75 U Amplitaq Gold (Applied Biosystems), and DEPC H_2O (Ambion). After an initial denaturing step at 95° for 10 minutes, a 2-step PCR (95° for 15 seconds followed by 60° for 1 minute) was performed for 40 cycles to amplify either a 127-bp product or a 93-bp product representing the most common splice variant. Products were visualized on agarose gels.

Statistical methods

χ^2 analysis or, where cell frequencies were small, Fisher exact tests were used to compare categorical variables between groups. Clinical and laboratory characteristics were categorized for analyses as shown in Table 2. Event-free survival (EFS) was measured from date of complete remission at the end of induction therapy to the first of any event, including induction failure, relapse, death from any cause, or censored at last contact; induction failure and induction death were considered events at time zero. Overall survival was defined as the time from randomization to death or censored at last contact. Survival distributions were estimated using the Kaplan-Meier method, and univariate associations were tested using log-rank tests. Cox proportional hazards regression was used to model the survival data on *TEL/AML1* status while adjusting for known prognostic factors and treatment. Manual stepwise model selection was performed with likelihood ratio tests using the variables in Table 2; all 2-way interactions between variables in the final model were examined separately. Given that WBC count and age were found to be highly collinear with risk group, the final multivariable modeling used risk group and excluded WBC and age. All reported *P* values were from 2-sided tests, with 5% type 1 error rates. No adjustments were made for multiple comparisons. Analyses of the data set were current as of December 2004.

Table 1. Therapy on DFCI ALL Consortium Protocol 95-01

Type of therapy, duration, and regimen
Induction therapy, 4 wk
Vincristine 1.5 mg/m^2 intravenously every week for 4 weeks
Prednisone 40 mg/m^2 by mouth every day for 28 days
Doxorubicin 30 mg/m^2 intravenously on days 0 and 1*
Methotrexate 4 g/m^2 intravenously on day 2
Leucovorin 200 mg/m^2 intravenous push hour 36, then 24 mg/m^2 intravenously every 6 hours until methotrexate level < 0.1 μM
Asparaginase 25 000 IU/m^2 intramuscularly on day 4†
IT-Ara-C at diagnosis, dosed by age‡
ITT on day 16 and 30, dosed by age§
CNS treatment, 3 wk
Vincristine 2 mg/m^2 intravenously for 1 dose
6-mercaptopurine 50 mg/m^2 by mouth for 14 days
Doxorubicin 30 mg/m^2 intravenously for 1 dose (HR only)*
SR: 1800 cGy cranial x-ray therapy (XRT) with IT every 18 weeks vs no XRT and intensive ITT every 9 weeks for 6 doses, then every 18 weeks
HR: IT every 18 weeks with 1800 cGy cranial XRT
Intensification therapy, 5-6 mo
Vincristine 2 mg/m^2 intravenously every 3 weeks
Prednisone 40 mg/m^2 by mouth for 5 days every 3 weeks (SR)
Prednisone 120 mg/m^2 by mouth for 5 days every 3 weeks (HR)
6-mercaptopurine 50 mg/m^2 by mouth for 14 days every 3 weeks
Asparaginase every week for 20 doses†
Methotrexate 30 mg/m^2 intramuscularly every week (SR only)
Doxorubicin 30 mg/m^2 intravenously every 3 weeks (HR only)*
Continuation therapy, 2 y of CCR
Vincristine as intensification
Prednisone as intensification
6-mercaptopurine as intensification
Methotrexate as intensification

Infants were treated as HR patients but received additional chemotherapy, including high-dose methotrexate and high-dose Ara-C.

*HR patients: doxorubicin given with or without dexrazoxane 300 mg/m^2. Total cumulative dose of doxorubicin was 300 mg/m^2 for HR and 60 mg/m^2 for SR patients.

†Asparaginase type randomized: *E coli* or *Erwinia* each at 25 000 IU/m^2 intramuscularly.

‡IT-Ara-C dosed by age: younger than 1 year, 15 mg; 1-1.99 years, 20 mg; 2-2.99 years, 30 mg; older than 3 years, 40 mg.

§ITT: triple intrathecal chemotherapy dosed per age: Ara-C dosing above, younger than 1 year, methotrexate 6 mg and hydrocortisone 6 mg; 1-1.99 years, methotrexate 8 mg and hydrocortisone 9 mg; 2-2.99 years, methotrexate 10 mg and hydrocortisone 12 mg; older than 3 years, methotrexate 12 mg and hydrocortisone 15 mg.

Results

Of the 491 eligible patients enrolled on Protocol 95-01, 396 (81%) had a diagnostic peripheral blood or a bone marrow sample obtained for *TEL/AML1* analysis. There was no difference in age, sex, risk group, immunophenotype, CNS involvement, or asparaginase treatment between patients whose diagnostic samples were submitted compared with those whose were not, though patients presenting with a higher leukocyte count (50 000/mm^3 or higher) were more likely to have a diagnostic sample submitted than patients with a lower leukocyte count (less than 50 000 mm^3) ($P = .002$). In addition, Hispanic patients were less likely to have submitted a diagnostic sample ($P = .01$) for unclear reasons (69% sample acquisition rate for Hispanic patients was similar at multiple consortium sites).

Reverse transcription–polymerase chain reaction (RT-PCR) analysis for the *TEL/AML1* fusion was performed successfully in 341 patients (86% of available samples), of whom 299 had B-precursor ALL. Seventy-seven (26%) of 299 B-precursor ALL patients were *TEL/AML1*-positive. None of the 42 patients with T-cell leukemia were *TEL/AML1*-positive. There were no significant differences between the clinical characteristics of patients who had successful PCR and those who did not. Because none of the T-cell patients were *TEL/AML1*-positive, all analyses of the association of *TEL/AML1* status with survival end points included only the 299 B-precursor patients with informative *TEL/AML1* results. Table 2 outlines the clinical and laboratory characteristics for the B-precursor *TEL/AML1*-positive and -negative patients at diagnosis. As shown, *TEL/AML1*-positive and -negative patients did not differ significantly with respect to any of these variables, with the exception of age ($P = .05$); *TEL/AML1*-positive patients were more likely to be between 1 and 10 years of age.

Follow-up remission data were lacking for 2 *TEL/AML1*-negative patients, and they were excluded from all EFS analyses. With a median follow-up of 5.2 years, 5-year EFS ($\pm$ SE) for *TEL/AML1*-positive patients was 0.89 ($\pm$ 0.04) compared with 0.80 ($\pm$ 0.03) for *TEL/AML1*-negative B-precursor patients ($P = .05$; Figure 2A), indicating a marginally significant association of *TEL/AML1* status with EFS. *TEL/AML1* status was not associated with EFS within each risk group ($P = .14$ for high risk and $P = .28$ for standard risk). When infants (all of whom were *TEL/AML1*-negative) were excluded from the analysis, the trend toward inferior EFS in *TEL/AML1*-negative patients was still observed (5-year EFS, 0.82), though the difference in EFS between *TEL/AML1*-negative and -positive patients did not reach the level of statistical significance ($P = .09$).

The median relapse time among the 7 *TEL/AML1*-positive patients who had relapses was 42.8 months (range, 27-53.6 months), compared with 28.8 months (range, 5-64.2 months) among the 37 *TEL/AML1*-negative B-precursor ALL patients who

Table 2. Presenting clinical features of B-precursor patients enrolled on DFCI 95-01 with *TEL/AML1* status at diagnosis

Presenting feature	*TEL/AML1* diagnostic status		χ^2 *P*
	Negative, no. (%)	Positive, no. (%)	
Age			.05
0 y to less than 1 y	10 (5)	0 (0)	
1 y to less than 10 y	174 (78)	69 (90)	
More than 10 y	38 (17)	8 (10)	
Sex			.65
Male	119 (54)	39 (51)	
Female	103 (46)	38 (49)	
Risk			.23
Standard	127 (57)	50 (65)	
High	95 (43)	27 (35)	
WBC count			.08
Less than 50 × 10^9/L	186 (84)	58 (75)	
More than 50 × 10^9/L	35 (16)	19 (25)	
Unknown	1 (0)	0 (0)	
CNS disease			.57
No	179 (81)	66 (86)	
Yes	30 (14)	7 (9)	
Traumatic	10 (4)	3 (4)	
Unknown	3 (1)	1 (1)	
Ethnicity			.75*
White	179 (81)	66 (86)	
Black	10 (4)	3 (4)	
Hispanic	24 (11)	7 (9)	
Other	9 (4)	1 (1)	
Asparaginase treatment			.14
Randomized *E coli*	59 (27)	26 (34)	
Randomized *Erwinia*	56 (25)	24 (31)	
Assigned *E coli*	107 (48)	27 (35)	

For *TEL/AML1*-negative patients, n = 222; for *TEL/AML1*-positive patients, n = 77.

*Fisher exact test.

had relapses. One *TEL/AML1*-positive patient and 3 *TEL/AML1*-negative patients experienced induction failure. No induction deaths occurred among the *TEL/AML1*-positive patients, but 2 *TEL/AML1*-negative patients died. One remission death occurred among the *TEL/AML1*-negative patients and none among the *TEL/AML1*-positive patients. Fourteen of the *TEL/AML1*-negative patients had relapses within the first year of treatment, whereas only one *TEL/AML1*-positive patient had a relapse within that time. The next failure among the *TEL/AML1*-positive patients occurred 2.25 years after the end of induction. Five-year overall survival was 0.97 (± .02) for *TEL/AML1*-positive patients compared with 0.89 (± 0.02) for *TEL/AML1*-negative patients ($P = .03$; Figure 2B).

There were no differences between *TEL/AML1*-positive and -negative patients with respect to the preparation of asparaginase received. Fifty-three (69%) *TEL/AML1*-positive patients and 166 (75%) *TEL/AML1*-negative patients received *E coli* asparaginase, either as a result of the randomization or direct assignment after the randomization was closed, compared with 24 (31%) *TEL/AML1*-positive patients and 56 (25%) of *TEL/AML1*-negative patients who received *Erwinia* asparaginase during the randomization ($P = .31$). When comparing patients who were assigned the same type of asparaginase (*E coli* or *Erwinia*), *TEL/AML1* status was not significantly associated with EFS ($P = .07$ and $P = .44$ for E coli and *Erwinia* groups, respectively), though there was a trend toward fewer events among *TEL/AML1*-positive patients in each asparaginase group. Moreover, within *TEL/AML1* status groups, the type of asparaginase assigned was not significantly associated with EFS ($P = .80$ comparing EFS between *E coli*- and *Erwinia*-assigned *TEL/AML1*-positive patients subset and $P = .77$ among the *TEL/AML1-negative* B-precursor patients).

In multivariable regression analyses examining the association of EFS with *TEL/AML1* status and the other known prognostic factors and treatment groups shown in Table 1, none of the following were statistically significant predictors of outcome: *TEL/AML1* status, sex, CNS status, or race. When controlling for other significant predictors of EFS in the final model, risk group ($P = .009$) and asparaginase treatment group ($P = .04$), but not *TEL/AML1* status ($P = .12$), were significantly associated with EFS. None of the interactions between these variables were significant, indicating that the effect of *TEL/AML1* status on EFS did not differ within treatment or risk groups. However, the power for these comparisons was low. When WBC count and age were included in the modeling instead of risk group, they were also highly statistically significant and overall results were similar. In all models, when controlling for risk group (or WBC count and age) and asparaginase treatment group, the effect of *TEL/AML1* on EFS was not statistically significant.

Discussion

One of the most important prognostic factors for patients with ALL is the intensity of treatment received.[15] Among the major determinants of treatment is the assignment of patients to risk groups at

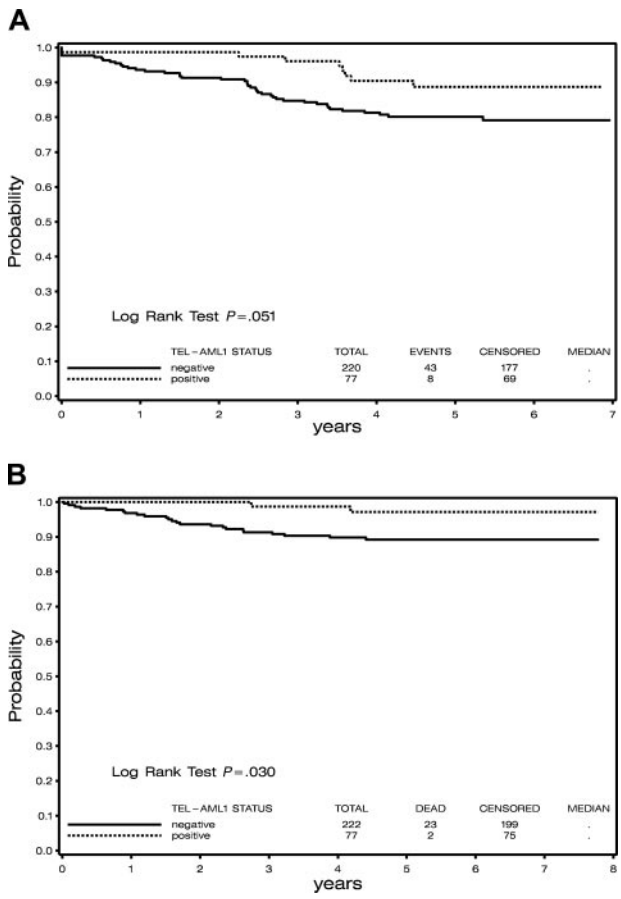

Figure 2. Survival in patients on Consortium Protocol DFCI 9501. (A) Event-free survival of *TEL/AML1*-positive compared with *TEL/AML1*-negative patients. (B) Overall survival of *TEL/AML1*-positive compared with *TEL/AML1*-negative patients.

diagnosis. The criteria used to assign patients to groups stratified according to risk have varied among national and international cooperative groups. In 1995, the Cancer Treatment and Evaluation Program gathered investigators to discuss unifying the approach to risk stratification, thus increasing the efficiency of clinical research in ALL.[16] In combination with data gathered from Rome, these joint efforts led to the widely and currently used NCI–Rome risk stratification, which uses the 2 parameters of age and white blood cell count at presentation as the basis for this grouping.

Therefore, when the outcome data for patients who have *TEL/AML1*-positive leukemia is interpreted, it is crucial to assess risk stratification and subsequent dose intensity received. The heterogeneity of reported outcomes of *TEL/AML1*-positive patients can be analyzed within this context. For instance, DFCI ALL Consortium protocols before 1995 used more stringent criteria for defining patients at lower risk (standard risk, age 2-9 years; presenting WBC count, less than 20×10^9/L; no CNS 2 or 3 disease; lack of T-cell immunophenotype; lack of anterior mediastinal mass).[11,17] All other patients were considered at high risk for relapse and received intensive therapy, including a higher cumulative dose of anthracycline and, often, cranial radiation. In fact, half the *TEL/AML1*-positive patients in the first retrospective report from the DFCI, and many of the patients reported by St Jude (who placed them in a higher risk group in light of a diploid DNA index) received intensive therapy, potentially explaining the superior outcomes for these groups.[1,11] In contrast, many of the patients reported to have relapsed disease in the initial BFM studies were classified as having low or intermediate risk features and might have had less intensive therapy. Indeed, findings from several studies by investigators in the Children's Cancer Group (CCG) and by institutions in Japan have supported this hypothesis indirectly; in these studies, the outcome of patients with *TEL/AML1*-positive disease has improved with more intensive therapy.[18]

A second factor to consider when evaluating the use of *TEL/AML1* as a prognostic indicator is evidence that the type of chemotherapy received by patients can make a difference in outcome. For example, *TEL/AML1*-positive lymphoblasts are exquisitely sensitive to steroids, vincristine, and asparaginase in vitro.[19,20] The mechanism for the increased sensitivity to asparaginase among *TEL/AML1*-positive lymphoblasts is unclear and does not appear to be related to the levels of asparagine synthetase, either at baseline or after exposure to the drug.[21] The favorable outcome of *TEL/AML1*-positive patients on DFCI ALL Consortium protocols may be the result of protracted use of asparaginase during intensification and frequent pulses of vincristine and steroids during the 2 years of treatment. The exposure to weekly L-asparaginase during the intensification phase in DFCI clinical trials has been closely linked with EFS.[22,23] In addition, investigators in Pediatric Oncology Group (POG) reported that patients with *TEL/AML1*-positive leukemia accumulate lower amounts of methotrexate polyglutamates. Such decreases have led to one speculation that these patients may be better treated by the increased doses of methotrexate used in these protocols.[24]

In the present report, there was no difference in EFS among patients who were *TEL/AML1*-positive with respect to the type of asparaginase preparation received. *Erwinia* asparaginase has a shorter half-life than *E coli* asparaginase and has been associated with higher relapse rates when used instead of *E coli* asparaginase.[25] Similarly, when analyzing all children enrolled on Protocol 95-01, *Erwinia* asparaginase was associated with an inferior EFS.[26] The lack of association between outcome and type of asparaginase used in *TEL/AML1*-positive patients supports that these patients have an increased sensitivity to asparaginase, resulting in adequate treatment even when a less effective preparation is used.

A third factor to consider when evaluating the outcome of *TEL/AML1*-positive patients is that relapsed disease might represent a novel "secondary" *TEL/AML1*-positive leukemia in which the original, preleukemic cell of origin was not completely eradicated by upfront chemotherapy. In support of this possibility, studies demonstrating the presence of low levels of the *TEL/AML1* fusion gene in bloodspots from newborns who later had leukemia establish that the initiating event of the *TEL/AML1* rearrangement occurs in utero and that subsequent events are required in early childhood to facilitate the full transformation to leukemia.[27] The secondary events that occur at the time of diagnosis of *TEL/AML1*-positive leukemia often include deletion of the wild-type *TEL* allele in most cases[28] and acquisition of trisomy 21.[29]

Analyses of cohorts of patients with relapsed *TEL/AML1*-positive leukemia indicate that patients remain sensitive to the same chemotherapeutic agents used at initial diagnosis.[30] Using microsatellite markers to study *TEL* gene rearrangements, immunoglobulin or TCR rearrangements, and fluorescence in situ hybridization (FISH) analysis, a number of patients experiencing late relapses had *TEL/AML1*-positive leukemia derived from, but not identical to, the dominant clone at diagnosis.[31,32] In addition, at least one patient who experienced relapse during therapy developed microsatellite instability in the relapsed subclone of the original leukemia. Taken together, these data support the hypothesis that preleukemic cells harboring the *TEL/AML1* fusion gene are not eradicated by initial therapy in some patients who later have relapses. A second, independent transforming event may then give rise to another *TEL/AML1*-positive leukemia, still marked by excellent response to chemotherapy.

In summary, we have confirmed in a prospective analysis that the *TEL/AML1* fusion gene occurs in approximately 26% of pediatric patients with B-precursor ALL and that these patients have excellent outcomes with currently available therapy from the DFCI ALL Consortium. The longer time to relapse for *TEL/AML1*-positive patients is consistent with other reports, and the excellent overall survival of these patients in this study suggests that those who have relapses can be successfully treated. Our results indicate that presenting age and leukocyte count, not *TEL/AML1* status, were independent predictors of outcome, suggesting that NCI–Rome risk group status should be considered when treating *TEL/AML1*-positive patients. Because the association of *TEL/AML1* status with EFS was a secondary objective, this study was not designed to guarantee sufficient power for examination of this end point. However, the lack of data in support of *TEL/AML1* as a prognostic factor independent of risk group in this clinical trial provides a cautionary note to avoid future decreases in the intensity of therapy for *TEL/AML1*-positive patients otherwise at high risk because of age or presenting leukocyte count.

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References

1. Shurtleff SA, Buijs A, Behm FG, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia. 1995;9:1985-1989.
2. Romana SP, Poirel H, Leconiat M, et al. High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. Blood. 1995;86:4263-4269.
3. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell. 1994;77:307-316.
4. Golub TR, Barker GF, Stegmaier K, Gilliland DG. The TEL gene contributes to the pathogenesis of myeloid and lymphoid leukemias by diverse molecular genetic mechanisms. Curr Top Microbiol Immunol. 1997;220:67-79.
5. Wang LC, Kuo F, Fujiwara Y, Gilliland DG, Golub TR, Orkin SH. Yolk sac angiogenic defect and intra-embryonic apoptosis in mice lacking the Ets-related factor TEL. EMBO J. 1997;16:4374-4383.
6. Golub TR, Barker GF, Bohlander SK, et al. Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 1995;92:4917-4921.
7. Romana SP, Mauchauffe M, Le Coniat M, et al. The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. Blood. 1995;85:3662-3670.
8. Pui CH, Campana D, Evans WE. Childhood acute lymphoblastic leukaemia—current status and future perspectives. Lancet Oncol. 2001;2:597-607.
9. Arico M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med. 2000;342:998-1006.
10. Loh ML, Rubnitz JE. TEL/AML1-positive pediatric leukemia: prognostic significance and therapeutic approaches. Curr Opin Hematol. 2002;9:345-352.
11. McLean TW, Ringold S, Neuberg D, et al. TEL/AML-1 dimerizes and is associated with a favorable outcome in childhood acute lymphoblastic leukemia. Blood. 1996;88:4252-4258.
12. Seeger K, Adams HP, Buchwald D, et al. TEL-AML1 fusion transcript in relapsed childhood acute lymphoblastic leukemia: the Berlin-Frankfurt-Munster Study Group. Blood. 1998;91:1716-1722.
13. Harbott J, Viehmann S, Borkhardt A, Henze G, Lampert F. Incidence of TEL/AML1 fusion gene analyzed consecutively in children with acute lymphoblastic leukemia in relapse. Blood. 1997;90:4933-4937.
14. Hann I, Vora A, Harrison G, et al. Determinants of outcome after intensified therapy of childhood lymphoblastic leukaemia: results from Medical Research Council United Kingdom acute lymphoblastic leukaemia XI protocol. Br J Haematol. 2001;113:103-114.
15. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med. 2004;350:1535-1548.
16. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol. 1996;14:18-24.
17. Loh ML, Silverman LB, Young ML, et al. Incidence of TEL/AML1 fusion in children with relapsed acute lymphoblastic leukemia. Blood. 1998;92:4792-4797.
18. Takahashi Y, Horibe K, Kiyoi H, et al. Prognostic significance of TEL/AML1 fusion transcript in childhood B-precursor acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 1998;20:190-195.
19. Krishna Narla R, Navara C, Sarquis M, Uckun FM. Chemosensitivity of tel-aml1 fusion transcript-positive acute lymphoblastic leukemia cells. Leuk Lymphoma. 2001;41:615-623.
20. Ramakers-van Woerden NL, Pieters R, Loonen AH, et al. TEL/AML1 gene fusion is related to in vitro drug sensitivity for L-asparaginase in childhood acute lymphoblastic leukemia. Blood. 2000;96:1094-1099.
21. Stams WA, den Boer ML, Beverloo HB, et al. Sensitivity to L-asparaginase is not associated with expression levels of asparagine synthetase in t(12;21)+ pediatric ALL. Blood. 2003;101:2743-2747.
22. Sallan SE, Hitchcock-Bryan S, Gelber R, Cassady JR, Frei E, Nathan DG. Influence of intensive asparaginase in the treatment of childhood non-T-cell acute lymphoblastic leukemia. Cancer Res. 1983;43:5601-5607.
23. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97:1211-1218.
24. Whitehead VM, Vuchich MJ, Cooley LD, et al. Accumulation of methotrexate polyglutamates, ploidy and trisomies of both chromosomes 4 and 10 in lymphoblasts from children with B-progenitor cell acute lymphoblastic leukemia: a Pediatric Oncology Group Study. Leuk Lymphoma. 1998;31:507-519.
25. Duval M, Suciu S, Ferster A, et al. Comparison of *Escherichia coli*-asparaginase with *Erwinia*-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. Blood. 2002;99:2734-2739.
26. Silverman LB, Levy DE, Dalton VK, Lipshultz SE, Gerber RD, Sallan SE. Outcome of Dana-Farber Cancer Institute ALL Consortium protocol 95-01 for children with newly diagnosed acute lymphoblastic leukemia. Presented at: 46th Annual Meeting of the American Society of Hematology; December 4-7, 2004; San Diego, CA.
27. Wiemels JL, Cazzaniga G, Daniotti M, et al. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet. 1999;354:1499-1503.
28. Raynaud S, Cave H, Baens M, et al. The 12;21 translocation involving TEL and deletion of the other TEL allele: two frequently associated alterations found in childhood acute lymphoblastic leukemia. Blood. 1996;87:2891-2899.
29. Loncarevic IF, Roitzheim B, Ritterbach J, et al. Trisomy 21 is a recurrent secondary aberration in childhood acute lymphoblastic leukemia with TEL/AML1 gene fusion. Genes Chromosomes Cancer. 1999;24:272-277.
30. Seeger K, Stackelberg AV, Taube T, et al. Relapse of TEL-AML1–positive acute lymphoblastic leukemia in childhood: a matched-pair analysis. J Clin Oncol. 2001;19:3188-3193.
31. Ford AM, Fasching K, Panzer-Grumayer ER, Koenig M, Haas OA, Greaves MF. Origins of "late" relapse in childhood acute lymphoblastic leukemia with TEL-AML1 fusion genes. Blood. 2001;98:558-564.
32. Zuna J, Ford AM, Peham M, et al. TEL deletion analysis supports a novel view of relapse in childhood acute lymphoblastic leukemia. Clin Cancer Res. 2004;10:5355-5360.