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Early Release Paper

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Excellent prognosis of late relapses of *ETV6/RUNX1*-positive childhood acute lymphoblastic leukemia: lessons from the FRALLE 93 protocol

Running title : Prognosis of ETV6/RUNX1 leukemia relapses

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ABSTRACT

Background.

The prognosis of relapses of *ETV6/RUNX1*-positive acute lymphoblastic leukemia remains to be evaluated, particularly with regards to the frequency of late relapses. We performed a long term follow-up a retrospective study to address the outcome of *ETV6/RUNX1*-positive leukemia relapses.

Design and Methods.

Among the 713 children tested for *ETV6/RUNX1* enrolled into the FRALLE 93 protocol, 43 *ETV6/RUNX1*-positive patients relapsed (19.4%). Most were initially stratified as low or intermediate risk groups. Median follow-up after relapse was 54.2 months. All but three received a second-line salvage therapy and 16 underwent an allogeneic transplantation.

Results.

ETV6/RUNX1 greatly impacted on overall survival after relapse (3 year-survival= 64.7 % for positive *versus* 46.5 % for negative cases) ($p= 0.007$). The 5-year cumulative incidence of relapse was 19.4% and testes were more frequently involved in *ETV6/RUNX1*-positive relapses ($p=0.04$). 81.4 % were late relapses, early combined or isolated extramedullar relapses. The 5-year survival rate of *ETV6-RUNX1*-positive acute lymphoblastic leukemia relapses reached 80.8% when relapse occurred after 36 months (*vs* 31.2%). In univariate analysis, female gender was associated with a poor survival, whereas site of relapse, age at diagnosis, leukocytosis and consolidation strategy had no effect. In multivariate analysis, only the duration of first remission remained associated with outcome.

Conclusions.

We found an excellent outcome for *ETV6/RUNX1*-positive leukemia relapses occurring over 36 months post-diagnosis. Duration of first complete remission may thus be a guide to define the treatment strategy of *ETV6/RUNX1*-positive leukemia relapse.

Introduction

Despite significant improvements in treatment, about 20% of children with acute lymphoblastic leukaemia (ALL) still suffer from relapse (1-5). Unfortunately the cure rate remains low with event-free survival (EFS) reaching only 30% at 10 years after relapse (6-9). Few prognostic factors have been identified (10). Blast phenotype (T *versus* B), site of relapse, and duration of the first remission are the main variables affecting outcome. The current relapse risk group stratifications are based on the system first described by the Berlin–Frankfurt–Munster (BFM) relapse group, which takes into account the time of relapse, the site of relapse (bone marrow and/or extramedullary), and the subsequent immunophenotype to categorize patients into one of four risk categories (S1 to S4) (11). This stratification has been adapted by the Medical Research Council (MRC, UK) and is also used by the Children’s Oncology Group which divided marrow relapse into early relapse occurring up to 36 months from diagnosis and late relapse which occurred greater than or equal to 36 months from initial diagnosis (7, 8). The Children’s Cancer Group has also divided relapses into early (< 18 months from diagnosis), intermediate (18-35 months) and late relapse (≥ 36 months) (12). In this context, *ETV6/RUNX1*-positive ALLs have a separate status. Indeed, the ALL subgroup is characterized by a good prognosis which is better than for B-ALL without this fusion transcript (2-5, 13). Nevertheless, some recent long-term results revealed late relapses in this group of patients (14, 15). It has been hypothesized that a relapse is essentially a *de novo* ALL originating from a preleukemic stem cell (16-19). This notion should be taken into account when considering the treatment options. The increased proportion of extramedullary relapses including testicular or ovarian relapses has also been discussed by a few authors (20, 21). To investigate the outcome of *ETV6/RUNX1*-positive ALL relapses, we report the long-term results of the *ETV6/RUNX1*-positive ALL relapses initially enrolled into the FRALLE93 protocol and we identify some prognostic factors which call into question the current therapeutic approach for treating late relapses.

Design and Methods

The FRALLE (FRench group for childhood ALL) 93 trial was open to children aged 0 to 20 years with untreated ALL, not including those with L3 ALL or Down's syndrome. Between June 1, 1993, and December 31, 1999, 1395 children were enrolled onto the FRALLE 93 trial in 18 French paediatric centers and one Belgian paediatric center. This study was approved by the ethics committee of the Hôpital Saint Louis, France (accepted April 29, 1993). All patients, or their parents, gave their informed consent in accordance with the Declaration of Helsinki. The diagnosis of ALL was based upon morphological, immunophenotypic, and cytogenetic analyses of bone marrow samples. From 1995, children were systematically screened for four fusion transcripts (*ETV6-RUNX1*, *BCR-ABL*, *E2A-PBX1*, *MLL-AF4*). The FRALLE 93 study population was stratified into three groups (low-risk, intermediate-risk, and high-risk) based on the following risk factors: age, white-cell count at diagnosis, haemoglobin level, immunophenotype, karyotype, and response to steroids. Treatment has been described in details by Dufourg *et al*, 2007 (22) (described in supplementary data 1).

Statistical analysis

Summary statistics were calculated, namely median (inter-quartile range, IQR) for continuous variables, and frequency (with percentages) for qualitative variables.

Distribution of qualitative variables was compared with the chisquare test, or —when inappropriate— the Fisher exact test; comparison of continuous variables was based on the nonparametric Wilcoxon rank-sum test.

We first compared the characteristics of tested and non-tested patients, to check for potential selection bias. We then focused on the *ETV6/RUNX1* positive children who relapsed. We compared the cumulative incidence of relapse in positive *versus* negative patients. Relapses were segregated into isolated central nervous system (CNS) relapse, isolated bone-marrow relapse, and others. We also considered bone-marrow relapses (whether isolated or combined), as well as testicular relapses, the latter only assessed in boys.

Finally, we focused on the outcome of *ETV6/RUNX1*-positive compared with negative patients. Relapsed patients were classified according to the risk groups defined in the REZ-BFM 95/96 study (11). This distinguished S1/S2 patients (isolated bone-marrow relapses occurring ≥ 6 months after completion of primary therapy, isolated extra-medullary relapses,

and combined bone marrow relapses occurring ≥ 18 months after completion of primary therapy) from S3/S4 patients (<6 months isolated bone marrow relapses and < 18 months after primary diagnosis combined bone marrow relapses).

Patient follow-up data were updated in October, 2010. The cumulative incidence of relapse was estimated from the date of complete remission, with death prior to relapse considered as a competing risk, and subgroup comparisons based on the Gray test. The Kaplan–Meier method was used to estimate the overall survival (OS) probabilities with 95% confidence intervals (CI) from the date of relapse until death, irrespective of cause. Comparisons of Kaplan–Meier curves were based on the log-rank test. Multivariate Cox stepwise forward-regression analysis (including testicular and non-testicular relapses) was performed to determine the independent set of prognostic variables.

All statistical tests were two-sided, with p-values of 0.05 or less denoting statistical significance. Statistical analysis was performed using the software SAS v9.2 (SAS Inc, Cary, NC), and R 2.10.1 (<http://www.R-project.org>) software packages.

Results

Total cohort

A flow chart representing the study design is shown in Figure 1. Among the 1,105 B lineage ALL excluding infants and patients with t (9;22) or t (4;11), 724 had a known *ETV6/RUNX1* status (most of the other cases were diagnosed before systematic screening). In terms of age, gender, blood prednisone response, day 21 marrow status, complete remission, and survival, there were no differences between these 724 patients and the 381 who were not tested for *ETV6/RUNX1*. However, the initial median leukocyte count was higher in the tested group ($10.10^9/l$ versus $6.4.10^9/l$ $p= 0.003$), and more patients were enrolled in the low-risk group of treatment among those not tested for *ETV6/RUNX1* ALL (22% versus 13% $p=0.001$).

Tested patients

A total of 186 (97.4%) out the 191 patients with t(12;21) achieved CR1, whereas 6 (1.1%) out of the 533 *ETV6/RUNX1*-negative patients did not achieve CR1 ($p= 0.17$).

Median follow-up after CR was 9.9 years [IQR: 8.3-11.4]. Overall, 162 (45 % of boys) of the 713 children who reached CR1 relapsed, including 43 with t(12;21).

The cumulative incidence of relapse did not significantly differ between *ETV6/RUNX1*-positive and negative ALL ($p=0.94$), with a 5-year estimate at 19.4% and 19.9%, respectively, nor according to gender with 17% of females *versus* 22% of males who had relapsed at 5 years ($p=0.10$) (Figures 2A, 2B). There was no difference in the type of relapse according to *ETV6/RUNX1* positivity, with 106 (65.4%) isolated bone-marrow relapses ($n=27$ in *ETV6/RUNX1*-positive patients and $n=79$ in *ETV6/RUNX1*-negative patients), 24 (14.8%) isolated but extra-medullary relapses ($n=6$ for *ETV6/RUNX1*-positive patients and $n=18$ of *ETV6/RUNX1*-negative patients), and 32 (19.8%) involving combined sites ($p=0.80$) (Figure 1). Among the 383 boys in CR1, 96 relapsed, with a 5-year cumulative incidence of relapse of 21.6% in *ETV6/RUNX1*-positive ALL and 22.4% in *ETV6/RUNX1*-negative ALL ($p=0.90$). Of note, 11 out of 26 (43%) were testicular relapses in the *ETV6/RUNX1*-positive ALL males *versus* 16 out of 70 (23%) in *ETV6/RUNX1*-negative ALL cases ($p=0.04$) (Table 2). As testicular relapses of *ETV6/RUNX1*-positive ALL occur mostly after 36 months (10 out of 11 cases), we distinguished the 5-year cumulative incidence of relapse in males and females. As depicted in Figure 2B, 5-year cumulative incidence of relapses did not differ significantly neither in males ($p=0.90$ by the Gray test) nor in females ($p=0.81$ by the Gray test) between *ETV6/RUNX1*-positive and *ETV6/RUNX1*-negative cases of ALL (21.6% *versus* 22.5% respectively) (Figure 2B). Nevertheless, in males, the cumulative incidence of testicular relapses appeared to be statistically increased in *ETV6/RUNX1*-positive patients ($p=0.03$, by the Gray test), with at 5-year, an estimated cumulative incidence of 11.3% compared to 4.9% in *ETV6/RUNX1*-negative patients. By contrast, non-testicular relapses were slightly decreased – though not significantly ($p=0.24$), in the *ETV6/RUNX1*-positive patients compared to those with *ETV6/RUNX1*-negativity (Figure 2C).

Median follow-up after relapse was 6.9 years [IQR: 5.7-8.6]. The presence of *ETV6/RUNX1* fusion transcript greatly impacted the outcome of relapses with a 5-year OS rate of 62.1% (95%CI: 49.0-78.7%) in *ETV6/RUNX1*-positive ALL compared with 37.2% (95%CI: 29.3-47.2%) for *ETV6-RUNX1*-negative ALL ($p=0.007$) (Figure 3A). The difference in survival estimates between *ETV6/RUNX1*-positive and negative ALL persisted though no longer significant in the S1/S2 subgroup with a 5-year OS rate of 67.7% (95%CI: 53.6-85.5%) in *ETV6/RUNX1*-positive and 47.0% (95%CI: 35.6-61.9%) in *ETV6/RUNX1*-negative ALL ($p=0.07$) (Figure 3B). S3/S4 relapses had a poor outcome, for the whole cohort with a 5-year OS rate of 27.6% (95%CI: 18.6-41.0%), not statistically different according to *ETV6/RUNX1*-positivity (37.5% (95%CI: 15.3-91.7%) and 26.2% (95%CI: 16.9-40.6%) for positive and

negative ALL, respectively; $p=0.45$ by the log-rank test) (Figure 3B). As the incidence of testicular relapses was high and could contribute to the favourable outcome *ETV6/RUNX1*-positive ALL, we investigated the outcomes according to testicular *versus* other sites of relapse. The improved OS of *ETV6/RUNX1*-positive relapses was observed whatever the site of relapses (but not reaching statistical significance) (Figure 3C).

***ETV6/RUNX1*-positive ALL relapses**

The initial clinical features of the 43 *ETV6/RUNX1*-positive ALL relapses are shown in Table 1 (details are provided in supplementary data 2). There were 26 (60.5%) males, with median age at diagnosis of 4.1 years [IQR: 2.7-5.4], a median leukocyte count of $15.5 \times 10^3/\text{mm}^3$ [IQR: 7.0-35.6] and no cases of CNS involvement. Thirty three (77%) patients were classified as low risk/intermediate risk and displayed good early responses to therapy.

Salvage therapy after relapse

According to the risk groups defined by the REZ-BFM 95/96 study, 35 (81.4%) patients were classified as S1/S2 and 8 (18.6%) patients were classified as S3/S4.

All but three patients received a second-line therapy protocol (three patients were treated according to a first-line protocol after a relapse occurring at 68, 39, and 32 months after diagnosis). Thirty-seven patients were included in the COOPRALL-97 study (23) which recommended a VANDA (VP-16, cytarabine, mitoxantrone, dexamethasone, asparaginase) induction regimen (except for the late extra-medullary relapses) followed by successive blocks (B1, B2, B3). If an HLA identical donor was available, stem cell transplantation (SCT) was performed after 2 or 3 blocks. Otherwise, alternative treatment consisted of 3 successive blocks repeated 3 times followed by autologous transplantation or —for late combined/isolated medullary or isolated extra medullary relapses— maintenance and CNS radiotherapy. Two patients were treated according to a Capizzi scheme (24) and the latest one according to the UKALL R2 (8).

Outcomes

Median follow-up after relapse was 7.7 years [IQR: 6.2-9], with only 1 child lost to follow-up at 12 months after first relapse.

All but one patient (due to early death) achieved CR2 ($n=42$, 98%). Eighteen children received a consolidation chemotherapy program and 24 children were transplanted (Flowchart of salvage therapy after relapse in supplementary data 3). Transplantations were performed at

4.2±1.2 months for the 16 allogeneic transplants (10 were classified as S1/S2 and 6 as S3/S4) and 7.6±1.2 months after relapse for the 8 autologous transplants.

A second relapse occurred in 13 patients, including 10 (77%) initially stratified as S1/S2. At 5 year, the cumulative incidence of second relapse was 28.8% (95%CI: 14.8-42.8%), 18.8% in allogeneic transplants, 37.5% in autografted patients, and 36.2% in those who received chemotherapy. Four out of 13 were still alive in CR3, three treated by chemotherapy in CR2 and one allotransplanted.

Finally, a total of 16 deaths were observed. The 5-year OS rate after first relapse was 62.1% (95%CI: 49.0-78.7%), with differences according to the type of relapse: 70.9% (95%CI: 57.3-87.9%) in S1/S2 versus 37.5% (95%CI: 15.3-91.7%) in the S3/S4 risk group (p=0.049).

Prognostic factors

Based on univariate analyses, the overall survival of *ETV6/RUNX1*-positive ALL after relapse was significantly affected by the duration of the first remission with a 5-year OS rate that was significantly better when relapse occurred after 36 months (80.8%, 95%CI:66.9-97.5% versus 31.2%, 95%CI:15.1-64.6%, p=0.0008). Female gender was also associated with a poor survival (p= 0.015), whereas the site of relapse (p= 0.13), age at initial diagnosis (p= 0.81), and leukocytosis (p=0.42) were all not found to be of any prognostic value (Table 2). Similarly, the consolidation strategy (allograft or chemotherapy ± autograft) had no effect on survival (p=0.18).

In multivariate Cox-regression analysis, only the duration of first remission remained associated with the outcome (Figure 4A). In particular, the site of relapse (testicular versus non-testicular relapse) did not provide any additional prognostic information (p=0.79). Although timing of relapse of *ETV6/RUNX1*-negative patients impacted also significantly prognosis (Figure 4B), the 5-year OS rate of the 73 tested patients who relapsed after 36 months appeared to be significantly different in the 27 *ETV6/RUNX1*-positive patients (4 extra-medullary, 9 combined and 14 isolated marrow relapses) compared to the 46 *ETV6/RUNX1*-negative ones (81% versus 50%, respectively; p= 0.015 by the log-rank test).

Discussion

After a long-term follow-up, we have reported here the results of the *ETV6/RUNX1*-positive ALL relapses which were initially enrolled in the FRALLE 93 protocol. Indeed, long-term results are now mandatory because very late relapses have been reported (20, 21). In agreement with previous studies, we found that relapses occurred in about 20 % of *ETV6/RUNX1*-positive ALL, and for males after a longer remission period than other ALL of B lineage (13, 15, 25, 26, 27). Indeed, the male cumulative incidence of relapses was impacted by the frequency of testicular relapses which occurred later than in other sites in boys. More recent protocols based on intensified chemotherapy schemes, especially with intensive use of L-asparaginase (28), report lower frequencies of relapse for *ETV6/RUNX1*-positive ALL but should be examined with longer follow-up (3, 5, 13, 29). We also highlight that boys are slightly over-represented which has been rarely reported except by Seeger *et al*, and more recently by the NOPHO group (15, 27). Moreover, we observed a specific increase of testicular relapses in *ETV6/RUNX1*-positive ALL without any increase of other extramedullary sites (mainly CNS). Whatever gender, when pooling all sites of relapses, we did not find any difference in cumulative incidence of relapses according to *ETV6/RUNX1*-positivity. To our knowledge, this finding has not been previously reported and highlights the interest of a primary treatment including drugs able to cross the testicular barrier like high dose methotrexate. Most of these testicular relapses occurred late and special attention to the testes, as well as maybe the ovary, should be required in long-term follow-up of *ETV6/RUNX1*-positive ALL. According to the responsiveness of the disease, *ETV6/RUNX1*-positive relapses are generally considered as well treatable (13, 15), and here we reported a similar good overall outcome of 62.1 % which is significantly better than *ETV6/RUNX1*-negative relapses. We also found that almost 98% of patients achieved a second complete remission which is significantly higher than that reported by the ALL-REZ BFM 90 and the MRC UKALL-R (6, 9) and also higher than FRALLE 93 *ETV6/RUNX1*-negative relapses. Unfortunately, in our retrospective study, we were unable to explore IgH/TCR rearrangements or sequence the *ETV6/RUNX1* genome to better understand treatment failures. The respective prognostic role of age, site, and time of relapse in the outcome of all types of relapses has been often debated (9, 12). In addition, the definition of extramedullary relapses could, in an early date, move considering the prognostic relevance of submicroscopic bone marrow involvement based on *in vitro* amplification sensitive methods (30). Age did not affect prognosis in our exclusively pediatric study but it should be noted that *ETV6/RUNX1*-

positive ALL can present a different clinical progress in young adults (14). We showed that only time-point of relapse had prognostic significance in a multivariate analysis for *ETV6/RUNX1* relapses. We thus highlight that the outcome of relapses occurring ≥ 36 months reached an OS rate of 80.8% which is excellent and similar to the prognosis of primary ALL. We could not show any contribution of the high incidence of testicular relapse to this very favourable outcome, possibly due to some lack of statistical power. In contrast, the prognosis of early recurrences (stratified as S3/S4 according to ALL REZ-BFM classification) remained poor despite the presence *ETV6/RUNX1* positivity. This timing of relapse is also significant for *ETV6/RUNX1*-negative relapses but not reaching such a high OS for late relapses. This finding may support the hypothesis that the majority of *ETV6/RUNX1* “late” relapses could be due to a novel leukemic clone arising from a persistent initial *ETV6/RUNX1*-positive preleukemic clone (16, 18, 31). Indeed, *ETV6/RUNX1* is an early event which is insufficient for leukemic development (32, 33) and requires the occurrence of additional events conferring proliferative advantages for full malignant transformation (19, 34). Bhojwani *et al* have even suggested that this model could be operating in non-*ETV6/RUNX1* subtypes of late relapses (35). Staal *et al*, in a genome-wide expression analysis of paired diagnosis-relapse samples, also found that relapses could result from therapy involving selection of minor clones present at diagnosis and from genetic alteration of the original tumor cells (36).

Although the mechanisms governing relapses remain unclear, the particular natural history of *ETV6/RUNX1*-positive ALL may lead to use intensive but first-line therapeutic approaches for late relapses. The impact of glucocorticoid resistance at relapse should also probably be explored in the future according to the recent data published by Kuster *et al* who have implicated glucocorticoid signalling in *ETV6/RUNX1* positive relapses (37). In this small study sample we failed to show any impact of the use of allogeneic transplantation, possibly because of an excess of transplantation-related mortality. However, the poor survival rate of early relapses encourages the use of a second-line treatment with cell therapy according to REZ-BFM. In both types of relapse (38), clinical decisions could also be helped by assessing the dynamics of treatment response using minimal residual disease quantification, which is a promising tool being validated in ongoing relapse trials (25, 39). Finally, our data on *ETV6/RUNX1*-positive ALL from the FRALLE 93 study can probably be extended to other first-line protocols because Freyer *et al* have recently shown that post-relapse survival in childhood acute lymphoblastic relapse was independent of initial treatment intensity (38).

In conclusion, relapses of *ETV6/RUNX1*-positive ALL occurring over 36 months post-diagnosis have an excellent prognosis. Testicular relapses are of especially high frequency and suggest using a primary treatment able to cross the testicular barrier and prolonging follow-up with particular interest for screening gonads. If early relapses need common relapse therapeutic approaches, the physiopathology of *ETV6/RUNX1*-positive ALL could allow the postponement of high-dose therapy for late relapses under cover of examining biological markers of responsiveness.

Authorship and Disclosures

All the authors have substantially contributed to the conception and design or acquisition of data or analysis and interpretation of the data in this multicentric study, and participated to drafting and revising the article. SC and MFA performed statistical analysis. GL and AB approved the version to be published. All authors read and approved the final manuscript.

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Table 1. Initial features of the 43 *ETV6/RUNX1* positive ALL relapses.

Median [IQR] N (%)	Stratification according to REZ-BFM		
	Overall n=43	S1/S2 n=35	S3/S4 n= 8
Presenting clinical features			
Median age [IQR] at primary dg	4.1 years [2.7-5.4]	4.1 [2.7-5.5]	4.1 [3.0-5.0]
< 5 years	n=29	23 (66%)	6 (75%)
≥ 5 years	n= 14	12 (34%)	2 (25%)
Male gender	26 (60%)	21 (60%)	5 (63%)
CNS involvement	0	0	0
Median WBC x 10 ³ / mm ³ [IQR]	15.5 [7.0-35.6]	12.4 [6.4-28.9]	23.1 [10.7-93.0]
<50	34 (79%)	29 (83%)	5 (63%)
≥ 50	9 (21%)	6 (17%)	3 (37%)
Initial risk group (FRALLE 93)			
LR	6 (14%)	5 (14%)	1 (12%)
IR	27 (63%)	24 (69%)	3 (38%)
HR	10 (23%)	6 (17%)	4 (50%)
Early response to therapy			
CR	43 (100%)	35 (100%)	8 (100%)
D8 PGR	42 (98%)	34 (97%)	8 (100%)
D21 marrow status			
M1	32 (74%)	28 (80%)	4 (50%)
M2	10 (23%)	7 (20%)	3 (38%)
M3	1 (2%)	0	1 (12%)
Relapse sites			
BM	27 (62%)	19 (54%)	8 (100%)
BM+ CNS	2 (5%)	2 (6%)	0
BM+testes	7 (27% of males)	7	0
Isolated	4 (15% of males)	4	0
Testis			
Isolated CNS	2 (4.6%)	2 (6%)	0
Others	2 (4.6%)	2 (6%)	0
Median relapse time after dg, months	42.5 [32-55]	48.4 [38.0-58.3]	24.3 [19.2-32.6]
Second relapse	13 (30%)	10 (29%)	3 (38%)

IQR: interquartile range

PGR: a good prednisone response was defined as a blast count <1000/μl blood after the first seven days on prednisone therapy (*i.e.* on day 8) and one triple-drug intrathecal injection.

D21 marrow status: a good early response to chemotherapy was defined by a blast count fewer than 5% in bone marrow smears on day 21 (M1) and a poor early chemotherapy response by a blast count equal to or more than 5% (with two categories: M2 = 5% to 25% and M3 = more than 25%).

CR: complete remission was defined by no physical evidence of disease, no detectable leukemic blasts on blood smears and less than 5% blasts on bone marrow smears, active haematopoiesis, and normal cerebrospinal fluid.

WBC: white blood cell count; BM: bone marrow; CNS: central nervous system; LR: low risk group; IR: intermediate risk group; HR: high risk group (details in supplementary data 1)

ALL REZ-BFM S1/S2 classification : isolated bone-marrow relapses ≥ 6 months after completion of primary therapy, isolated extra-medullary relapses, and combined bone-marrow relapses ≥ 18 months after completion of primary therapy). ALL REZ-BFM S3/S4 classification: <6 months isolated bone marrow relapses and < 18 months after primary diagnosis combined bone-marrow relapses.

Table 2. Univariate analyses of factors influencing survival after first relapse of *ETV6/RUNX1*-positive ALL.

	Number of patients (n=43)	3y-OS (%), 95%CI	P
Age at diagnosis			
< 5 years	29	61.3 (45.7-82.2)	0.81
≥ 5 years	14	71.4 (51.3-95.1)	
WBC (x 10³/ mm³)			
<50	34	61.8 (47.4-80.5)	0.42
≥ 50	9	77.8 (54.9-100)	
Gender			
male	26	80.3 (66.2-97.4)	0.015
female	17	41.2 (23.3-72.7)	
Duration of first remission			
< 36 months	16	31.2 (15.1-64.6)	0.0008
≥ 36 months	27	85.0 (72.5-99.7)	
Classification of relapse			
S1-S2	35	70.9 (57.3-87.9)	0.049
S3-S4	8	37.5 (15.3-91.7)	
Sites of relapse			
Isolated BM	27	54.7 (38.7-77.5)	0.13
Isolated extra medullary	6	66.7 (37.9-100)	
Others	10	90.0 (73.2-100)	
Consolidation treatment*			
Chemotherapy/autologous SCT	26	73.3 (58.2-92.4)	0.18
Allogeneic SCT	16	50.0 (30.6-81.6)	

WBC: white blood cell count; BM: bone marrow; SCT: stem cell transplantation

ALL REZ-BFM S1/S2 classification: isolated bone-marrow relapses ≥ 6 months after completion of primary therapy, isolated extra-medullary relapses, and combined bone-marrow relapses ≥ 18 months after completion of primary therapy). ALL REZ-BFM S3/S4 classification: <6 months isolated bone marrow relapses and < 18 months after primary diagnosis combined bone-marrow relapses.

* 1 patient dead during induction

Figure legends

Figure 1. Flow chart of study design.

43 and 119 relapses respectively occurred in ETV6/RUNX1-positive and -negative cases of ALL. More testicular relapses ($p=0.04$) characterized ETV6/RUNX1-positive ALL without any increase in other extra-medullary sites. Second complete remission was achieved in 98% of ETV6/RUNX1-positive ALL and in 82% of ETV6/RUNX1-negative ALL.

Figure 2. Cumulative incidence of relapse according to *ETV6/RUNX1* positivity (negative versus positive): overall (A), according to gender (B), according to site of relapse (testicular or non testicular) for males(C).

Figure 3. Kaplan-Meier curves of overall survival of FRALLE 93 relapses according to *ETV6/RUNX1* positivity (negative versus positive) overall (A), in REZ-BFM risk group (B), and according to the site of relapse (testicular or non-testicular) (C).

ALL REZ-BFM classification: S1/S2 = isolated bone-marrow relapses occurring ≥ 6 months after completion of primary therapy, isolated extra-medullary relapses, combined bone marrow relapses occurring ≥ 18 months after completion of primary therapy; S3/S4 = < 6 months isolated bone marrow relapses or < 18 months after primary diagnosis combined bone marrow relapses

Figure 4. Prognostic value of time elapsed, in months, since diagnosis in terms of survival after relapse according to *ETV6/RUNX1* positivity

Figure 1: Flow chart of study design

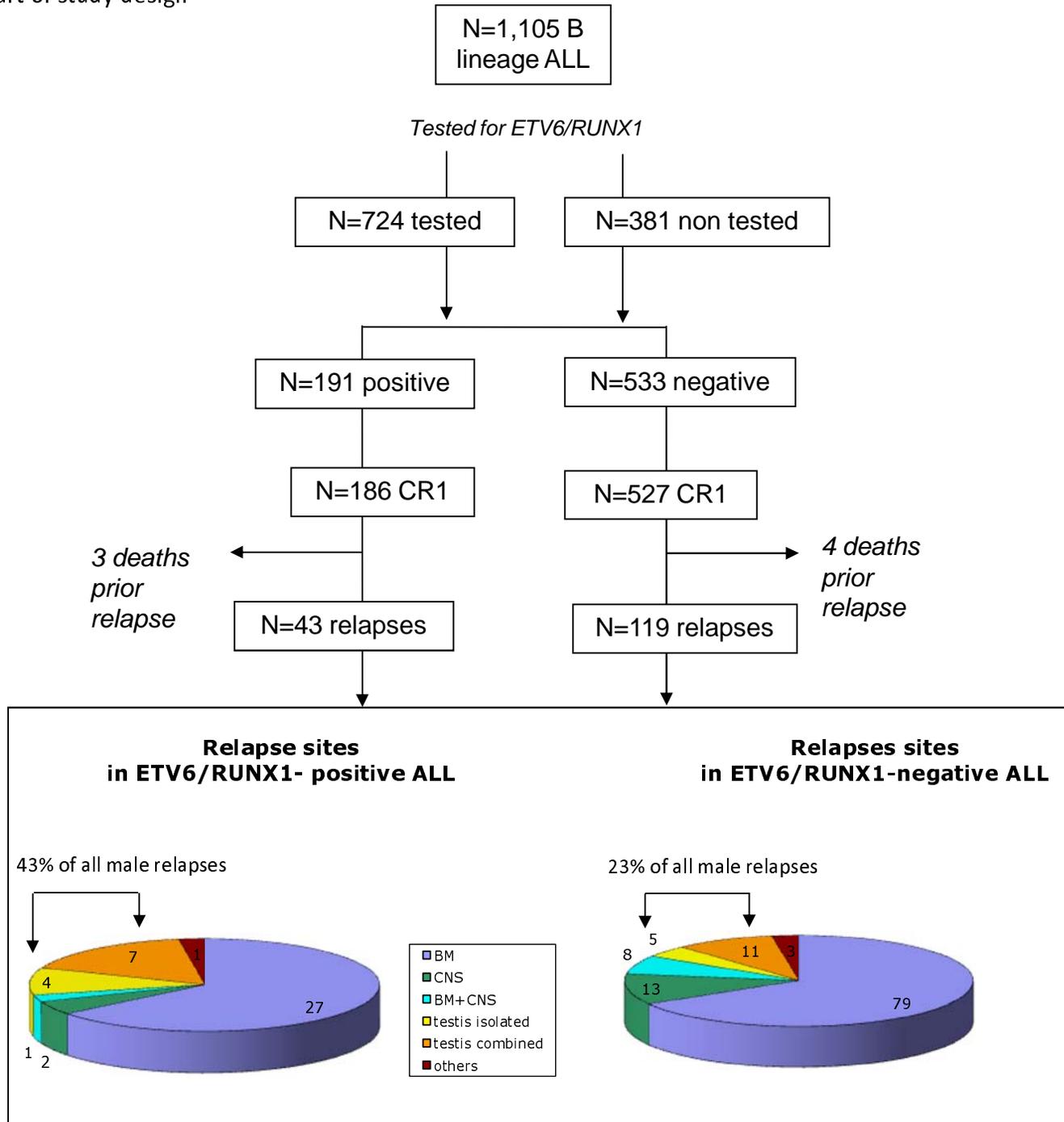


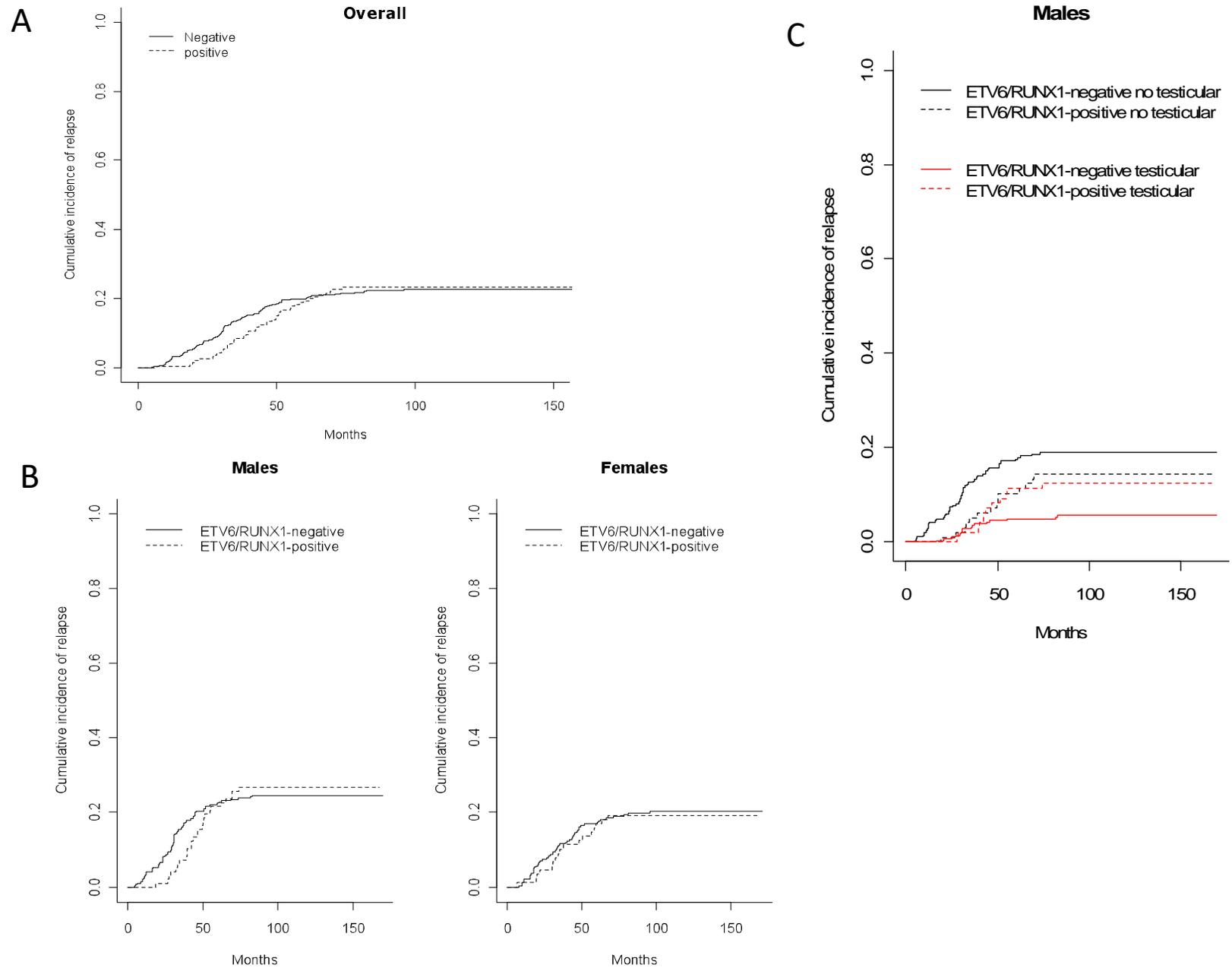
Figure 2: Cumulative incidence of relapse according to *ETV6/RUNX1* positivity

Figure 3 : Kaplan-Meier curves of overall survival of FRALLE 93 relapses according to *ETV6/RUNX1* positivity

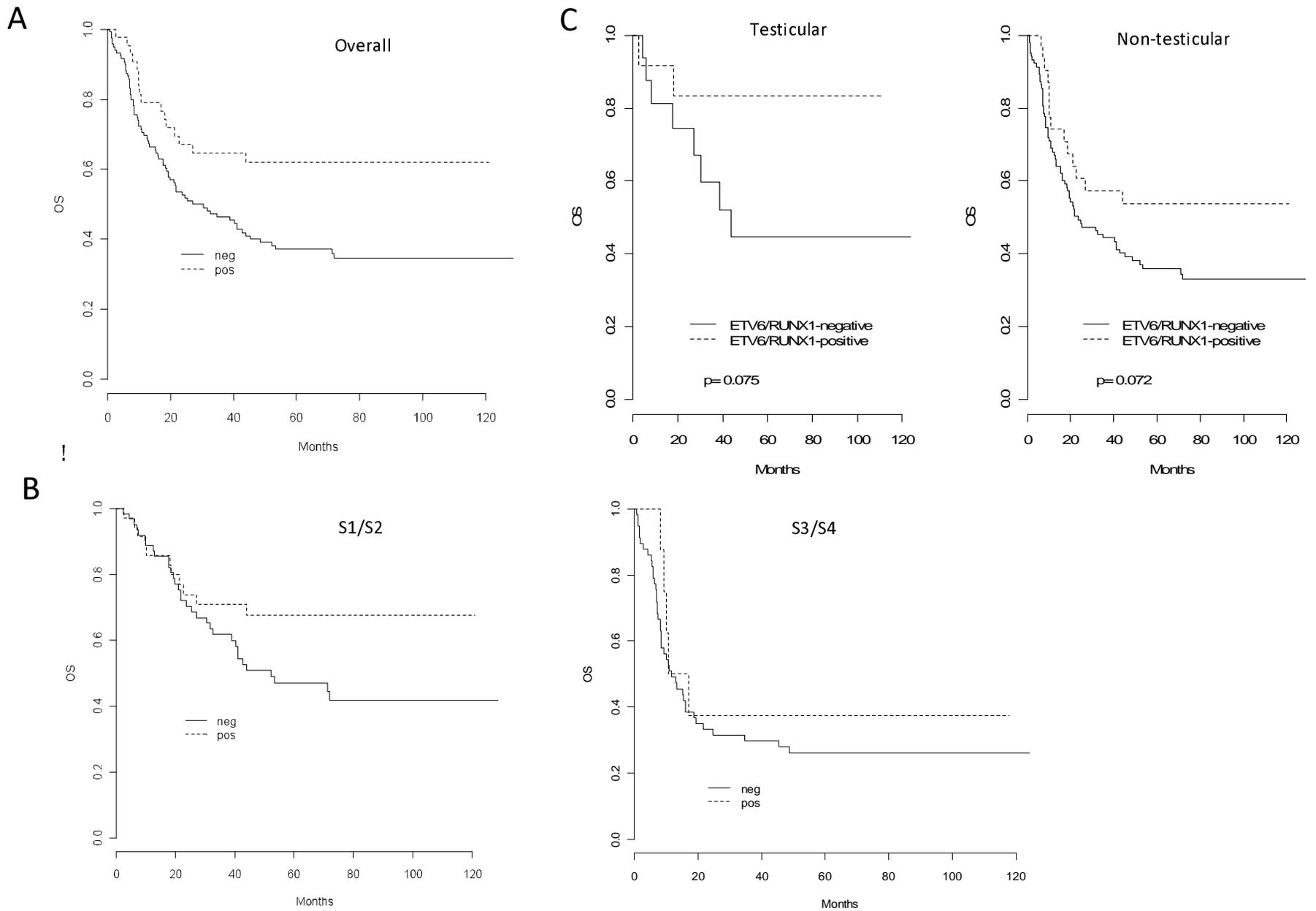
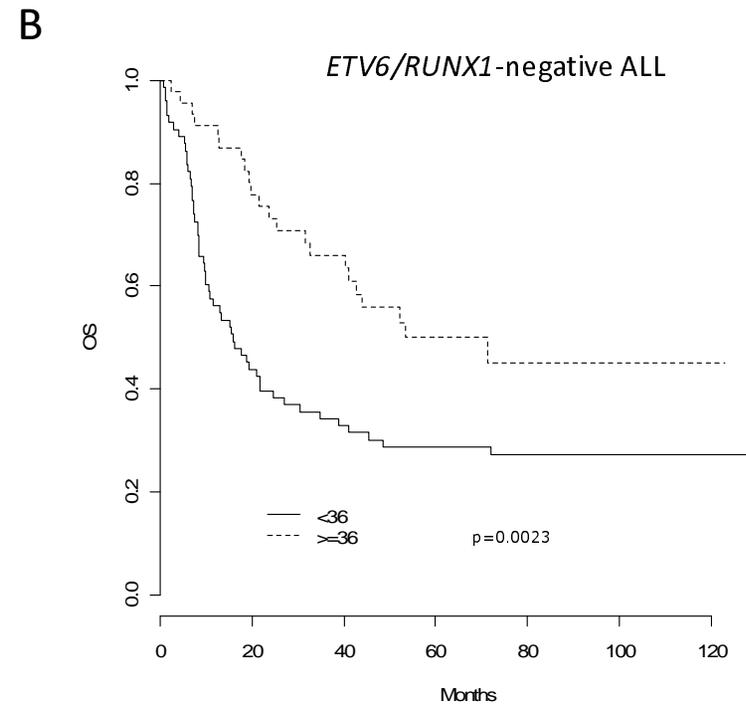
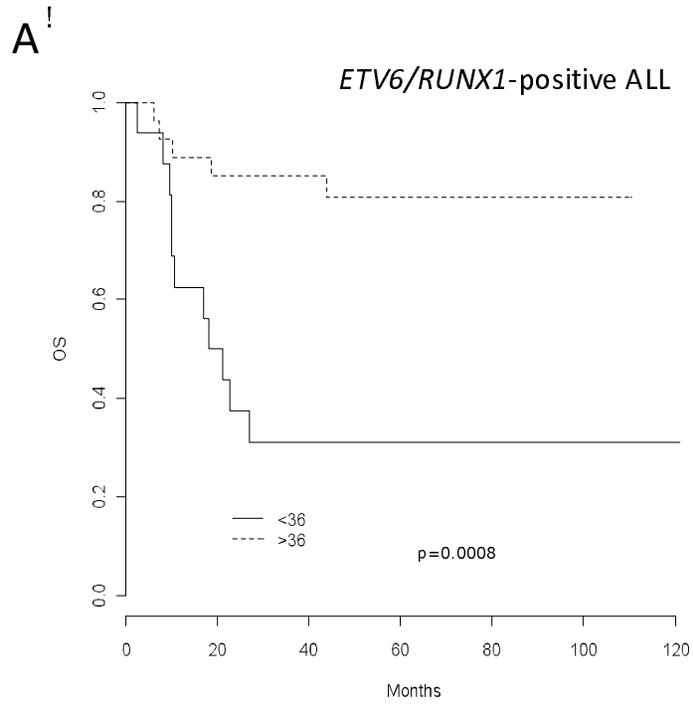


Figure 4: Prognostic value of time elapsed (in months) since diagnosis in terms of survival after relapse according *ETV6/RUNX1* positivity



Supplementary data 1. Summary of the FRALLE 93 stratification.

The FRALLE 93 study population was stratified into three groups (low-risk [LR], intermediate-risk [IR], and high-risk [HR]) based on the following risk factors: age, white-cell count at diagnosis, haemoglobin level, immunophenotype, karyotype, and response to steroids.

- LR was defined by age between 1 and 7 years, white-cell count $<10 \cdot 10^9/l$, B lineage c-ALL CD 10 positive, and no adverse cytogenetic findings.
- HR was defined by at least one major criteria: age below 1 year or >15 years, white-cell count $>100 \cdot 10^9/l$, t(9;22) or t(4;11) or hypoploid or tetraploid karyotype, T-cell lineage, or at least 2 minor criteria: age >10 years, tumour syndrome, haemoglobin >10 G/l, white-cell count $>50 \cdot 10^9/l$, expression of two myeloid antigens such as CD13, CD33, or CD14.
- IR was defined by exclusion of LR and HR.

Patients received an initial treatment comprised of a prednisone prophase and a triple-drug intrathecal injection. Induction treatment then included prednisone, vincristine, L-asparaginase, daunorubicin (except for the LR group), and one or two more triple-drug intrathecal injections (TIT). The main treatment features of the SR and IR protocol were induction, consolidation, delayed intensification, and maintenance (total treatment time 26 and 38 months for girls and boys respectively). Treatment of the HR patients consisted of induction, consolidation, two delayed intensifications, and maintenance with a total treatment time of 2 years. LR patients received 16 TIT and IR patients received 18 TIT. HR children over 4 years received 12 TIT before central nervous system (CNS) radiotherapy, whereas younger children received 18 TIT, without radiotherapy. Very high risk patients (steroid-resistant on day 8 or poor bone marrow early response to chemotherapy on day 21) received intensification with six cycles of chemotherapy alternating with methotrexate and high-dose cytarabine, followed by autografting. Allogeneic stem-cell transplantation was recommended for very high risk patients with related matched donors and for all patients with t(9;22) or t(4;11) or hypoploid karyotype.

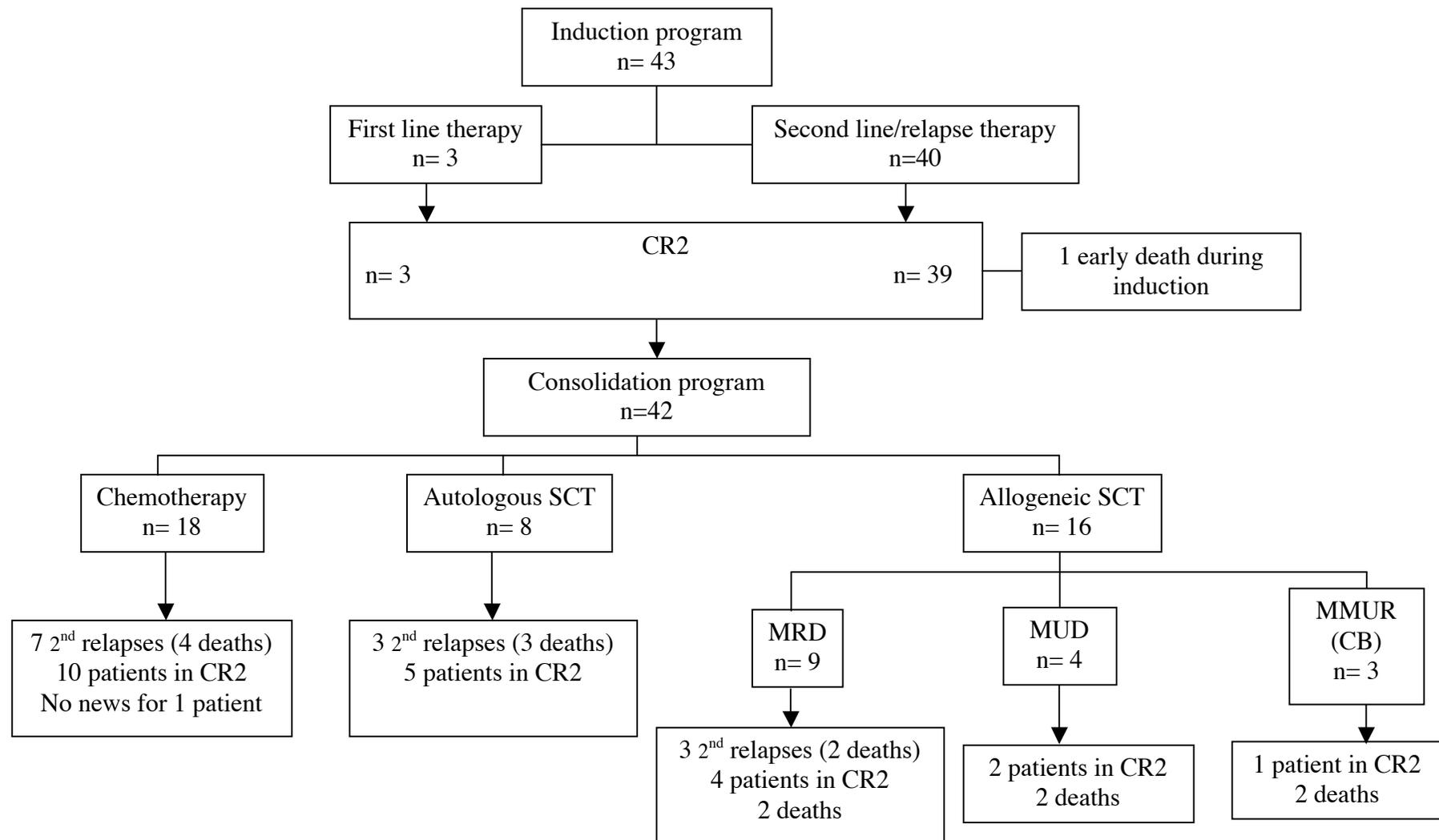
Patient	Years at dg	Gender	WBC x10 ³ /mm ³	D8 PGR	D21 M status	End of induction	FRALLE 93 treatment risk group	Time of relapse (months)	Site of relapse	REZ-BFM	Salvage therapy	Post CR treatment	2 nd relapse status	
1	3.7	male	15.5	yes	M1	CR	IR	74	testis	S1-S2	COOPRALL	CT	alive	
2	4.8	male	11	yes	M1	CR	IR	32	BM	S3-S4	NFL	autoSCT	BM	dead
3	4.5	female	4.7	yes	M1	CR	LR	51	BM	S1-S2	COOPRALL	CT	alive	
4	5.5	female	27.2	yes	M2	CR	IR	49	BM	S1-S2	COOPRALL	CT	alive	
5	2.8	female	35.6	yes	M1	CR	IR	34	BM	S1-S2	COOPRALL	autoSCT	alive	
6	7.3	male	10.4	yes	M1	CR	IR	70	BM	S1-S2	COOPRALL	CT	BM+CNS	dead
7	5.3	male	2.3	yes	M1	CR	IR	43	BM+testis	S1-S2	COOPRALL	CT	CNS	alive
8	5.8	male	12.4	yes	M1	CR	IR	55	BM+testis	S1-S2	COOPRALL	CT		alive
9	2.6	male	229	yes	M1	CR	HR	28	BM+CNS	S1-S2	Capizzi	No CR2		dead
10	7.1	female	16.1	yes	M2	CR	IR	60	BM	S1-S2	COOPRALL	alloSCT		dead
11	5.4	male	24.6	yes	M1	CR	IR	47	BM+testis	S1-S2	COOPRALL	autoSCT		alive
12	11.4	male	10.3	yes	M2	CR	HR	35	BM	S3-S4	COOPRALL	alloSCT		alive
13	2.6	female	6.4	yes	M2	CR	LR	64	BM	S1-S2	COOPRALL	alloSCT		dead
14	1.7	female	50	yes	M1	CR	IR	58	BM	S1-S2	COOPRALL	alloSCT		alive
15	4.7	male	80	yes	M3	CR	HR	19	BM	S3-S4	COOPRALL	alloSCT		Alive
16	2.3	female	28.9	yes	M1	CR	IR	32	BM	S1-S2	COOPRALL	alloSCT		Dead
17	1.9	male	5.1	yes	M1	CR	HR	28	testis	S1-S2	COOPRALL	CT	BM	Dead
18	7.7	male	9.1	yes	M1	CR	IR	51	BM	S1-S2	COOPRALL	CT	BM	Dead
19	8.6	male	4.2	yes	M2	CR	HR	62	BM	S1-S2	COOPRALL	alloSCT		Alive
20	4.1	female	8.2	yes	M1	CR	IR	57	BM	S1-S2	COOPRALL	CT		Alive
21	3.9	female	8	yes	M1	CR	IR	20	CNS	S1-S2	COOPRALL	autoSCT	CNS	Dead
22	3.1	female	36.8	yes	M1	CR	IR	32	BM	S1-S2	COOPRALL	alloSCT	BM	Dead
23	4.2	male	19.3	yes	M1	CR	IR	44	BM+testis	S1-S2	COOPRALL	alloSCT	testis	Alive
24	12.3	female	27.8	no	M2	CR	IR	68	other	S1-S2	NFL	CT		Alive
25	4.5	female	11.7	yes	M2	CR	IR	32	BM	S1-S2	COOPRALL	CT	BM	Dead
26	4.0	male	6.3	yes	M1	CR	IR	39	CNS	S1-S2	NFL	alloSCT		alive
27	3.4	male	6.1	yes	M2	CR	LR	27	BM	S3-S4	COOPRALL	alloSCT	BM	dead
28	3.7	male	5.2	yes	M1	CR	LR	40	BM+testis	S1-S2	COOPRALL	CT		alive
29	2.0	male	51.3	yes	M1	CR	HR	40	BM+testis	S1-S2	COOPRALL	autoSCT		alive
30	2.2	male	26.7	yes	M1	CR	IR	66	BM	S1-S2	COOPRALL	CT	testis	alive
31	2.7	male	61.3	yes	M1	CR	HR	47	BM	S1-S2	COOPRALL	autoSCT		alive
32	5.3	female	223	yes	M1	CR	HR	22	BM	S3-S4	COOPRALL	alloSCT	BM	dead
33	3.8	male	19.8	yes	M1	CR	IR	50	BM	S1-S2	COOPRALL	CT	BM	alive
34	1.7	male	106	yes	M1	CR	HR	33	BM	S3-S4	COOPRALL	alloSCT		alive
35	3.4	male	7	yes	M2	CR	IR	56	testis	S1-S2	COOPRALL	CT		Alive
36	2.6	female	24	yes	M1	CR	IR	7	BM	S3-S4	COOPRALL	alloSCT		Dead
37	3.5	female	22.2	yes	M2	CR	IR	20	BM	S3-S4	COOPRALL	alloSCT		Dead
38	5.0	female	6.1	yes	M1	CR	LR	38	BM	S1-S2	COOPRALL	alloSCT		Dead
39	5.8	male	8	yes	M1	CR	IR	52	testis	S1-S2	COOPRALL	CT		Alive
40	5.8	male	7	yes	M1	CR	IR	70	BM+CNS	S1-S2	COOPRALL	CT		Alive
41	3.3	male	181	yes	M1	CR	HR	50	BM	S1-S2	UKALLR2	CT		?
42	2.1	female	3.8	yes	M1	CR	LR	35	BM	S1-S2	COOPRALL	autoSCT		Alive
43	4.8	male	63	yes	M1	CR	IR	43	BM+testis	S1-S2	Capizzi	alloSCT		Alive

Supplementary data 2. Details of characteristics of the 43 ETV6/RUNX1-positive ALL relapses.

D8 PGR: a good prednisone response was defined as a blast count $<1000/\mu\text{l}$ blood after the first seven days on 60 mg/m²/d of prednisone (*i.e.* on day 8) and one triple-drug intrathecal injection. D21 M status: a good early response to chemotherapy was defined by a blast count fewer than 5% in bone marrow smears on day 21 (M1) and a poor early chemotherapy response by a blast count $\geq 5\%$ (with two categories: M2 = 5% to 25% and M3 = more than 25%). CR: complete remission was defined by no physical evidence of disease, no detectable leukemic blasts on blood smears and less than 5% blasts on bone marrow smears, active haematopoiesis and normal cerebrospinal fluid.

WBC: white blood cell count; BM: bone marrow; CNS: central nervous system; LR: low risk group; IR: intermediate risk group; HR: high risk group; dg: diagnosis; CT: chemotherapy; auto/allo SCT: stem-cell transplantation; NFL: new first line therapy.

ALL REZ-BFM S1-S2 classification is defined as isolated bone marrow relapses ≥ 6 months after completion of primary therapy, isolated extra medullary relapses, and combined bone marrow relapses ≥ 18 months after completion of primary therapy). ALL REZ-BFM S3-S4 classification is defined as <6 months isolated bone marrow relapses and < 18 months after primary diagnosis combined bone marrow relapses.



Supplementary data 3. Flowchart of salvage therapy after relapse.

SCT: stem cell transplantation.

CR: complete remission.

MRD: matched related donor; MUD: matched unrelated donor ; MMUR : mismatched unrelated donor ; CB: cord blood.