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CME Article

International cooperative study identifies treatment strategy in childhood ambiguous lineage leukemia

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KEY POINTS

- **The largest cohort of ambiguous leukemias to date reveals a better prognosis of children who started on lymphoid-directed treatment.**
- **Myeloid-type primary treatment correlated with dismal outcomes in CD19⁺ leukemias.**

Despite attempts to improve the definitions of ambiguous lineage leukemia (ALAL) during the last 2 decades, general therapy recommendations are missing. Herein, we report a large cohort of children with ALAL and propose a treatment strategy. A retrospective multinational study (International Berlin-Frankfurt-Münster Study of Leukemias of Ambiguous Lineage [iBFM-AMBI2012]) of 233 cases of pediatric ALAL patients is presented. Survival statistics were used to compare the prognosis of subsets and types of treatment. Five-year event-free survival (EFS) of patients with acute lymphoblastic leukemia (ALL)–type primary therapy (80% ± 4%) was superior to that of children who received acute myeloid leukemia (AML)–type or combined-type treatment (36% ± 7.2% and 50% ± 12%, respectively). When ALL- or AML-specific gene fusions were excluded, 5-year EFS of CD19⁺ leukemia was 83% ± 5.3% on ALL-type primary treatment compared with 0% ± 0% and 28% ± 14% on AML-type and combined-type primary treatment, respectively. Superiority of ALL-type treatment was documented in single-population mixed phenotype ALAL (using World Health Organization and/or European Group for Immunophenotyping of Leukemia definitions) and bilineal ALAL. Treatment with ALL-type protocols is recommended for the majority of pediatric patients with ALAL, including cases with CD19⁺ ALAL. AML-type treatment is preferred in a minority of ALAL cases with CD19⁻ and no other lymphoid features. No overall benefit of transplantation was documented, and it could be introduced in some patients with a poor response to treatment. As no clear indicator was found for a change in treatment type, this is to be considered only in cases with ≥5% blasts after remission induction. The results provide a basis for a prospective trial. (*Blood*. 2018;132(3):264-276)

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Disclosures

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Learning objectives

Upon completion of this activity, participants will be able to:

1. Describe survival in children with different types of ambiguous lineage leukemia (ALAL) receiving different types of treatment, based on a multicenter international study
2. Describe treatment recommendations for children with ALAL, as developed from findings of an international study
3. Describe recommendations for changing treatment and for use of transplantation in children with ALAL, as developed from findings of an international study

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Introduction

Cases with acute leukemias (ALs) are typically diagnosed as belonging to either the lymphoid or myeloid lineage. However, 2% to 5% of AL cases fulfill the definition of ambiguous lineage leukemia (ALAL). The following 4 different types of lineage ambiguities have been described: (1) single-population mixed-phenotype leukemias (MPALs; also known as biphenotypic ALs), which share immunophenotypic features of lymphoblastic and myeloid AL (acute lymphoblastic leukemia [ALL] and acute myeloid leukemia [AML], respectively); (2) bilineal ALs, in which 2 separate clones of different lineages coexist; (3) undifferentiated ALs, in which the criteria of ALL and AML are not met due to a paucity of positive markers of any of the 2 diseases; and (4) ALs with an early switch to a different lineage, in which the phenotype of leukemic cells changes to a different lineage during treatment (usually during the early phases).[1-3] This type of ALAL was not included in this study, unless this switch was combined with 1 of the 3 aforementioned ALAL presentation features.

Most of these leukemias fulfill 1 of the 2 international definitions of MPALs, both of which principally rely on flow cytometry. An older definition by the European Group for Immunophenotyping of Leukemia (EGIL) uses a spectrum of antigens weighted by their presumed significance.[4,5] A newer definition by World Health Organization (WHO) utilizes fewer antigens[6,7] (supplemental Table 1, available on the *Blood* Web site). In general, the prognosis of MPALs is slightly worse than that of non-MPALs on the same type of treatment.[8-11]

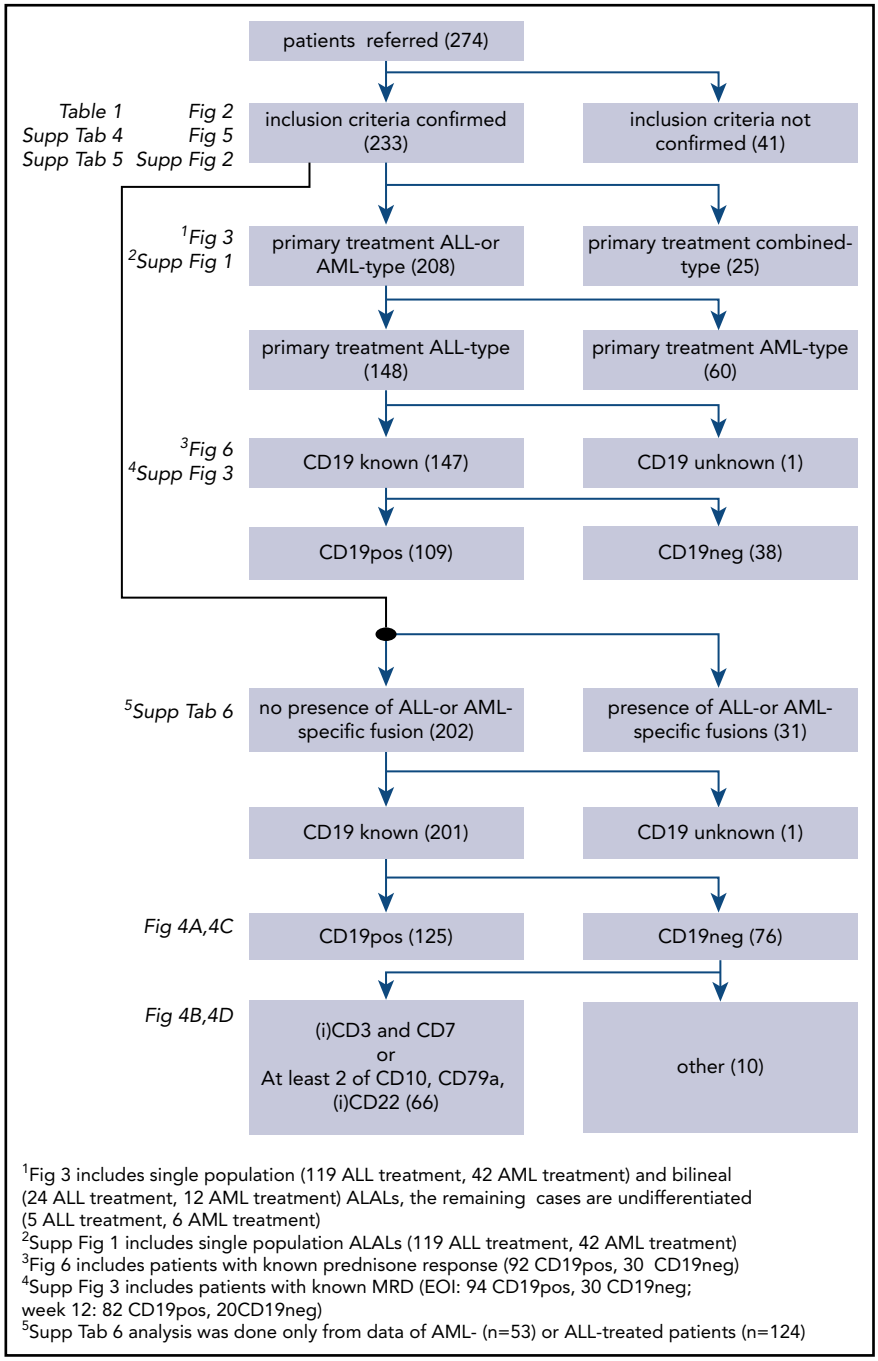
The spectrum of underlying genetic aberrations is wide. Whereas fusion genes with *KMT2A* (*MLL*) contribute to more myeloid or lymphoid phenotypes partly depending on the fusion partner,[12] other fusions are typically lymphoid with a few myeloid markers and only occasionally have a full ambiguous phenotype (*ETV6/RUNX1*) or analogically with myeloid lineage (*RUNX1/RUNX1T1*). These fusion genes, however, are present only in a minority of ALALs,[9] and the majority of ALALs are genetically heterogeneous. Next-generation sequencing techniques are likely to discover not only further heterogeneity but also more recently identified recurrent genetic aberrations such as the *ZNF384* gene involving fusions, which occur especially in B-myeloid ALAL.[13-15,36] Mutations in *TP53*, which are generally more frequent in adult leukemias, were expectedly found mostly in adult ALALs.[16]

The aim of the present study was to collect a detailed data on biological features, treatment, and outcome of ALALs in order to set up treatment recommendations that can be tested prospectively.

Patients and methods

Patient accrual

Eighteen centers participated in the iBFM-AMBI2012 study. These centers were located in Australia (single hospital), Austria, Brazil (single hospital), Czechia, Germany, Greece (single hospital), Israel (single hospital), Italy, Netherlands, the NOPHO group (representing Denmark, Estonia, Finland, Norway, Sweden, Iceland, and Lithuania), PINDA (Chile), Poland, SAHOP (Argentina), Slovakia, St. Jude Children's Research Hospital and Baylor College of Medicine (United States, 2 institutions), Ukraine, and the United Kingdom. Each center supplied data on consecutive children to a Web-based database. The periods of referral differed between the centers and lasted 1 to 12 years, except for Greece, which reported 3 isolated cases (supplemental Table 2). Patients diagnosed before 2002 or after 1 June, 2015 were excluded from this study. The fulfillment of the inclusion criteria was then verified by 3 of the authors (V.d.H.,



[1]Fig 3 includes single population (119 ALL treatment, 42 AML treatment) and bilineal (24 ALL treatment, 12 AML treatment) ALALs, the remaining cases are undifferentiated (5 ALL treatment, 6 AML treatment)
[2]Supp Fig 1 includes single population ALALs (119 ALL treatment, 42 AML treatment)
[3]Fig 6 includes patients with known prednisone response (92 CD19pos, 30 CD19neg)
[4]Supp Fig 3 includes patients with known MRD (EOI: 94 CD19pos, 30 CD19neg; week 12: 82 CD19pos, 20CD19neg)
[5]Supp Tab 6 analysis was done only from data of AML- (n=53) or ALL-treated patients (n=124)

Figure 1. Flowchart. Rectangles contain selection criteria in each step, followed by the number of patients in parentheses. Tables or figures mentioned next to each rectangle depict all patients from the respective node unless a selection is specified in a footnote.

J. Stancikova, and O.H.). Except when excluded patients are explicitly mentioned, all analyses address included patients only.

Inclusion criteria

Patients with AL diagnosed or referenced in one of the participating centers within the study period before 18 years of age were considered for analysis. Definition of a single-population biphenotypic/mixed lineage leukemia or bilineal leukemia or undifferentiated leukemia was fulfilled. For single-population ALAL, the immunophenotypic criteria of EGIL and/or WHO were met. Cases with >1 significant population, each belonging to a different lineage, were considered bilineal. Blast populations that did not fulfill the criteria of B, T, or myeloid lineages were considered undifferentiated. Subsequently, we excluded patients who had a genetic disease that predisposes to leukemia. Lastly, we excluded patients who received only palliative treatment or other treatment not considered as ALL type, AML type, or combined type (supplemental Table 3).

Statistics

For event-free survival (EFS) calculations, the uncensored event was death of any cause, relapse of leukemia, and secondary malignancy. The censoring times included time to a treatment type change unless we explicitly mention that treatment changes are disregarded. In other instances, censoring times reflected the end of follow up without an event. For univariate comparisons of EFS, a log-rank (Mantel-Cox) test was used. For the analysis of possible benefit of stem cell transplantation

Table 1. Patient characteristics and primary choice of treatment

	Primary protocol type				Type of ambiguity			
	ALL	Combined	AML	χ^2 P*	Bilineal	Single population	Undifferentiated	χ^2 P*
Total	148 (100)	25 (100)	60 (100)		45 (100)	176 (100)	12 (100)	
Sex								
Female	62 (42)	12 (48)	30 (50)	.53	19 (42)	79 (45)	6 (50)	.88
Male	86 (58)	13 (52)	30 (50)		26 (58)	97 (55)	6 (50)	
WBC count, ×10⁹/L								
<20	88 (59)	8 (32)	24 (40)	.002	19 (42)	96 (55)	5 (42)	<.0001
20 to 100	42 (28)	7 (28)	18 (30)		6 (13)	57 (32)	4 (33)	
≥100	18 (12)	10 (40)	18 (30)		20 (44)	23 (13)	3 (25)	
Age at diagnosis, y								
Infant	1 (0.68)	13 (52)	8 (13)	<.0001	8 (18)	11 (6)	3 (25)	.087
1 to <6	50 (34)	4 (16)	15 (25)		10 (22)	57 (32)	2 (17)	
6 to <11	45 (30)	4 (16)	11 (18)		12 (27)	44 (25)	4 (33)	
11 to <18	52 (35)	4 (16)	26 (43)		15 (33)	54 (31)	3 (25)	
CNS leu								
No	133 (90)	15 (60)	47 (78)	.0001	30 (67)	156 (89)	9 (75)	.0002
Yes	10 (6.8)	9 (36)	10 (17)		14 (31)	14 (8)	1 (8.3)	
Not known*	5 (3.4)	1 (4)	3 (5)		1 (2.2)	6 (3.4)	2 (17)	
Type of ambiguity								
Bilineal	24 (16)	9 (36)	12 (20)	.048	N/A	N/A	N/A	N/A
Single population	119 (80)	15 (60)	42 (70)		N/A	N/A	N/A	
Undifferentiated	5 (3.4)	1 (4)	6 (10)		N/A	N/A	N/A	
Follow-up status								
Alive with leukemia	0 (0)	0 (0)	3 (5)	<.0001	0 (0)	3 (1.7)	0 (0)	.0001
CR1	115 (78)	14 (56)	22 (37)		21 (47)	126 (72)	4 (33)	
CR2 or higher	3 (2)	3 (12)	6 (10)		1 (2.2)	9 (5.1)	2 (17)	
Death from leukemia	17 (11)	6 (24)	22 (37)		12 (27)	28 (16)	5 (42)	
Death from toxicity	11 (7.4)	2 (8)	5 (8.3)		9 (20)	8 (4.5)	1 (8.3)	
Death from unknown reason	1 (0.7)	0 (0)	1 (1.7)		2 (4.4)	0 (0)	0 (0)	
Lost to follow-up*	1 (0.68)	0 (0)	1 (1.7)		0 (0)	2 (1.1)	0 (0)	

Values are presented as n (%) of patients, unless otherwise indicated.

CNS leu, leukocyte (including pathological cells) presence in cerebrospinal fluid at diagnosis; N/A, not applicable.

*For χ^2 calculations, unknown items are not considered.

(SCT), a delayed-entry statistics was applied, in which all events (deaths, relapses, or secondary malignancy onsets) preceding the median time to SCT for the analyzed subcohort were considered censored times.

For multivariate Cox regression, the following variables were considered: referring center, age, sex, number of white blood cells (WBCs) per liter, central nervous system infiltration, fulfillment of WHO/EGIL criteria, categorization to single population/bilineal/undifferentiated, estimate of lineage by Association of Pediatric Hematology Oncology/Berlin-Frankfurt-Münster flow,[17] positivity of *KMT2A/AFF1*, lineages involved using EGIL or WHO criteria, frequency of blasts, and positivity of CD1a, CD2, CD3, intracellular (i) CD3, CD5, CD7, CD8, CD10, CD117, CD11c, CD13, CD14, CD15, CD19, CD20, CD22, iCD22, CD24, CD33, CD64, CD65, iCD79a, intracellular immunoglobulin M, iLysozyme, intracellular myeloperoxidase (iMPO), T-cell receptor αβ, T-cell receptor γδ, and intracellular terminal deoxynucleotidyltransferase. Factors with no correlation with the outcome in univariate pre-assessment ($P > .2$) were considered irrelevant, leaving WBC count and positivity of *KMT2A/AFF1*, CD2, CD10, CD11c, CD22, iCD22, CD24, CD64, iMPO, and intracellular terminal deoxynucleotidyltransferase included in the analysis for ALL-treated patients. For AML-treated patients, the following factors were considered relevant and included in the analysis: age at diagnosis, WBC count, and positivity of *KMT2A/AFF1*, CD2, iCD3, CD7, CD14, CD19, CD22, iCD79a, and iMPO.

Results

Characteristics of the included patients

In total, data on 274 patients were available. A CONSORT diagram of the entire study is shown in Figure 1. Of these, 233 cases fulfilled

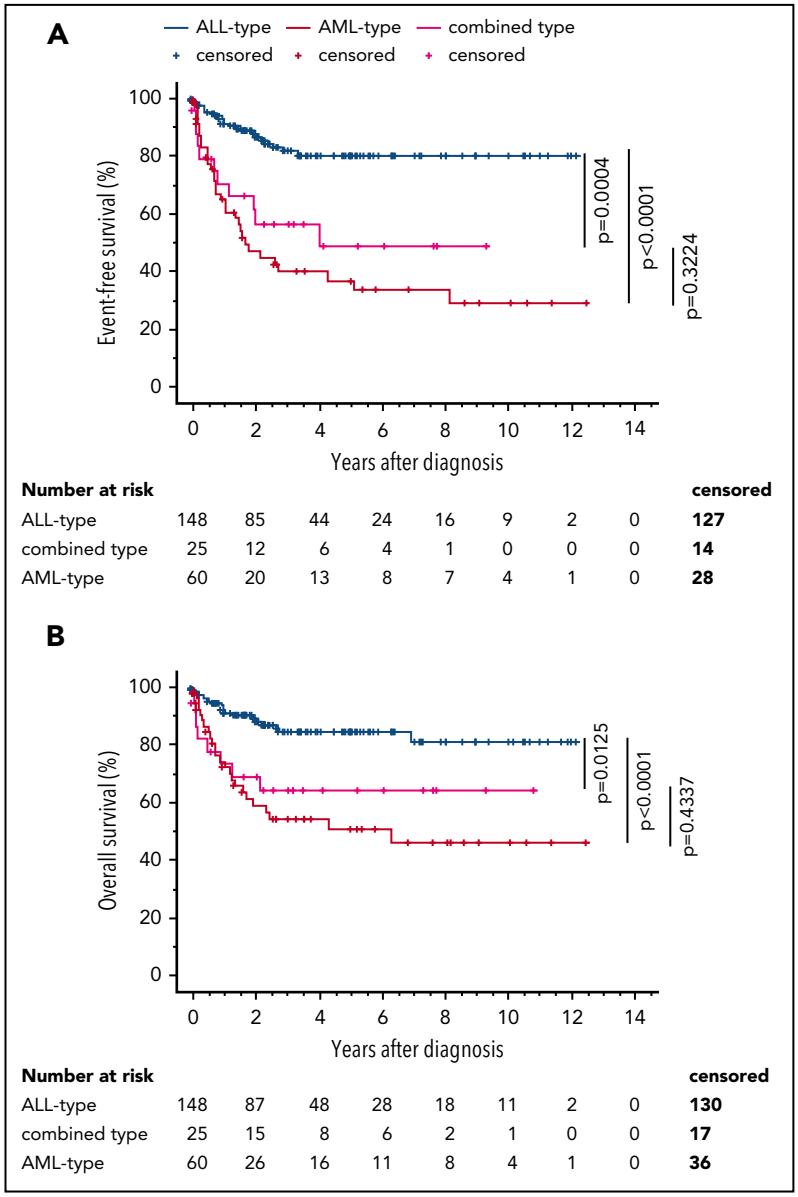


Figure 2. Outcome of patients with ALAL by the primary type of treatment. (A) EFS. (B) Overall survival. A log-rank (Mantel-Cox) statistical test was used.

the inclusion criteria. The exclusion of noneligible cases (n = 41) did not noticeably affect the 5-year EFS of the entire cohort (62% ± 3.6% and 65% ± 3.8% before and after exclusion, respectively). The predisposing genetic diseases that resulted in exclusion from the cohort (n = 8) comprised Fanconi anemia (n = 2), Down syndrome (n = 4), and *GATA2* mutations (n = 2). Patients excluded for not having received or not having been assigned to any type of therapy (n = 4) or with a missing information regarding primary therapy (n = 3) partially overlapped with the ones excluded for predisposing genetic features. The rest of the article describes only the included patients. Demographic and diagnostic information is listed in Table 1, together with the primary chemotherapy. In total, 176 patients had single-population ALAL, 45 had bilineal ALAL, and 12 had undifferentiated leukemia. Of the single-population ALAL, 103 fulfilled WHO and EGIL definitions, 42 fulfilled EGIL only, and 31 fulfilled WHO only. Distribution of cases into genetic categories demonstrates the heterogeneity of ALAL and the correlation with the physicians' choice of primary treatment type or lineages involved (supplemental Table 4).

Selection of the treatment type

Due to the specific nature of ALAL, various treatment protocols or experimental therapies were applied. Supplemental Table 5 lists the different choices for initial therapy.

The outcome of patients who started on ALL-type treatment was superior (5-year EFS, 80% ± 4.0%) to that of patients who started on AML-type or combined-type (ALL/AML) treatment (5-year EFS, 36% ± 7.2% and 50% ± 12%, respectively; Figure 2). No significant prognostic difference was observed between AML- and combined-type primary therapies. Treatment toxicity was in general relatively high, accounting for 18 out of 63 deaths, and was seemingly evenly distributed between ALL-, combined-, or AML-type primary therapy (7.4%, 8%, and 8.3% patients on the respective primary treatment). In total, 69 children underwent SCT, and 20 of them (29%) died (4 [5.8%] from toxicity and 16 [23%] from leukemia progression).

Genetic categories

The genetic categories of the patients document a diversity of ALAL. The genetic findings of the patients in the study are shown

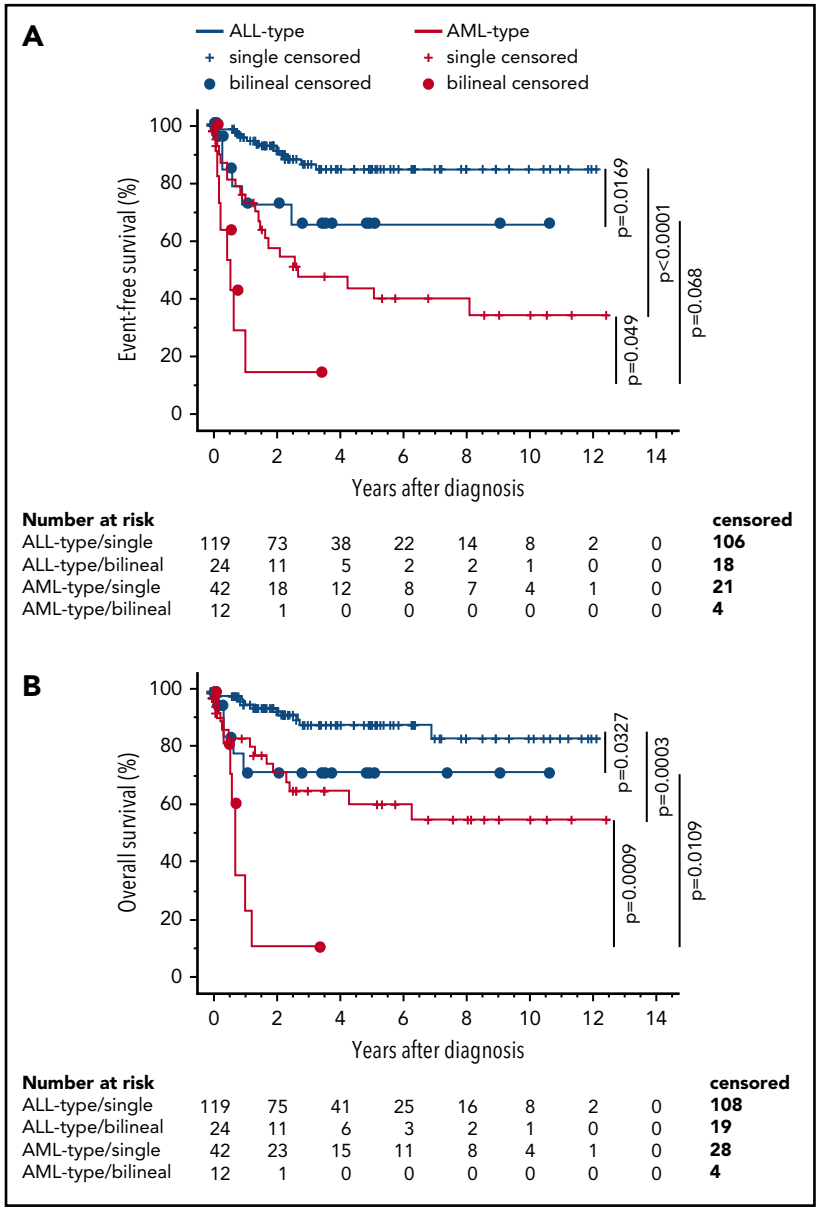


Figure 3. Outcome of patients with ALAL on different treatment types. Single-population ALAL vs bilineal ALAL. (A) EFS. (B) Overall survival. A log-rank (Mantel-Cox) statistical test was used.

in correlation to the primary type of therapy and type of ambiguity in supplemental Table 4.

Prognostic impact of ambiguity types

Patients with bilineal ALAL (Figure 3) fared better on ALL therapy than on AML therapy (5-year EFS, 65% ± 12% and 14% ± 13%) and in general fared worse than single-population ALAL (5-year EFS, 84% ± 4.2% and 43% ± 8.9% for ALL and AML therapy, respectively). Supplemental Figure 1 demonstrates that the better prognosis on ALL-type primary therapy holds true for ALAL defined by the EGIL criteria only and for ALAL defined by WHO criteria or both.

Typical ALL- or AML-specific gene fusions

As patients with molecular genetic findings that are considered to be unequivocally ALL (*ETV6/RUNX1* or *TCF3/PBX1*) or AML (*RUNX1/RUNX1T1*, *PML/RARA*, or *CBFB/MYH11*)[12,18] should be guided as such in treatment recommendations, we have not included these patients in the subsequent analyses. In patients with *BCR/ABL1* fusion, ALL-type treatment with tyrosine kinase inhibitors is indicated,[19] and *BCR/ABL1*$^+$ cases were also excluded from further analyses. Typically, *TCF3/PBX1* leads to nonambiguous ALL and *CBFB/MYH11* leads to nonambiguous AML. In line with this rule, no patient in our study was identified with *TCF3/PBX1* or *CBFB/MYH11*. In total, 31 (13%) patients had one of the above-named gene fusions. For the remaining 202 patients (87%), we searched for the most significant parameters that would correlate with prognosis.

Multivariate analysis pointed to CD19 positivity

Among the patients without the gene fusions specified in the previous paragraph, 124 and 53 cases received ALL- and AML-type treatment, respectively. For each of these major types of treatment separately, a multivariate Cox regression analysis was performed. Of all factors considered (see Patients and methods for list of variables and their univariate preassessment), WBC count and iMPO were prognostically significant in ALL-type primary treatment and WBC count and CD19 were prognostically

Table 2. Multivariate Cox regression analysis of risk factors after ALL-type primary therapy

Parameter	Hazard ratio	LCL	UCL	*P*
WBC	1.01	1.005	1.02	.0004
iMPO	0.27	0.090	0.83	.022

Results of the multivariate Cox regression analysis of EFS in cases without evidence of a preexisting genetic disease or the specified gene fusions (*ETV6/RUNX1*, *RUNX1/RUNX1T1*, *PML/RARA*, or *BCR/ABL1*) primarily treated with ALL-type therapy. For the treatment type, 11 factors that withstood the univariate prescreening (listed in "Patients and methods") were included in the model.

LCL, lower limit of confidence interval; UCL, upper limit of confidence interval.

significant in AML-type primary treatment (Tables 2 and 3, respectively).

After identifying factors associated with differences in risks of events within one type of treatment, we searched for the ones that correlated with the most significant prognostic difference between ALL- and AML-type therapy (supplemental Table 6). Out of these parameters, the most striking difference between ALL- and AML-type primary therapy was found among $CD19^+$ cases (Figure 4A,C). Among patients with positivity of CD19 (which included a positivity or a partial positivity in ≥1 blast population in cases with bilineal ALAL), the outcome strongly correlated with type of treatment. Whereas the 5-year EFS of children who received ALL type primary therapy was 83% ± 5.3%, the 5-year EFS of children who started with AML or combined type of treatments was inferior (0% ± 0% and 28% ± 14%, both $P < .0001$). After AML-type primary therapy, no patient was alive and without an event beyond 1.4 years after the diagnosis. We thus consider cases with CD19 indicated for a non-AML primary treatment. Details on $CD19^+$ cases with AML-type primary treatment are described in supplemental Information 1. Among patients who started on a combined-type treatment, there was a high proportion of *KMT2A/AFF1*$^+$ ALAL (supplemental Table 4), which largely drives the poorer prognosis of this subset. Among *KMT2A/AFF1*$^-$ cases with CD19, the 5-year EFS did not differ between children with a combined-type treatment (71% ± 17%, n = 8) and ALL-type treatment (84% ± 5.2%, n = 84; $P = .38$).

Primary treatment options for $CD19^-$ patients

If we consider patients with above-listed gene fusions, and/or those with $CD19^+$ blasts assigned to recommended primary treatments, 33% remain to be addressed (76 out of 233 patients in our cohort). The primary therapy was of the ALL, combined, and AML type in 35 (46%), 9 (12%), and 32 (42%) cases, respectively. In total, 18 events occurred in this subset of patients with an ALL-, combined-, and AML-type primary therapy (4, 1, and 13 events, respectively).

The 2 main types of primary therapy (ALL and AML type) were compared for $CD19^-$ cases (n = 67). As we sought to find a parameter that would positively define a biologic subset, we analyzed the impact of the remaining (after exclusion of CD19) antigens that define lymphoid involvement in both the EGIL and WHO classification. This means that cases were selected with positive CD7 and CD3 (intracellular or surface) or with at least 2 positive antigens of CD10, intracellular CD79a, and CD22 (intracellular or surface). This selected subcohort had a very good outcome on either ALL- or combined-type primary therapy (Figure 4B,D).

Among the remaining 10 patients, 5 children fulfilled criteria for undifferentiated leukemia. Although the number of patients was too low to make reliable conclusions, the ALL-type primary treatment appeared particularly inefficient; of the 3 patients on ALL-type primary treatment, the type of treatment was changed between days 15 and 19 of treatment in 2 of them, and both died within <3 months of the diagnosis. The third patient died 2 years after diagnosis. In contrast, 4 of 5 patients who received AML-type primary treatment were alive, although only 1 of them in the first complete remission (CR1). The treatment was changed from AML type in 2 patients (at days 15 and 34 of treatment). Finally, both patients on combined-type treatment remained on it and were in CR1.

Major changes of treatment

In 29 patients, the treatment type (ALL/combined/AML) was changed after 8 to 126 days of therapy (median, 32 days; supplemental Figure 2). The reasons for these treatment-type changes mostly reflected response to the primary treatment. Although, as expected, the outcome of patients whose type of treatment changed was poorer, some of the patients whose treatment type changed were brought to durable remission in each of the types of primary treatment.

Another major treatment factor was SCT. In total, 69 patients underwent SCT, and 58 of them were transplanted in the CR1. Using a delayed-entry survival analysis, no significant prognostic benefit could be demonstrated in the ALL, AML, or combined type of primary treatment (Figure 5).

We then analyzed specifically patients with a high leukemia load at the end of induction (ALL-, AML-, or combined-type primary treatment). In total, 31 patients had 5% leukemic cells or greater. This relatively low number of patients did not allow separation by type of primary treatment. Of the 31 patients, 12 underwent SCT (irrespective of the remission status at the time of SCT). The 5-year EFS of these transplanted children was 83% ± 15%, compared with 29 ± 16% among nontransplanted patients ($P > .05$); in a delayed-entry analysis, 5-year EFS was 83% ± 15% and 36% ± 19%, respectively ($P > .05$).

Response to ALL treatment, and when to consider treatment changes

Patients who started on ALL-type therapy would be considered for a treatment change if their estimated outcome was poorer on

Table 3. Multivariate Cox regression analysis of risk factors after AML-type primary therapy

Parameter	Hazard ratio	LCL	UCL	*P*
WBC	1.005	1.002	1.007	.002
CD19	3.17	1.39	7.21	.006

Results of the multivariate Cox regression analysis of EFS in cases without evidence of a preexisting genetic disease or the specified gene fusions (*ETV6/RUNX1*, *RUNX1/RUNX1T1*, *PML/RARA*, or *BCR/ABL1*) primarily treated with AML-type therapy. For treatment type, 11 factors that withstood the univariate prescreening (listed in "Patients and methods") were included in the model.

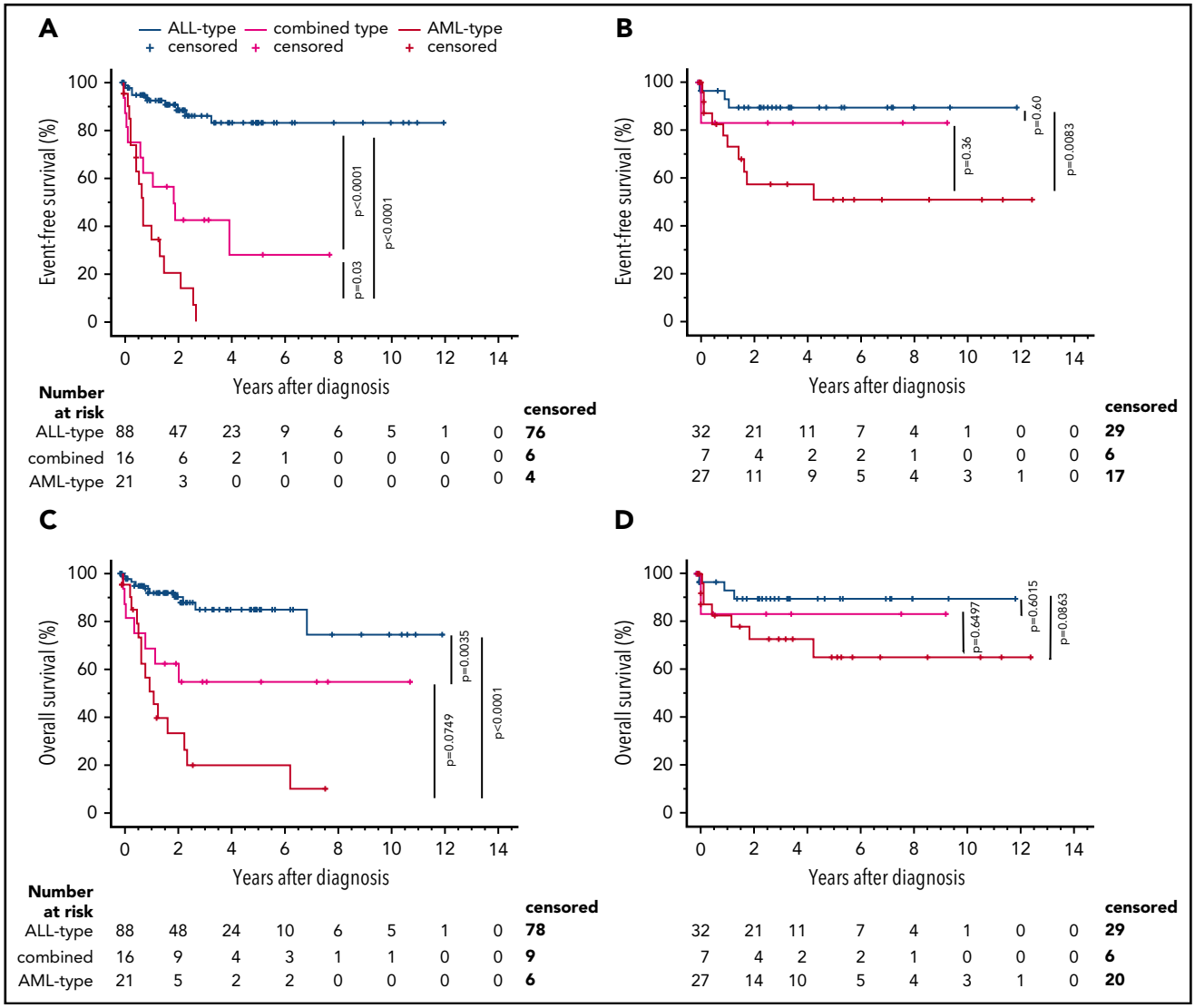


Figure 4. Outcome of children with CD19⁺ ALAL and CD19⁻ ALAL with other lymphoid-specific antigens by the type of primary therapy. Patients without *ETV6/RUNX1*, *RUNX1/RUNX1T1*, *PML/RARA*, or *BCR/ABL1* gene fusions. A log-rank (Mantel-Cox) statistical test was used. (A) EFS of CD19⁺ ALAL. (B) EFS of CD 19⁻ ALAL with other lymphoid-specific antigens. (C) Overall survival of CD19⁺ patients. (D) Overall survival of CD19⁻ ALAL with other lymphoid-specific antigens. Other lymphoid-specific antigens are listed in "Results."

the continued ALL treatment than on the changed treatment type. Such a poor response predictor could be anticipated especially if the ALAL patients with a poorer response to initial treatment had a markedly poorer prognosis than similar patients with nonambiguous ALL. Therefore, we evaluated the proportion and EFS of ALAL patients with a poor treatment response. Among CD19⁺ and CD19⁻ ALAL cases, the proportion of patients with a poorer leukemia clearance was 25% and 50% at day 8 (poor prednisone responders [PPRs]; outcome is shown in Figure 6), 32% and 60% at the end of induction (minimal residual disease [MRD] ≥0.1%; outcome is shown in supplemental Figure 3A-B,E-F), and 13% and 10% at week 12 (MRD ≥0.01%; outcome is shown in supplemental Figure 3C-D,G-H), respectively.

By definition, a poorer blast clearance at day 8 is found in PPRs. The 5-year EFS of CD19⁺ PPRs with ALAL was 47% ± 14%, which appears inferior to that of nonambiguous precusor B (pB) ALL (68% ± 4.6%[20]). As the principal question was whether the type of treatment should be changed, we analyzed the number and outcome of children whose type of treatment changed at any time after PPR status was set. Of the 23 CD19⁺ ALAL PPRs, the treatment type was changed in 11 and remained ALL type in 12, leading to continued CR1 in 4 and 6 cases, respectively. The 5-year EFS of CD19⁻ PPRs was (90% ± 9.5%), which is definitely not inferior to that reported in nonambiguous T ALL (63% ± 5.7%[20]).

At the end of induction, the EFS of CD19⁺ and CD19⁻ cases with MRD ≥0.1% was 60% ± 13% and 79% ± 11% (supplemental Figure 3A-B), respectively, which appears to show an outcome noninferior to that reported for nonambiguous ALL cases (cumulative incidence of relapses, 36% ± 3.6% and 32% ± 3.6% for pB at 5 years[21] and T ALL at 7 years,[22] respectively) and possibly reflects increased treatment intensities in the MRD higher-risk patients treated by contemporary protocols.

At week 12, the 5-year EFS of CD19⁺ cases with MRD ≥0.01% was 50% ± 19%, but both CD19⁻ patients with MRD ≥0.01% died (one from toxicity and one from leukemia) (supplemental Figure 3C-D).

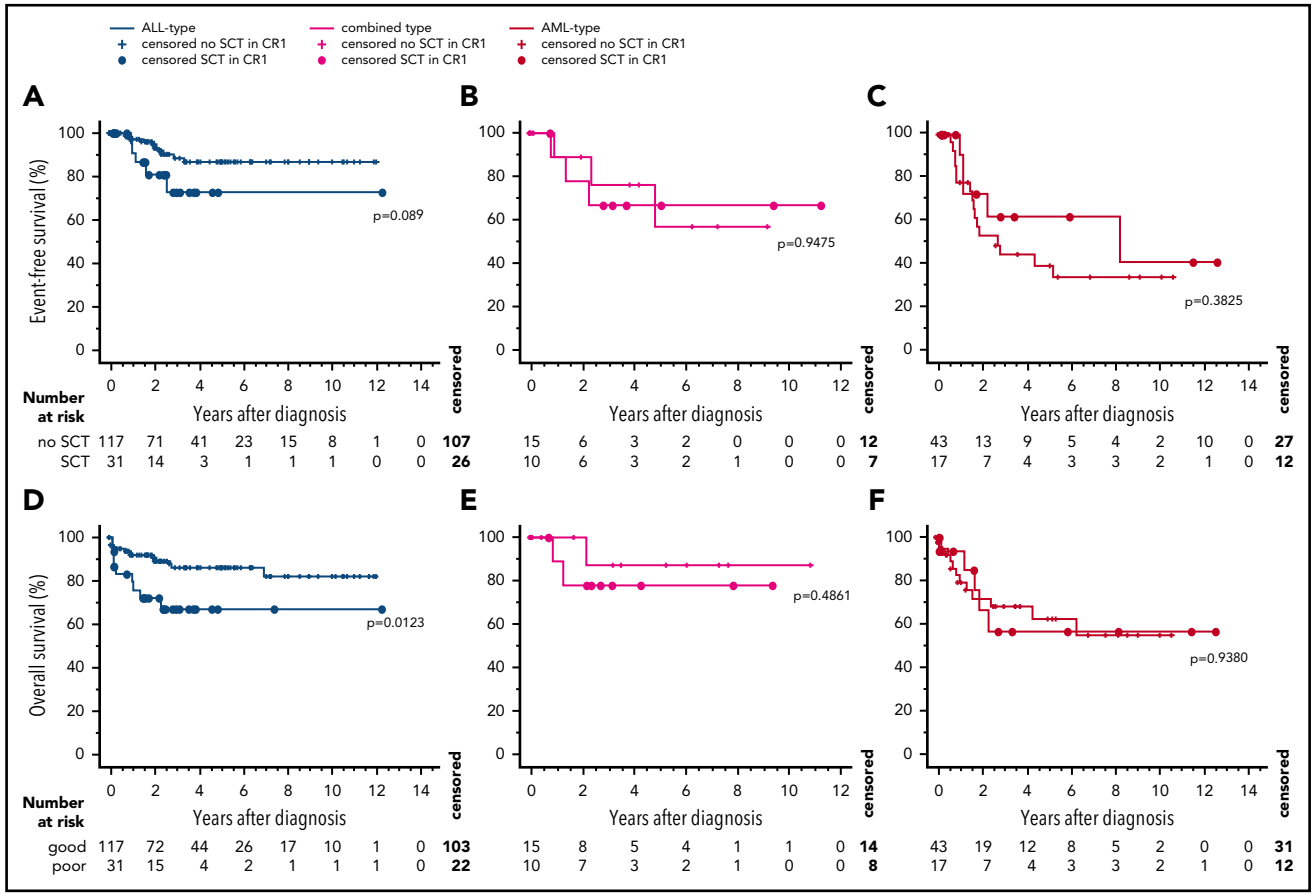


Figure 5. Outcome of ALAL patients transplanted in the first complete remission compared with other patients. In each type of primary treatment, events occurring prior to the median time to transplant were neglected. A log-rank (Mantel-Cox) statistical test was used. (A) ALL treatment type EFS. (B) Combined-type treatment EFS. (C) EFS. (D) ALL treatment type overall survival. (E) Combined-type treatment overall survival. (F) AML treatment type overall survival.

Whereas the data of CD19$^+$ ALAL patients do not indicate a marked difference of nonambiguous pB ALL with high MRD,[23] the grim prognosis of the 2 CD19$^-$ cases calls for more data.

Discussion

Diagnosis of ALAL

We present the largest study to date on childhood ALAL involving children with 3 types of ambiguity. Keeping these patients together in one cohort allows for overlapping cases; eg, it depends on the investigator whether they consider cells with distinct phenotype as 2 separate populations (and thus a bilineal leukemia) or they consider one leukemic population that differentiates into 2 phenotypic states. We have shown previously that even quite phenotypically distinct populations might result from one clone.[24]

Before the first attempts to standardize the ALAL definitions by immunophenotyping in the 1990s, the value of morphological examinations was also investigated.[25] While ALAL typically presents with ambiguous morphological features, there are cases with typical ALL or AML appearance. In an early study by Pui et al,[25] which used morphologic and cytochemical diagnostic criteria for treatment selection, 2 of the 4 nonresponders to AML-type treatment later responded to ALL-type therapy. However, morphology was not the focus of more recent studies, including this one.

Optimal therapy for ALAL

So far, treatment recommendations are mostly based on scarce observations or on assumptions based on the biological similarities of leukemic cells to physiological hematopoiesis. In physiological granulocytic development, MPO is a prominent molecule and serves as a traditional marker of myeloid differentiation. However, its expression was documented in ALL cells on the messenger RNA and protein levels,[26] and although it was missing in more mature B or T cells, it was also found in messenger RNA of sorted physiological pre-B cells (recalculated data from Novershtern et al[27]). Moreover, there is no evidence that MPO expression is directly connected to a better response to AML-type therapy. In fact, cases of MPO$^+$ leukemia that respond well to ALL-type therapy are documented in the literature[26,28] and in this study. Given the variability of MPO expression, it is not surprising that there has been a debate over the cutoff for the diagnostic MPO positivity.[29-31]

This study documents the diagnostic heterogeneity of ALAL and demonstrates that the therapeutic approaches vary. These results, together with the retrospective nature of our study, led us to interpret our data with caution. We observed a superior outcome of children treated with ALL-type therapy, which is in line with previous studies.[8,9,32-35] This finding should not automatically imply that ALL-type therapy is superior for any subset of ALAL. However, the striking failure of AML-type therapy

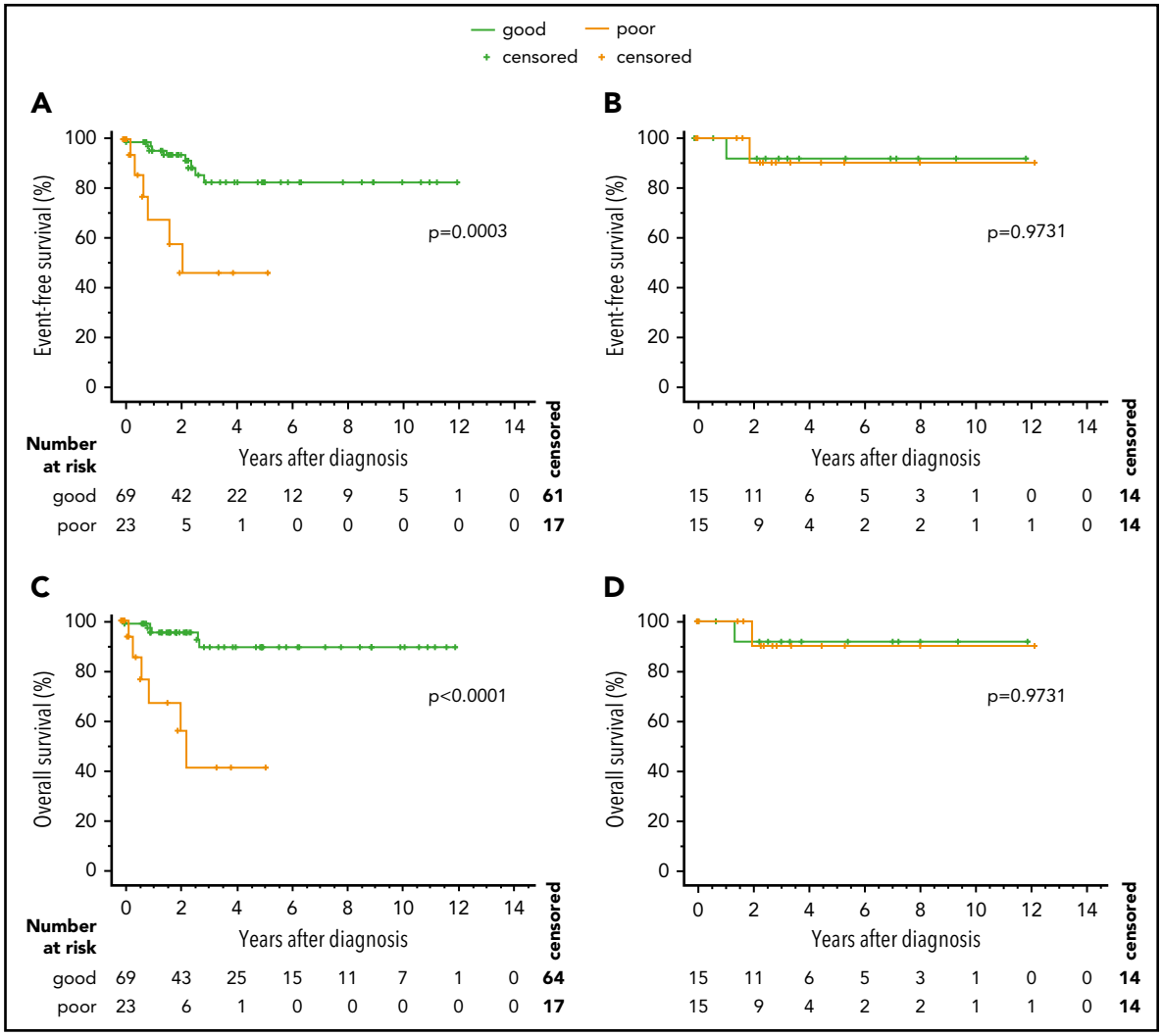


Figure 6. Outcome of ALAL patients in relation to their response to prednisone. (A) CD19$^+$ EFS. (B) CD19$^-$ EFS. (C) CD19$^+$ overall survival. (D) CD19$^-$ overall survival. A log-rank (Mantel-Cox) statistical test was used.

to cure any child with CD19$^+$ leukemia in the absence of AML-specific gene fusions leads us to recommend other types of chemotherapy in this relatively large subset of ALAL. However, without a prospective study, we are unable to estimate the prognosis of these ALAL patients on ALL-type treatment. For the remaining 33% of patients, the support for our recommendation is less clear, and the ultimate answer could only come after being tested prospectively. We have established an international platform, which has allowed testing the prognosis of children with ALAL treated according to predefined rules.

Given that the phenotype of ALAL is in between classical ALL and AML, it has always been tempting to speculate that these leukemias would respond best to therapy that comprises elements of both ALL and AML types of treatment. This speculation assumes that what has been proven empirically for "pure" ALL or AML would analogically hold true for borderline cases. One problem is that this assumption might be wrong. Second, both ALL and AML types of treatment are close to maximum tolerable intensity. Therefore, by intending to combine these treatments, we either compromise their efficacy or increase the risk of toxicity beyond acceptable margins.

Primary treatment algorithm

Collectively, the data lead to an algorithm, the details of which can be tested prospectively (Figure 7). After addressing ALL- and AML-specific fusions (13% of our cohort), positivity of CD19 should be considered. Due to the dismal outcome of CD19$^+$ cases on AML-type primary treatment, this primary treatment type is not recommended. Although the EFS of CD19$^+$ patients on combined-type primary therapy was significantly poorer than that of patients on ALL-type primary treatment, this difference was largely driven by a high proportion of *KMT2A/AFF1*$^+$ cases; among *KMT2A/AFF1*$^-$ cases, the outcome corresponding to the combined-type treatment is not different from the outcome of ALL-type primary treatment. Thus, combined-type primary treatment is a possible alternative for CD19$^+$ ALAL. For the CD19$^-$ patients, the bases for recommendations were less straightforward. In presence of lymphoid markers, the outcome on ALL-type primary therapy was excellent, which should be considered. However, a minority of children on AML-type primary therapy had an outcome similar to that of nonambiguous AML, leaving this option open of AML-type therapy for cases with additional myeloid features. Very rarely, ALAL presented without CD19 or the listed lymphoid features, and the outcome was poor, especially on ALL-type treatment, making the other 2 types of

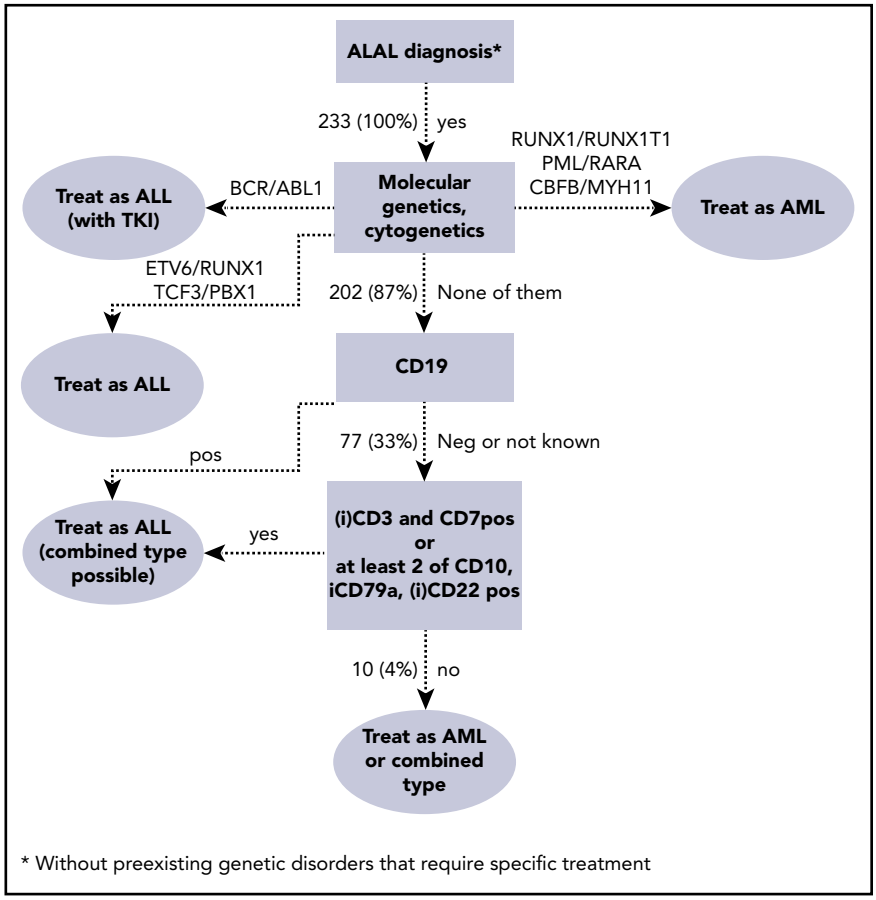


Figure 7. Treatment recommendation algorithm. After each decision node, the number (percentage) of patients who still remain to be categorized is given. Note that the algorithm shows a recommendation to be tested prospectively and does not refer to the way in which patients in this study were treated. Numbers (percentages) include all 233 patients in the study with confirmed inclusion criteria. Neg, negative; pos, positive (or partly positive). TKI, tyrosine kinase inhibitor.

primary treatment preferred. Altogether, these treatment recommendations should be tested prospectively in the setting of multidisciplinary leukemia diagnostics.

Changing the treatment type, transplantation

In addition to the selection of the primary treatment type, another key question is whether and when to change the type of treatment. The outcome of ALAL patients with a poorer blast clearance at day 8 ($CD19^-$ PPRs) or at the end of induction (both $CD19^+$ and $CD19^-$ cases) was not inferior to what is expected for nonambiguous ALL. Although $CD19^+$ PPRs among ALAL do appear to have a worse prognosis than analogous nonambiguous ALL cases, in half of these cases, the type of treatment changed after day 8; while this change did not bring a clear benefit in a low number of patients, a similarly low number of patients stayed on ALL-type therapy. Notably, our data show that proportion of PPRs is higher (23 of 92 [25%] patients with $CD19^+$ ALAL and 15 of 30 [50%] patients with $CD19^-$ ALAL on ALL-type treatment with known prednisone response) than in nonambiguous ALL (6.4% and 34% in B-cell precursor ALL and T ALL, respectively).[20] Children who remain on ALL-type treatment should thus often be treated in high-risk strata.

For evaluation of SCT benefit specifically among patients with a high leukemia burden at the end of induction, the number of patients in our cohort was limited, and the possible benefit was not significant. With all these limitations, our observation points to the fact that patients with very poor response to induction treatment may be transplanted and rescued. Major treatment-type modifications are thus to be considered only in children who do not achieve CR1 by the end of induction. Such modifications comprise either SCT or, after re-evaluation of blast phenotype, change of treatment type. The possibility to transplant children with a poor treatment response is in line with a recent smaller study, which found a benefit of SCT in adult ALAL, although not using delayed-entry analysis.[32]

In summary, a multicenter international study was used to outline treatment recommendations for children with ALAL. These recommendations should be tested prospectively in a multinational study, and eventually, each patient with ALAL will be assigned to their optimal treatment.

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Authorship

Contribution: O.H. and J. Stary designed the study; O.H., V.d.H., E.M., J.T., M.Z., J.I., B.K., H.I., A.V.M., J.G.R., M.S.F., L.D.-P., M.D., B.B., G.B., M.C., M.E.C., N.M., S.E., S.I., D.L., T.F., A. Kolenova, P.S., O.K., K.R.R., S.P., A. Kattamis, E.d.C., R.R., D.R., J.K.C., M.S., and J. Stary performed primary or reference diagnostics and/or treatment of the patients; O.H., V.d.H., I.J., and A.L. performed data collection; V.d.H., I.J., K.B., A.M.,

B.K., H.I., K.S., S.S., M.S.F., J.M., M.D., B.B., M.C., M.E.C., S.E., A. Kolenova, O.K., S.P., and D.R. performed clinical data generation and processing; O.H., V.d.H., J. Stancikova, I.J., E.M., V.C., and B.V. performed data analysis (including statistics); O.H., V.d.H., J. Stancikova, B.V., I.J., M.Z., K.B., A.M., T.B.A., Z.Z., J.T., K.S., A.V.M., M.S., and J. Stary interpretation data; O.H., J. Stancikova, J. Stary, and B.V. wrote the manuscript; and all authors edited and revised the manuscript.

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Footnotes

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