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Original Research

Outcome of adolescent patients with acute lymphoblastic leukaemia aged 10–14 years as compared with those aged 15–17 years: Long-term results of 1094 patients of the AIEOP-BFM ALL 2000 study

Anna Maria Testi [a,1,**], Andishe Attarbaschi [b,*,1], Maria Grazia Valsecchi [c], Anja Möricke [d], Gunnar Cario [d], Felix Niggli [e], Daniela Silvestri [c,f], Peter Bader [g], Michaela Kuhlen [h], Rosanna Parasole [i], Maria Caterina Putti [j], Peter Lang [k], Christian Flotho [l], Georg Mann [b], Carmelo Rizzari [f], Elena Barisone [m], Franco Locatelli [n,o], Christin Linderkamp [p], Melchior Lauten [q], Meinolf Suttorp [r], Martin Zimmermann [p], Guiseppe Basso [s], Andrea Biondi [f], Valentino Conter [f], Martin Schrappe [d] on behalf of the AIEOP-BFM (Associazione Italiana di Ematologia e Oncologia Pediatrica & Berlin-Frankfurt-Münster) Study Group

[a] *Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy*
[b] *Department of Pediatric Hematology and Oncology, St. Anna Children's Hospital, Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria*
[c] *Center of Biostatistics for Clinical Epidemiology, Department of Health Science, University of Milano-Bicocca, Milano, Italy*
[d] *Department of Pediatrics, Christian-Albrechts-University Kiel and University Medical Center Schleswig-Holstein, Kiel, Germany*
[e] *Department of Pediatric Oncology, University Children's Hospital, Zürich, Switzerland*
[f] *Pediatric Hematology-Oncology Unit, Department of Pediatrics, University of Milano-Bicocca, MBBM Foundation/ASST Monza, Monza, Italy*
[g] *Department of Children and Adolescent Medicine, Children's Hospital, Goethe University, Frankfurt, Germany*
[h] *Department of Pediatric and Adolescent Medicine, University Children's Hopsital Augsburg, Swabian Children's Cancer Center, Augsburg, Germany*
[i] *Department of Pediatric Hemato-Oncology, A.O.R.N. Santobono-Pausilipon, Naples, Italy*
[j] *Department of Woman and Child Health, Laboratory of Haematology-Oncology, University of Padova, Padova, Italy*
[k] *Department of Pediatric Hematology and Oncology, Children's Hospital, Eberhard Karls University, Tübingen, Germany*
[l] *Department of Pediatric Hematology and Oncology, Children's Hospital, Albert Ludwigs University, Freiburg, Germany*
[m] *Pediatric Onco-Hematology, Regina Margherita Children's Hospital, AOU Città della Salute e della Scienza, Turin, Italy*

* *Corresponding author*: Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy.
** *Corresponding author*: St. Anna Children's Hospital, Kinderspitalgasse 6, 1090 Vienna, Austria. Fax: 0043 1 40170-7000.
E-mail addresses: testi@bce.uniroma1.it (A.M. Testi), andishe.attarbaschi@stanna.at (A. Attarbaschi).
[1] share the first authorship.

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[n] Department of Pediatric Hematology-Oncology, IRCCS "Bambino Gesù" Children's Hospital, Rome, Italy
[o] Department of Pediatrics, Sapienza, University of Rome, Rome, Italy
[p] Department of Pediatric Hematology and Oncology, Children's Hospital, Medical School Hannover, Germany
[q] Department of Pediatric and Adolescent Medicine, Children's Hospital, University of Lübeck, Germany
[r] Department of Pediatrics, Pediatric Hemato-Oncology Unit, Children's Hospital "Carl Gustav Carus", Dresden, Germany
[s] Italian Institute for Genomic Medicine, Turin, Italy

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Abstract ***Background:*** Adolescents (aged 10–17 years) with acute lymphoblastic leukaemia (ALL) have unfavourable disease features and an inferior outcome when compared with younger children, but it is still unclear if differences in disease biology and prognosis exist between adolescents older or younger than 15 years.
Methods: We retrospectively analysed outcomes of 1094 adolescents with ALL, aged 10–17 years, treated within the AIEOP-BFM ALL 2000 trial, overall and by the age groups 10–14 and 15–17 years.
Findings: Compared with younger children (aged 1–9 years, n = 3647), adolescents had a statistically inferior 5-year event-free survival (EFS) [74.6% (1.3) vs. 84.4% (0.6)] and overall survival (OS) [83.4% (1.1) vs. 92.7% (0.4); $p < 0.001$]. Clinical and biological disease characteristics did not differ between the two subgroups of adolescents, including minimal residual disease (MRD) results during initial therapy, except for *ETV6-RUNX1* frequency and gender. With a median follow-up of 8.8 years, the 5-year EFS and OS were 76.2% (1.5) and 84.9% (1.3), respectively, for those aged 10–14 years and 70.0% (2.8) and 78.8% (2.5) for those aged 15–17 years ($p = 0.06$; 0.05). There was no significant difference in the cumulative incidence of relapses [17.8% (1.4) and 18.3% (2.4); $p = 0.98$], while the incidence of treatment-related deaths as a first event was 2.6% (0.6) versus 7.4% (1.6) ($p < 0.001$) with, in particular, a higher incidence in the high-risk arm.
Interpretation: Further prospective studies and biological investigations are required to define optimal treatment for adolescents, in particular for those aged 15–17 years. Newer agents (immunotherapy, targeted therapy) in early treatment phases of patients at higher risk of treatment failure could replace most toxic treatment elements, with the aim of reducing both toxicity and the risk of relapses.

1. Introduction

Despite the improvement of cure rates in pediatric acute lymphoblastic leukaemia (ALL), age at diagnosis continues to emerge as a prognostic factor: children older than 10 years have a worse prognosis than their younger counterparts [1–4]. This is partially related to the different biology of the disease with a higher incidence of T-ALL, Philadelphia chromosome-positive (Ph+) and Ph-like leukaemia subtypes and lower frequencies of *ETV6-RUNX1* gene fusions and high-hyperdiploid karyotypes in the older patients [1,3,5]. Poor early response to treatment is also more frequent in adolescents, with a higher frequency of patients with prednisone poor response (PPR) on day 8 of therapy and non-remission or higher minimal residual disease (MRD) levels after induction and/or consolidation therapy [1,6–9]. Consequently, there is a higher proportion of high-risk (HR) patients among older children, requiring a more intensive post-induction treatment, including allogeneic haematopoietic stem cell transplantation (HSCT) in the first complete remission (CR). Therapy-related side-effects and deaths in the first CR are thus also more frequent in this age group, in spite of the improvement of results obtained by treating teenagers with pediatric-based trials [3,10–13]. However, it is doubtful whether there are presenting clinical and biological differences, and consequently prognostic differences, between the two groups of patients with ALL aged 10–14 and 15–17 years.

Herein, we report the analysis of clinical and biological characteristics and treatment outcomes of a large cohort of older children with Ph-negative (Ph–) ALL, separated by the age, 10–14 and 15–17 years, enrolled into the AIEOP-BFM (Associazione Italiana di

Ematologia e Oncologia Pediatrica - Berlin-Frankfurt-Münster) ALL 2000 trial. Of note, the cut-off of 10 years was used for a first analysis because the National Cancer Institute (NCI) criteria, being used for stratification and having a robust prognostic relevance in most international ALL trials, still define their HR B-cell precursor ALL group by an age ≥10 years (in addition to a white blood cell [WBC] count ≥50.000/μl) [1–5,10–13]. Moreover, the World Health Organisation (WHO) defines adolescence by an age range of 10–20 years. The cut-off of 15 years was used for a second analysis to allow for a comparison with other international ALL trials reporting the outcome on adolescents with ALL and due to the fact that most toxicities, including treatment-related deaths as a first event, seem to occur significantly more frequently in older adolescents [1–5,10–13]. Although one could argue that still the definition of our age groups is arbitrary, we also used age as a continuous variable in our analysis to explore the impact of age on outcomes in adolescents with ALL.

2. Patients and methods

Between July 1 (September 1 for AIEOP) 2000 and July 31 (June 30 for BFM) 2006, a total of 4741 children and adolescents (AIEOP, n = 1999; BFM, n = 2742) with Ph− ALL, aged 1–17 years, were enrolled into the AIEOP-BFM ALL 2000 trial (Austria, Germany, Italy and Switzerland).

ALL was diagnosed according to standard cyto-morphological and cytochemical criteria, when ≥25% of lymphoblasts were present in the bone marrow (BM). Immunophenotypic as well as conventional genetic and molecular genetic analyses were centrally performed at the national reference laboratories of each participating country. Immunophenotyping by flow cytometry and analysis for *ETV6-RUNX1*, *BCR-ABL1* and *KMT2A-AFF1* fusion genes and MRD detection were performed according to consensus protocols as previously described [14–16]. Treatment details, stratification, randomisations, response criteria and indications for HSCT in the first CR of the AIEOP-BFM ALL 2000 trial have been previously published [6–8]. Complete remission was defined as a BM with active haematopoiesis and fewer than 5% leukaemic blast cells and absence of extramedullary disease. Resistance was defined as failure to achieve CR by the end of the third HR block.

All patients were stratified into three risk groups: standard-risk (SR), intermediate-risk (IR) and HR groups according to diagnostic biological features and early response to therapy, including MRD assessment. In brief, patients with PPR and no CR after completion of induction phase IA or *KMT2A-AFF1* were allocated to the HR group. In this protocol, MRD was used for final risk stratification. MRD detection was based on immunoglobulin and T-cell receptor gene rearrangements and performed centrally at two different protocol time points (TPs): TP1, at completion of phase IA (day 33), and TP2, at completion of consolidation phase IB (day 78). Those with MRD levels $\geq 5 \times 10^{-4}$ at TP2 were considered HR patients; in the absence of other HR features, patients with MRD positivity at either of the two time points but with MRD levels $< 5 \times 10^{-4}$ at TP2 were defined as IR group patients and patients with MRD negativity at TP1 and TP2, with at least two markers with a sensitivity of 10^{-4} or less, were defined as SR group patients. Central nervous system (CNS) status and testicular involvement at diagnosis were not used for risk stratification.

Informed consent was obtained from patients' parents or legal guardians before starting treatment. The study was conducted in accordance with the Declaration of Helsinki after the approval by each centre's ethical committee. The trial was registered at clinicaltrials.gov (NCT00430118 for BFM and NCT00613457 for AIEOP).

3. Supportive care

Supportive care recommendations included measures for management of hyperleukocytosis and bulky mediastinal mass, treatment and prophylaxis of tumour lysis syndrome, prevention and treatment of *Pneumocystis jirovecii* pneumonia with cotrimoxazole, Varicella zoster virus prophylaxis and guidelines for the support with blood products. Prophylaxis and treatment of febrile neutropenia and fungal infections were also recommended but performed according to each centre's local policy; responsibility was always left to the discretion of the individual treating centre.

4. Statistical analysis

Event-free survival (EFS) was calculated from the date of diagnosis to the date of the 1st event. Events considered were resistance, relapse, death or second malignant neoplasms, whichever occurred first. Time was censored at the date of the last follow-up if no event was reported. Overall survival (OS) was defined as the time from diagnosis to death from any cause or to the date of the last follow-up. EFS and OS curves were estimated according to the Kaplan-Meier method, with standard errors (SE) calculated according to the Greenwood formula; differences between groups were evaluated using the log-rank test. Cumulative incidence functions for competing events were constructed by the method of Kalbfleisch and Prentice and were compared using the Gray test. The Cox proportional hazard model was used for multivariate analyses on EFS and on the cause-specific hazard of death in the first continuous

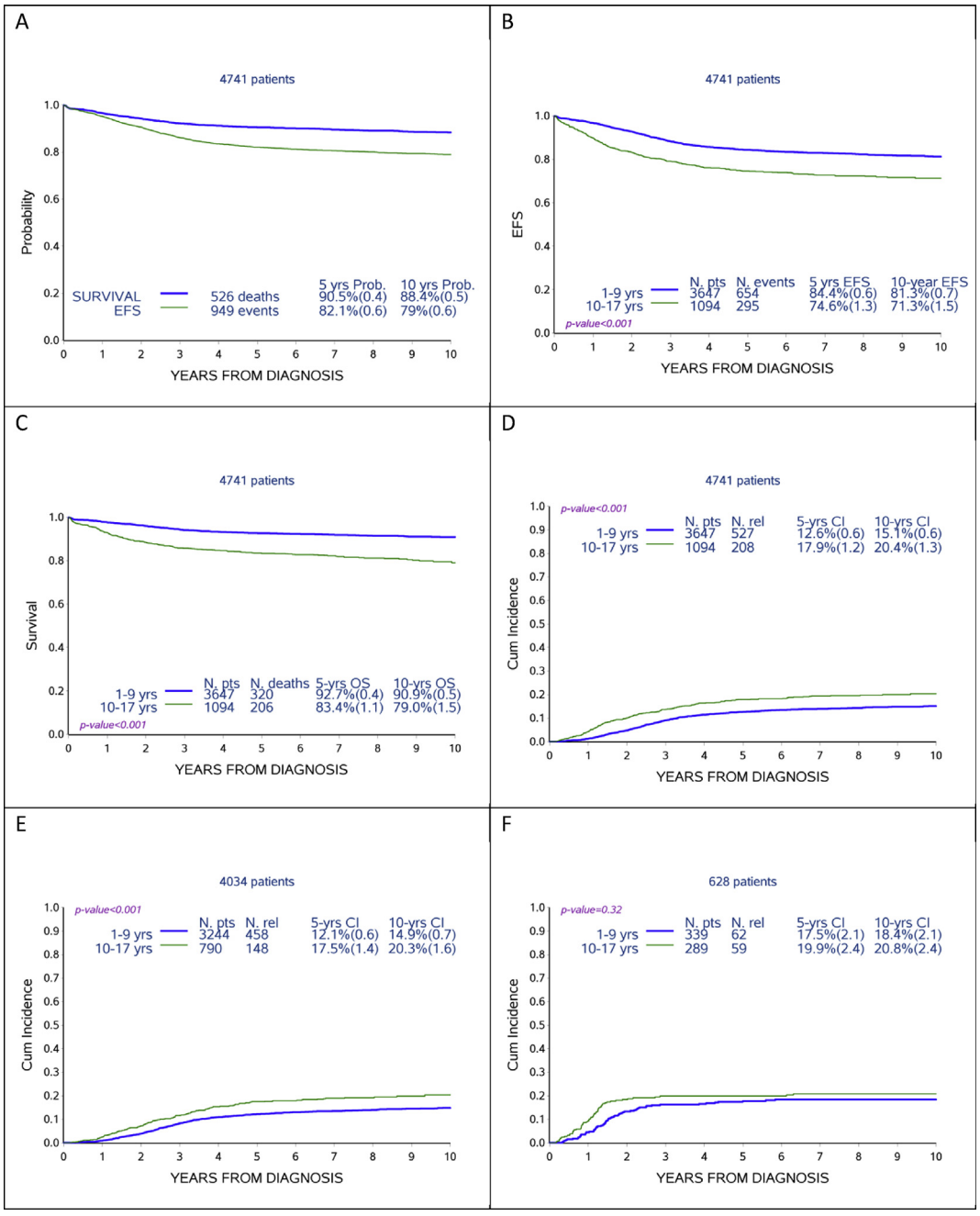

Fig. 1. **(A)** Event-free and overall survival (EFS, OS) of all 4741 patients, **(B)** EFS by age, **(C)** OS by age, **(D)** cumulative incidence of relapse (CIR) by age, **(E)** CIR by age in B-lineage ALL, **(F)** CIR by age in T-ALL.

complete remission (CCR); results were reported for all covariates considered (in particular, a time-dependent variable was used for the transplant indicator). In a separate analysis, age was used as a continuous variable to study the impact of age on EFS and death in CCR in the whole cohort of adolescents. Differences in the distribution of categorical variables were analysed using the Chi-square test. Follow-up was updated in January 2014, with a median follow-up of 8.8 years. Analyses were carried out using the SAS software, version 9.4, (SAS Institute, Cary, NC, USA).

5. Results

5.1. Comparison of adolescents (aged 10–17 years) and children (aged 1–9 years) with acute lymphoblastic leukaemia

For the entire cohort of 4741 patients enrolled in the AIEOP-BFM ALL 2000 trial, the 5-year and 10-year EFS (SE) rates were 82.1% (0.6) and 79.0% (0.6), while OS rates were 90.5% (0.4) and 88.4% (0.5), respectively (Fig. 1A). Outcomes in adolescents (n = 1094) as

compared with children aged 1–9 years (n = 3647) were significantly worse; 5-year EFS was 74.6% (1.3) vs. 84.4% (0.6) (p-value<0.001; Fig. 1B); OS was 83.4% (1.1) vs. 92.7% (0.4) (p-value<0.001; Fig. 1C) and the 5-year cumulative incidence of relapse (CIR) was 17.9% (1.2) vs. 12.6% (0.6) (p-value<0.001; Fig. 1D). Analysed by immunophenotype, the 5-year CIR was 17.5% (1.4) vs. 12.1% (0.6) in B-lineage ALL (p-value<0.001) and 19.9% (2.4) vs. 17.5% (2.1) in T-ALL (p = 0.32; Fig. 1E and F). Cumulative incidence of death in the 1st CR at 5 years was 4.0% (0.6) and 1.1% (0.2) for patients aged 10–17 years vs. those aged 1–9 years (p-value<0.001).

We compared clinical and biological characteristics and early response to treatment of the group of 1094 adolescents with those of younger children (Table 1). Adolescents differed from younger patients by showing a male predominance (58.7% vs. 54.7%; p-value = 0.02), higher proportion of hyperleukocytosis (≥100.000/μl) at diagnosis (14.5% vs. 8.6%; p-value<0.001), higher incidence of CNS involvement (4.7% vs. 3.1%; p-value<0.001) and higher frequency of T-cell immunophenotype (27.0% vs. 9.5%; p-value<0.001). The frequency of *ETV6-RUNX1* positivity was significantly lower (7.7% vs. 26.0%; p-value<0.001), and the presence of *KMT2A-AFF1* fusion genes was significantly higher (1.5% vs. 0.4%; p-value<0.001) in adolescents. Statistically significant differences were also observed for treatment response: the PPR rate was higher (14.6% vs. 8.2%; p-value<0.001) and the CR rate at the end of phase IA was lower (95.7% vs. 98.7%; p-value<0.001) in the group of adolescents. A significantly higher rate of patients with MRD-HR results or stratified to the final HR group was also observed in adolescents (MRD-HR, 13.2% vs. 6.1%, p-value<0.001; HR group, 21.0% vs. 12.0%, p < 0.001) than in younger children. A comparison of the type of events in both age groups is shown in Supplementary Table 1. Moreover, we also compared adolescent patients who all are per definition NCI HR with the younger NCI HR patients who qualified for HR by the presence of a WBC count ≥50.000/μl only (n = 646; Supplementary Table 2). Intriguingly, there was no significant difference in outcomes, with respect to neither EFS nor OS or CIR (Supplementary Fig. 1).

5.2. Comparison of adolescents aged 10–14 years and 15–17 years with acute lymphoblastic leukaemia

Of the 1094 adolescents enrolled into the trial, 811 were aged 10–14 years and 283 were aged 15–17 years (Table 1). Most of the clinical and biological characteristics, *i.e.* hyperleukocytosis and CNS involvement at diagnosis, immunophenotype, *KMT2A-AFF1* positivity and DNA index, were not significantly different between younger and older adolescents; incidence of male gender was, however, 56.6% vs. 64.7% (p = 0.02), and the proportion of patients having an *ETV6-RUNX1* gene fusion was 8.7% vs. 4.7% (p = 0.03).

Table 1
AIEOP-BFM ALL 2000 trial: Patients' characteristics and early treatment response by age.

Parameters	1–9 years		10–17 years		10–14 years		15–17 years	
	N	%	N	%	N	%	N	%
Total n. of patients	3647	*76.9*	1094		811		283	
Gender								
Male	1994	*54.7*	642	*58.7*	459	*56.6*	183	*64.7*
Female	1653	*45.3*	452	*41.3*	352	*43.4*	100	*35.3*
p-value	*0.02*				*0.02*			
WBC, × 10^9/L								
<20.0	2388	*65.5*	666	*60.9*	495	*61.1*	171	*60.4*
20.0–100.0	946	*25.9*	269	*24.6*	203	*25.0*	66	*23.3*
≥100.0	313	*8.6*	159	*14.5*	113	*13.9*	46	*16.3*
p-value	*<0.001*				*0.59*			
Immunophenotype								
B-lineage ALL	3244	*90.5*	790	*73.0*	596	*74.5*	194	*69.5*
T-ALL	339	*9.5*	289	*27.0*	204	*25.5*	85	*30.5*
Not known	64		15		11		4	
p-value	*<0.001*				*0.11*			
CNS involvement								
CNS1/2	3491	*96.9*	1004	*95.3*	747	*95.4*	257	*94.8*
CNS3	80	*3.1*	50	*4.7*	36	*4.6*	14	*5.2*
Not known	76		40		28		12	
p-value	*<0.001*				*0.70*			
ETV6-RUNX1								
Positive	865	*26.0*	76	*7.7*	64	*8.7*	12	*4.7*
Negative	2459	*74.0*	916	*92.3*	670	*91.3*	246	*95.3*
Not known	323		102		77		25	
p-value	*<0.001*				*0.03*			
KMT2A-AFF1								
Positive	13	*0.4*	15	*1.5*	11	*1.4*	4	*1.5*
Negative	2459	*99.6*	1012	*98.5*	749	*98.6*	263	*98.5*
Not known	323		67		51		16	
p-value	*<0.001*				*0.95*			
DNA index								
<0.8	9	*0.3*	4	*0.4*	2	*0.3*	2	*0.9*
0.8–1.16	2175	*77.5*	759	*88.9*	570	*89.2*	189	*87.9*
1.16–1.6	602	*21.4*	70	*8.2*	49	*7.7*	21	*9.8*
≥1.6	22	*0.8*	21	*2.5*	18	*2.8*	3	*1.4*
Not known	839		240		172		68	
p-value	*<0.001*				*0.32*			
PDN response								
Good	3326	*91.8*	921	*85.4*	689	*86.1*	232	*83.2*
Poor	299	*8.2*	158	*14.6*	111	*13.9*	47	*16.8*
Not known	22		15		11		4	
p-value	*<0.001*				*0.23*			
Response at the end of phase IA								
Remission	3560	*98.7*	1025	*95.7*	763	*96.2*	262	*94.2*
Resistant	47	*1.3*	46	*4.3*	30	*3.8*	16	*5.8*
Not appl/not known	40		23		18		5	
p-value	*<0.001*				*0.16*			
MRD stratification								
Standard	1228	*42.5*	224	*27.2*	172	*27.7*	52	*25.6*
Intermediate	1484	*51.4*	491	*59.6*	374	*60.2*	117	*57.6*
High	177	*6.1*	109	*13.2*	75	*12.1*	34	*16.8*
Not stratified	758		270		190		80	
p-value	*<0.001*				*0.23*			
Final risk								
Standard	1193	*32.7*	217	*19.8*	167	*20.6*	50	*17.7*
Intermediate	2018	*55.3*	647	*59.2*	485	*59.8*	162	*57.2*
High	436	*12.0*	230	*21.0*	159	*19.6*	71	*25.1*
p-value	*<0.001*				*0.13*			

Abbreviations: N/n, number; WBC, white blood cells; ALL, acute lymphoblastic leukaemia; CNS, central nervous system; PDN, prednisone; appl, applicable; MRD, minimal residual disease.

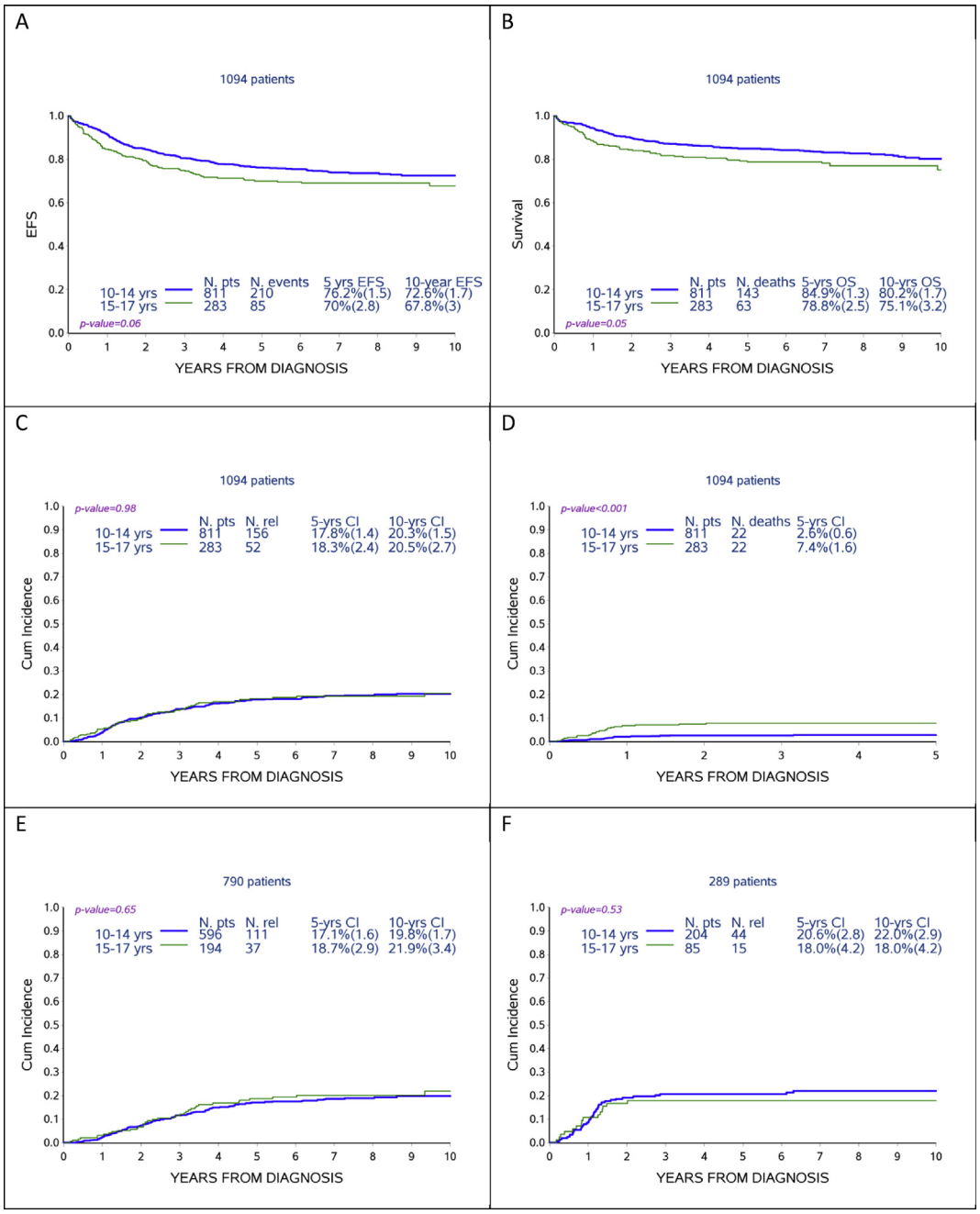

Fig. 2. **(A)** Event-free survival (EFS) by age, **(B)** overall survival (OS) by age, **(C)** cumulative incidence of relapse (CIR) by age, **(D)** cumulative incidence of death in remission as a first event by age, **(E)** CIR by age in B-lineage ALL, **(F)** CIR by age in T-ALL.

Prednisone response on day 8 was available for 1079 of 1094 (98.6%) adolescents; a PPR was detected in 13.9% and 16.8% (p-value = 0.23) of patients aged 10–14 and 15–17 years, respectively, (Table 1); a total of 1071 adolescents were evaluable for response to induction therapy: 3.8% and 5.8% of patients had ≥5% blasts at the end of phase IA in the two age groups (p = 0.16; Table 1). Eight hundred twenty-four adolescents (75%) were evaluable for MRD at TP1 and TP2: The proportion of patients with MRD-HR results were 12.1% and 16.8% (p = 0.23), and the rates of patients stratified to the final HR group were 19.6% and 25.1% (p = 0.13), respectively, in the cohorts aged 10–14 and 15–17 years (Table 1).

The probability of EFS at 5 years was 76.2% (1.5) and 70.0% (2.8) for patients aged 10–14 years and 15–17 years, respectively, (p-value = 0.06), and the probability of 5-year OS was 84.9% (1.3) for the younger and 78.8% (2.5) for the older adolescents (p-value = 0.05) (Fig. 2A and B). The rate of induction deaths was 2.3% (n = 19) and 1.8% (n = 5) for the age groups 10–14 and 15–17 years, respectively (Table 2);

Table 2
AIEOP-BFM ALL 2000 trial. Type of events by age in adolescents.

Parameters	10–14 years		15–17 years		TOTAL	
	N	%	N	%	N	%
Total n. of patients	811		283		1094	
Deaths before CR	19	*2.3*	5	*1.8*	24	*2.2*
Resistant disease	2	*0.3*	4	*1.4*	6	*0.5*
Patients in CR	790	*97.4*	274	*96.8*	1064	*97.3*
Relapses	**156**	***19.2***	**52**	***18.4***	**208**	***19.0***
BM	97	*12.0*	36	*12.7*	133	*12.2*
CNS	25	*3.1*	5	*1.8*	30	*2.7*
Testis	5	*0.6*	0		5	*0.5*
BM + other	20	*2.5*	7	*2.5*	27	*2.5*
Other	8	*1.0*	4	*1.4*	12	*1.1*
Not known	1	*0.1*	0		1	*0.1*
Deaths in CCR	**22**	***2.7***	**22**	***7.8***	**44**	***4.0***
After chemotherapy[a]	14	*1.7*	13	*4.6*	27	*2.5*
After HSCT	8	*1.0*	9	*3.2*	17	*1.5*
SMN	11	*1.4*	2	*0.7*	13	*1.2*
Alive in CCR	601	*74.1*	198	*70.0*	799	*73.0*
Months from diagnosis to relapse						
<18	66	*8.1*	24	*8.5*	90	*8.2*
18–30	33	*4.1*	11	*3.9*	44	*4.0*
≥30	57	*7.0*	17	*6.0*	74	*6.8*

Abbreviations: N/n, number; CR, complete remission; BM, bone marrow; CNS, central nervous system; CCR, continuous complete remission; SMN, second malignant neoplasm; HSCT, haematopoietic stem cell transplantation (76 and 33 transplanted patients among patients aged 10–14 (9.4%) and 15–17 (11.7%) years, respectively).

[a] Phases of deaths: in patients aged 10–14 years, 4 occurred in HR patients [after phase IA (n = 2), during block therapy (n = 1) or re-induction (n = 1)] and 10 in non-HR patients [after phase IA (n = 2), after re-induction (n = 7) and after off-therapy (n = 1)]; in patients aged 15–17 years, 6 occurred in HR patients [after phase IA (n = 2), during block therapy (n = 3) or maintenance (n = 1)] and 7 in non-HR patients [after phase IA (n = 3) and after re-induction (n = 4)].

the cause of death (unknown in 2 patients) was infection (n = 16), pancreatitis (n = 1), cerebral thrombosis (n = 1), intracranial bleeding (n = 1), multi-organ failure (n = 1), heart failure (n = 1) and gastrointestinal bleeding (n = 1). Resistant disease occurred in 0.3% (n = 2) and 1.4% (n = 4), while CR was achieved in 97.4% and 96.8% in the two age groups (Table 2).

Two-hundred eight adolescents (19%) suffered from relapse; the CIR was not significantly different in the two adolescent ALL groups, with a 5-year estimate of 17.8% (1.4) and 18.3% (2.4), respectively, for patients aged 10–14 and 15–17 years (p-value = 0.98; Fig. 2C), with no difference in both B-lineage or T-ALL (Fig. 2E and F). Type and time of relapse are shown in Table 2. Most relapses occurred in the BM (160 BM relapses: isolated, n = 133; combined, n = 27); isolated extramedullary relapses were observed in 35 patients (CNS, n = 30; testis, n = 5); 12 other extramedullary relapses occurred. In the groups of adolescents aged 10–14 and 15–17 years, BM (isolated or combined) was involved in 14.5% and 15.2% and CNS (isolated) in 3.1% and 1.8% of patients, respectively. Relapses occurred very early (<18 months from diagnosis) in 90 patients, early (18–30 months) in 44 and late (≥30 months) in the remaining 74 patients. The distribution by time of relapse was similar in the two age groups (Table 2). In T-ALL, most relapses were observed very early in 50/59 patients (84.7%), while in B-lineage ALL, they occurred very early in 40 of 148 (27.0%), early in 39 of 148 (26.4%) and late in 69 of 148 (46.6%) patients, with no difference between the two age groups (Supplementary Tables 3A and 3B).

Forty-four patients died in CR due to infections (n = 22), multi-organ failure (n = 2), cerebral thrombosis (n = 1), gastrointestinal bleeding (n = 1), car accident (n = 1) or stem cell transplantation (n = 17). Cumulative incidence of death in CR at 5 years was 2.6% (0.6) and 7.4% (1.6) in the two age groups (p-value<0.001; Fig. 2D). Of 27 non-transplanted patients who died in CCR, 10 occurred in the 230 HR patients: 4 deaths occurred in 159 patients aged 10–14 years [cumulative incidence: 2.5% (1.2)] and 6 among 71 patients aged 15–17 years [cumulative incidence: 8.5% (3.3), p-value = 0.04]. The remaining 17 deaths occurred in the 864 non-HR patients: 10 deaths occurred in 652 patients aged 10–14 years [cumulative incidence: 1.5% (0.5)] and 7 in 212 patients aged 15–18 years [cumulative incidence: 3.3% (1.2), p-value = 0.10]. When performing a detailed analysis of the HR patients only, evaluation of deaths in CCR shows similar rates for patients aged 1–9 and 10–14 years (3.2% and 4.7% after chemotherapy

Table 3
AIEOP-BFM ALL 2000 trial: Death in CCR as a first event by age among the cohort of HR group patients only.

Parameters	1–9 years, N = 436		10–14 years, N = 159		15–17 years, N = 71	
	N	%	N	%	N	%
Total n. of chemo patients	**311**		**85**		**38**	
Deaths in CCR after chemo	10	*3.2*	4	*4.7*	6	*15.8*
Consolidation therapy	2		2		2	
Block therapy	6		1		3	
Re-induction therapy	0		1		0	
Maintenance therapy	2		0		1	
Total n. of patients who underwent HSCT	125		74		33	
Deaths in CCR after HSCT	12	*9.6*	8	*10.8*	8	*24.2*

Abbreviations: N/n, number; CCR, continuous complete remission; chemo, chemotherapy; HSCT, haematopoietic stem cell transplantation.

and 9.6% and 10.8% after HSCT), while for patients aged 15–17 years, death rates were 15.8.% and 24.2%, respectively (Table 3).

Secondary malignancies occurred in 11 of 811 (1.4%) and 2 of 283 (0.7%) patients aged 10–14 and 15–17 years, respectively (Table 2), with a 5-year cumulative incidence of 0.7% (0.3) and 0.8% (0.6), respectively.

5.3. Prognostic features in adolescents with acute lymphoblastic leukaemia

A Cox model was fitted on EFS, separately on adolescents with B-lineage and T-ALL, as shown in Table 4. In both analyses, age 15–17 vs. age 10–14 years did not have a significant impact on EFS when adjusted for other prognostic features. In particular, in patients with B-lineage ALL, a significant impact was found for hyperleukocytosis (≥100,000 vs. <20,000/μl: hazard ratio = 2.46, p-value<0.001), stratification to the final HR group (HR vs. non-HR: hazard ratio = 2.06, p-value<0.001) and *ETV6-RUNX1* (positive vs. negative: hazard ratio = 0.48, p-value = 0.03). For T-ALL, the presence of HR criteria (HR vs. non-HR: hazard ratio = 2.56, p-value<0.001) and male gender (hazard ratio = 2.03, p-value = 0.02) were associated with a significantly worse outcome. When age was considered as a continuous variable (by year) among the whole cohort of adolescent patients (aged 10–17 years), the estimated hazard ratio for each additional year was 1.05 for B-lineage ALL and 1.01 for T-ALL, both not significantly different from 1 (p-value = 0.19 and p-value = 0.81, respectively). The results of the remaining variables were superimposable.

A Cox model was fitted on the whole group of adolescents to analyse the hazard of death in CCR (44 fatalities). This was associated with age (15–17 vs. age 10–14: hazard ratio = 2.69, p-value = 0.001), presence of HR criteria (HR vs. non-HR: hazard ratio = 2.98, p-value = 0.008) and HSCT in the first CR (transplanted vs. non-transplanted: hazard ratio = 6.14, p-value<0.001), while immunophenotype did not have a significant impact on mortality in CCR (T-ALL vs. B-lineage ALL: 0.58, p-value = 0.11, Supplementary Table 4). When age was considered as a continuous variable (by year) among the whole cohort of adolescent patients (aged 10–17 years), the estimated hazard ratio for each additional year was 1.31 (p-value<0.001) and the results of the remaining variables were superimposable.

6. Discussion

Our data confirm previously published findings that patients with ALL older than 10 years are more likely to present with hyperleukocytosis, a T-cell immunophenotype, and poor early response to therapy and a lower frequency of favourable prognostic genetic features such as *ETV6-RUNX1* positivity as compared with those aged 1–9 years [1,4,17,18]. Accordingly, adolescents end up significantly more often in the HR group, necessitating the most intensive (*i.e.*, block) chemotherapy and eventual HSCT in the 1st CR.

In the present study, we have retrospectively analysed an adolescent population of 1094 patients treated with the AIEOP-BFM ALL 2000 protocol, stratifying patients into three age groups: 1–9, 10–14 and 15–17 years. Clinical and biological characteristics at initial

Table 4
Multivariate Cox regression analysis on EFS in adolescent patients with B-lineage (n = 717) and T-ALL (n = 289) with known covariates.

Parameters	B-lineage ALL			T-ALL		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Age[a]						
10–14 years	1			1		
15–17 years	1.27	0.93–1.74	0.14	0.99	0.62–1.62	0.99
Gender						
Male	1.10	0.83–1.47	0.49	2.03	1.11–3.73	0.02
Female	1			1		
WBC × 10^9/L						
<20.0	1			1		
20.0–100.0	1.04	0.73–1.49	0.82	1.15	0.63–2.10	0.65
≥100.0	2.46	1.60–3.77	<0.001	1.23	0.67–2.26	0.50
ETV6-RUNX1						
Positive	0.48	0.24–0.95	0.03			
Negative	1					
FINAL RISK						
Non-high risk	1			1		
High	2.06	1.45–2.93	<0.001	2.56	1.58–4.12	0.001

Abbreviations: ALL, acute lymphoblastic leukaemia; CI, confidence interval; WBC, white blood cells.

[a] When age was considered as a continuous variable (by year) among the whole cohort of adolescent patients (aged 10–17 years), the estimated hazard ratio for each additional year was 1.05 for B-lineage ALL and was 1.01 for T-ALL, both not significantly different from 1 (p-value = 0.19 and p-value = 0.81, respectively). The results of the remaining variables were superimposable.

diagnosis did not differ between the two adolescent age groups, except for gender and *ETV6-RUNX1* positivity. No statistically significant difference was observed with regards to early response to therapy as measured by the rate of PPR, resistance to induction phase IA and MRD-HR levels.

Overall results obtained in this study in terms of EFS (76.2% vs. 70.0%) and OS (84.9% vs. 78.8%), for age groups 10–14 vs. 15–17 years, confirm the favourable outcome reported for adolescents until the age of 18 years, treated with pediatric ALL protocols [4,17–22]. In smaller series of adolescents (n = 159) enrolled into two consecutive pediatric Dana-Farber Cancer Institute (DFCI) Consortium Protocols, the 5-year EFS rates were 77% and 78% for patients aged 10–14 and 15–18 years, respectively, with no difference in the percentage of treatment-related complications between the two age groups [17]. More recently, the Tokyo Children's Cancer Study Group (TCCSG) reported a similar probability of EFS and OS in younger adolescents (n = 332; 66.4% and 74.2%, aged 10–14 years) and older adolescents (n = 41; 67.5% and 70.7%, aged 15–18 years), treated with 3 consecutive pediatric protocols from 1995 to 2006, including BFM-type intensive blocks for HR patients [18]. Both studies conclude that intensified treatment improves the prognosis in adolescents with ALL, although the outcome in this age group remains inferior to that of children younger than 10 years.

Our study on a large cohort of homogenously treated patients confirms the aforementioned findings and shows that the outcome in adolescents (aged 10–17 years) is statistically inferior to that obtained in younger children owing to an increased rate of both relapse and death in CCR. In adolescents, tolerance to intensive treatment was particularly poor in the older age group, in particular during post-induction/consolidation chemotherapy and because of transplant-related complications (death in CCR including transplant-related mortality: 7.8% vs 2.7% in patients aged 15–17 vs. 10–14 years for all risk groups), and this was even more evident when restricting the analysis to the HR group only. On the other hand, the relapse rate was similar (CIR at 5 years about 18–19%) in both age groups. Toxicity data clearly indicate that strict monitoring/vigilance and supportive therapy must be maximised in adolescents undergoing intensive therapy or HSCT and that further intensification of conventional treatment to improve results in adolescent age may not be tolerable and/or useful. Of note, life-threatening serious adverse events in the trial AIEOP-BFM ALL 2000 were twice or even more than twice as much in the group of patients aged 10–14 and 15–17 years, respectively, than in the group of patients aged 1–9 years among both the non-HR (9.7% vs. 12.7% vs. 5.1%) and HR groups (15.7% vs. 18.3% vs. 8.0%; Supplementary Table 5).

In recent years, several new therapies have emerged in the treatment of ALL and are currently used in the relapsed/refractory setting, such as proteasome inhibitors, antibody- or cell-mediated immunotherapy [*i.e.* blinatumomab, inotuzumab or chimeric antigen receptor (CAR)–T-cell therapy] and other targeted therapies [23–25]. These agents should be investigated in front-line regimens, particularly for patients with primary resistant disease or persisting high MRD levels, for eradicating the disease. These treatments may be less toxic than intensive chemotherapy and may improve survival as shown in adult ALL, while reducing the need for HSCT [24–27]. Our Cox regression analysis shows that the age of 15–17 years (vs. 10–14 years) is not independently associated with a statistical difference in EFS when other prognostic factors are considered, while it is for the risk of death in the first CR, confirming that also for this subgroup of patients alternative treatment strategies definitely merit to be investigated.

Interestingly, in an adolescent population, an increased incidence of Ph-like ALL (20–25%), a novel subtype of HR B-lineage ALL showing a gene expression profile similar to Ph + ALL, with a diverse range of genetic alterations that can activate kinase signalling, has been recently reported [28]. The feasibility of defining Ph-like ALL in real time and the impact of adding tyrosine kinase inhibitors to chemotherapy, however, need to be investigated [28,29].

According to the data on treatment-related fatalities presented herein, and also with the aim to further improve prognosis of all HR B-lineage patients per se, the AIEOP-BFM study group has decided to randomise 2 courses of blinatumomab against the 2nd and 3rd HR block in the AIEOP-BFM ALL 2017 trial (ClinicalTrials.gov NCT03643276), considering that blinatumomab-related toxicities are significantly less and mostly correlating with tumour burden which at that time point of therapy is usually very low (molecularly refractory disease) or undetectable. Moreover, we suppose that patients randomised to blinatumomab and having an indication for HSCT may also benefit from a reduced burden of toxicity when entering the transplant phase. In parallel, the role of CAR-T-cell therapy in the front-line setting is also going to be studied for HR B-lineage patients as an alternative to HR blocks and HSCT (ClinicalTrials.gov NCT0391128), namely by the ALLTogether consortium within their first multinational trial. Outcome and toxicity data of both large-scale trials will hopefully show where the best position of such immunotherapies in primary disease is and, in particular, whether adolescents with ALL suffering the most from high-risk conventional chemotherapy may have a benefit from them with respect to both toxicity and prognosis.

In conclusion, among a cohort of >1000 adolescent patients with ALL, our data indicate that the clinical characteristics and disease outcomes of adolescents aged 10–14 years are similar to those of adolescents aged 15–17 years except for treatment-related deaths in the

first CR which are significantly more frequent in the older adolescent age group. Further prospective studies and biological investigations are required for treatment optimisation in this subgroup. The continued refinement of MRD shall allow the decrease of treatment intensity for lower risk patients and the identification of patients who may benefit most from newer agents to improve both outcome and decrease toxicity. Continued understanding of the molecular biology of the disease will facilitate the application of appropriate targeted therapies.

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Conflict of interest statement

The authors declare no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.09.004.

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