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ORIGINAL REPORT

Use of Minimal Residual Disease Assessment to Redefine Induction Failure in Pediatric Acute Lymphoblastic Leukemia

David O'Connor, Anthony V. Moorman, Rachel Wade, Jeremy Hancock, Ronald M.R. Tan, Jack Bartram, John Moppett, Claire Schwab, Katharine Patrick, Christine J. Harrison, Rachael Hough, Nick Goulden, Ajay Vora, and Sujith Samarasinghe

Corresponding author: David O'Connor, MD, Department of Haematology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, WC1N 3JH, United Kingdom; e-mail: david.o'connor@gosh.nhs.uk.

ABSTRACT

Purpose

Our aim was to determine the role of end-of-induction (EOI) minimal residual disease (MRD) assessment in the identification and stratification of induction failure in patients with pediatric acute lymphoblastic leukemia (ALL) and to identify genetic abnormalities that drive disease in these patients.

Patients and Methods

Analysis included 3,113 patients who were treated in the Medical Research Council UKALL2003 multicenter randomized trial (NCT00222612) between 2003 and 2011. MRD was measured by using standardized real-time quantitative PCR. Median follow-up was 5 years 9 months.

Results

Fifty-nine patients (1.9%) had morphologic induction failure with 5-year event-free survival (EFS) of 50.7% (95% CI, 37.4 to 64.0) and 5-year overall survival of 57.7% (95% CI, 44.2 to 71.2). Of these, a small proportion of patients with M2 marrow (6 of 44) and a low EOI MRD level ($< 0.01\%$) had 5-year EFS of 100%. Conversely, among patients with morphologic remission 2.3% (61 of 2,633) had high MRD ($\geq 5\%$) and 5-year EFS of 47.0% (95% CI, 32.9 to 61.1), which was similar to those with morphologic induction failure. Redefining induction failure to include morphologic induction failure and/or MRD $\geq 5\%$ identified 3.9% (120 of 3,133 patients) of the trial cohort with 5-year EFS of 48.0% (95% CI, 39.3 to 58.6). Induction failure (morphologic or MRD $\geq 5\%$) occurred most frequently in T-ALL (10.1%; 39 of 386 T-ALL cases) and B-other ALL, that is, lacking established chromosomal abnormalities (5.6%; 43 of 772 B-other cases). Genetic testing within the B-other group revealed the presence of *PDGFRB* gene fusions, particularly *EBF1-PDGFRB,* in almost one third of B-other ALL cases.

Conclusion

Integration of EOI MRD level with morphology identifies induction failure more precisely than morphology alone. Prevalence of *EBF1-PDGFRB* fusions in this group highlights the importance of genetic screening to identify abnormalities that may be targets for novel agents.

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INTRODUCTION

Overall survival in children with acute lymphoblastic leukemia (ALL) who are treated with contemporary regimens is now $> 90\%$.[1] Whereas the vast majority of patients experience rapid response to chemotherapy, a minority do not achieve morphologic remission by the end of induction (EOI). A recent international analysis of induction failure (IF) demonstrated that, although most patients do eventually achieve a remission, long-term outcome is poor[2]; however, these data were based on historical cases that were treated between 1985 and 2000, and the outcome of IF in contemporary protocols has not been examined in detail.

Of importance, the previous analysis predates the era of minimal residual disease (MRD) monitoring, which is now considered the most important prognostic factor in pediatric ALL.[3,4] Studies in pediatric acute myeloid leukemia have demonstrated flow cytometry–based MRD to be more accurate than morphology in defining complete remission[5,6]; however, at present, the role of MRD in defining and stratifying IF in pediatric ALL remains unknown.

Furthermore, although it is known that IF is increased in those patients with high-risk

chromosomal abnormalities,[2] the role of newly described ABL-class and Janus kinase (JAK)–signal transducers and activators of transcription (STAT) genetic abnormalities[7] has not been fully explored. Identification of such lesions could provide an opportunity for the use of targeted therapy in this high-risk group.

To address these issues, we analyzed all cases of morphologic IF in the recently reported large, multicenter, randomized controlled trial, Medical Research Council UK ALL 2003 (MRC UKALL 2003). In addition, we investigated whether EOI MRD should be incorporated into the definition of IF and whether a targeted genetic screening strategy identifies patients with lesions that are amenable to treatment with novel agents.

PATIENTS AND METHODS

Participants and Trial Protocol

This study included all patients who were enrolled in the MRC UKALL 2003 trial, as previously reported.[4,8] An overview of the protocol, including treatment regimens (Appendix Table A1, online only), can be found in the Appendix (online only).

MRD Assessment

Bone marrow MRD was measured within five laboratories in the United Kingdom using standardized real-time quantitative PCR for immunoglobulin and T-cell receptor antigen gene rearrangements.[9] Quantitative range was 0.01%. An exact MRD cutoff of 0.01% was used to stratify patients.

Definitions

IF was defined as failure to achieve morphologic complete remission (< 5% bone marrow blasts) at EOI or persistent extramedullary disease. Patients with M2 marrow (5% to 25% blasts) were allocated to the high-risk arm, regimen C. Patients with M3 marrow (> 25% blasts) were deemed to have experienced treatment failure and were taken off protocol, although this was not defined as an event for the purpose of survival analyses. Microscopic assessments of marrow were done locally with no central review.

Genetic Analysis

Cytogenetic analysis, including fluorescence in situ hybridization (FISH) for detection of chromosomal abnormalities of prognostic significance, was carried out in the regional cytogenetics laboratories as part of routine diagnosis but was reviewed and collated by the Leukaemia Research Cytogenetics Group, as previously described.[10] A representative cohort of patients with B-other ALL—those cases lacking established chromosomal abnormalities—were screened for rearrangements that involved *ABL1, ABL2, PDGFRB, CSF1R, CRLF2,* and *JAK2* by using commercially available or home-grown[7,11] break-apart FISH probes (Cytocell, Cambridge, UK; Leica Microsystems, Milton Keynes, UK) or, in the case of *P2RY8-CRLF2,* by using multiplex ligation-dependent probe amplification (SALSA MLPA kit P335 IKZF1; MRC, Holland, the Netherlands).[12] *EBF1-PDGFRB* fusion was confirmed and validated by FISH and RT-PCR as recently described.[13]

Statistical Analysis

Categorical variables were compared with standard χ^2 tests or Fisher's exact tests as appropriate. Time-to-event outcomes were defined from start of treatment. Event-free survival (EFS) was the primary end point and was defined as time to relapse, secondary tumor, or death. Overall survival (OS) was time to death. Kaplan-Meier curves were produced and compared with the log-rank method. Multivariable analysis used Cox proportional

hazards regression models to test whether effects of prognostic factors were independent, with tests of proportional hazards using an interaction with time variable for significant factors. Graphs were plotted to 8 years to show as much information as possible; data presented in the text relate to 5-year outcome. All P values were two-sided. Linear trend P values were presented for variables with a natural ordering, such as white blood cell count (WCC), on the basis of suitable tests with one degree of freedom, for example, Mantel-Haenszel χ^2, log-rank test for trend.

Statistical analyses were performed in SAS (SAS/STAT User's Guide, Version 9.3; SAS Institute, Cary, NC) or with in-house programs. Figures were created with R (version 3.0.1; The R Foundation, Vienna, Austria). Median follow-up was 5 years 9 months (range, 1 month to 10 years 1 month).

RESULTS

Patient Characteristics

Of 3,207 patients registered for the trial, 3,113 were included in this analysis after exclusion of those with Philadelphia-positive ALL, withdrawal of consent, or misdiagnosis (Fig 1). Patient characteristics are listed in Table 1.

Fifty-nine patients (1.9%) were classified as IF; 44 had M2 marrow and 15 M3 marrow. No patients had persistent extramedullary disease. In keeping with previous reports, this group experienced poor outcomes with a 5-year EFS of 50.7% (95% CI, 37.4 to 64.0) and a 5-year OS of 57.7% (95% CI, 44.2 to 71.2). Outcome correlated with level of bone marrow blasts; those with M3 marrows (> 25% blasts) had a worse outcome than did those with M2 marrows (5-year EFS, 33.3% [95% CI, 9.4 to 57.2] *v*
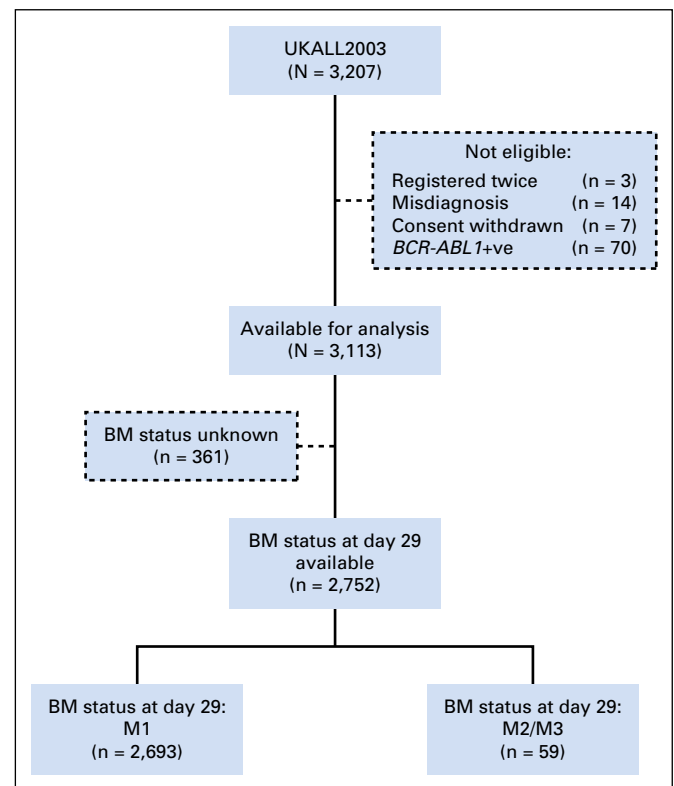
Fig 1. CONSORT diagram demonstrating selection of groups by end of induction (day 29) bone marrow (BM) status.

Table 1. Patient Characteristics on the Basis of Bone Marrow Status at Day 29

| Characteristic | Total (N = 3,113) | Bone Marrow Status at Day 29 | | | M1 v M2/M3 P* |
		Unknown (n = 361)	M1 (n = 2,693)	M2/M3 (n = 59)	
Patient					
Sex					
Male	1,767 (57%)	206 (57%)	1,530 (57%)	31 (53%)	.5
Female	1,346 (43%)	155 (43%)	1,163 (43%)	28 (47%)	
Age group at treatment start, years					
< 10	2,278 (73%)	249 (69%)	2,008 (75%)	21 (36%)	< .001
10-15	608 (20%)	70 (19%)	515 (19%)	23 (39%)	
≥ 16	227 (7%)	42 (12%)	170 (6%)	15 (25%)	
Median (range)	5 (1-24)	5 (1-24)	5 (1-24)	13 (1-23)	< .001
Ethnicity					
Black	74 (2%)	8 (2%)	65 (2%)	1 (2%)	.8
White	2,525 (81%)	282 (78%)	2,192 (81%)	51 (86%)	(.3†)
Asian	232 (7%)	32 (9%)	197 (7%)	3 (5%)	
Other	151 (5%)	21 (6%)	128 (5%)	2 (3%)	
Unknown	131 (4%)	18 (5%)	111 (4%)	2 (3%)	
Down syndrome					
No	3,026 (97%)	347 (96%)	2,625 (97%)	54 (92%)	.02
Yes	87 (3%)	14 (4%)	68 (3%)	5 (8%)	
Disease					
WCC at diagnosis					
< 20	1,904 (61%)	229 (63%)	1,648 (61%)	27 (46%)	< .001
20-	524 (17%)	57 (16%)	460 (17%)	7 (12%)	
50-	313 (10%)	36 (10%)	268 (10%)	9 (15%)	
100-	199 (6%)	21 (6%)	172 (6%)	6 (10%)	
≥ 200	173 (6%)	18 (5%)	145 (5%)	10 (17%)	
Median (range)	12 (0-881)	12 (1-609)	12 (0-881)	30 (1-582)	.0009
NCI risk group					
Standard	1,812 (58%)	203 (56%)	1,596 (59%)	13 (22%)	< .001
High	1,301 (42%)	158 (44%)	1,097 (41%)	46 (78%)	
Immunophenotype					
B-precursor	2,727 (88%)	311 (86%)	2,374 (88%)	42 (71%)	< .001
T cell	386 (12%)	50 (14%)	319 (12%)	17 (29%)	
Cytogenetic risk group‡					
Good	1,586 (58%)	182 (59%)	1,395 (59%)	9 (21%)	< .001§
Intermediate	860 (32%)	87 (28%)	752 (32%)	21 (50%)	
High	121 (4%)	14 (5%)	101 (4%)	6 (14%)	
Unknown	160 (6%)	28 (9%)	126 (5%)	6 (14%)	
CNS disease at diagnosis					
No	3,061 (98%)	351 (97%)	2,652 (98%)	58 (98%)	0.6
Yes	52 (2%)	10 (3%)	41 (2%)	1 (2%)	
Treatment					
Slow early response					
No	2,751 (88%)	322 (89%)	2,403 (89%)	26 (44%)	< .001
Yes	362 (12%)	39 (11%)	290 (11%)	33 (56%)	
Bone marrow status day 29					
M1	2,693 (87%)	—	2,693 (100%)	0 (0%)	N/A
M2	44 (1%)	—	0 (0%)	44 (75%)	
M3	15 (< 0.5%)	—	0 (0%)	15 (25%)	
Unknown	361 (12%)	361 (100%)	0 (0%)	0 (0%)	
MRD level day 29, %					
< 0.01	1,616 (52%)	176 (49%)	1,434 (53%)	6 (10%)	< .001
≥ 0.01	1,062 (34%)	108 (30%)	915 (34%)	39 (66%)	
Unknown	435 (14%)	77 (21%)	344 (13%)	14 (24%)	

Abbreviations: ALL, acute lymphoblastic leukemia; MRD, minimal residual disease; N/A, not applicable; NCI, National Cancer Institute; WCC, white blood cell count.
*P value for trend for ordered groups: age group, WBC, cytogenetic risk group; otherwise, P value for heterogeneity. 'Unknown' category excluded.
†White v black, Asian, and other.
‡Risk groups defined for B-precursor ALL. Good risk cytogenetics = ETV6-RUNX1, high hyperdiploidy; high risk = near haploidy, low hypodiploidy, KMT2A(MLL), iAMP21, t(17;19); intermediate = all others.
§B-precursor ALL only: good v intermediate v high.

57.1% [95% CI, 41.8 to 72.4]; P = .1; Fig 2), although this was not significant, likely as a result of the small number of cases.

The majority of patients with IF (51 of 59; 86%) did eventually achieve remission. Patients with M2 marrow were escalated to regimen C and 40 of 44 achieved complete remission (CR) after consolidation therapy. The majority of patients with M2 marrow who achieved CR continued on regimen C, whereas those who failed to achieve remission were treated off protocol, as were those
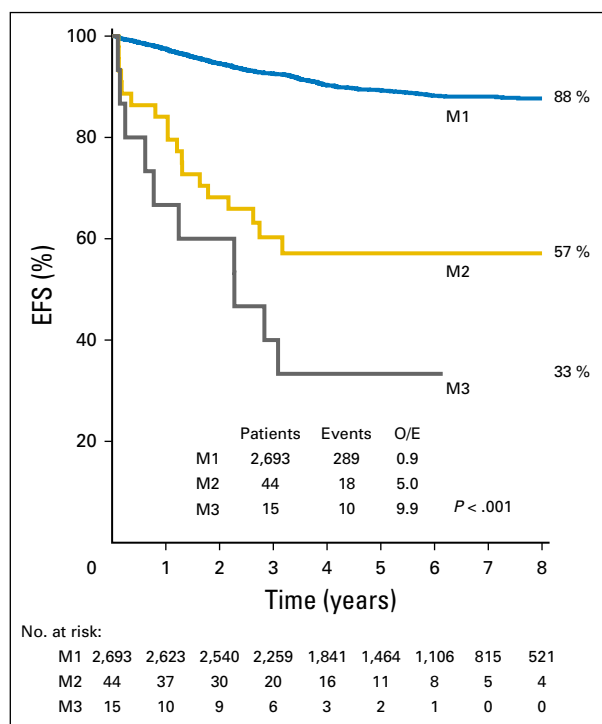
Fig 2. Comparison of event-free survival (EFS) stratified by end of induction morphologic marrow status. Data indicate 8-year EFS estimates. Numbers in each group are indicated in the risk table. M1 < 5% blasts; M2 5% to 25% blasts; M3 > 25% blasts. O/E, observed/expected.

Table 2. Univariable Analysis of Factors Influencing EFS in Morphologic Induction Failure

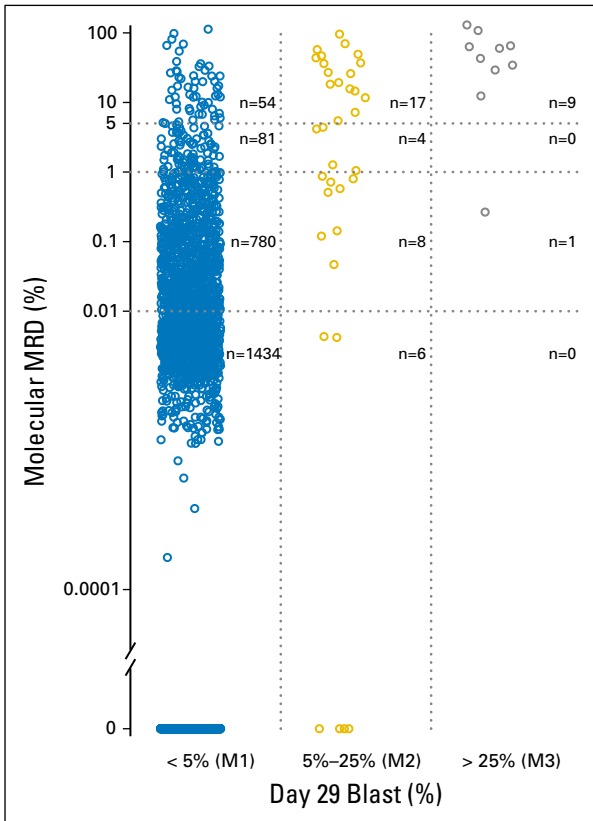| Characteristic	Events/ Patients	5-Year EFS (95% CI)	P (log rank)
Overall	28/59	50.7 (37.4 to 64.0)	
Patient			
Sex			
Male	16/31	45.5 (26.9 to 64.1)	.5
Female	12/28	56.0 (37.2 to 74.8)	
Age			
< 10	6/21	69.7 (49.3 to 90.1)	.007*
10-15	12/23	45.1 (23.7 to 66.5)	
≥ 16	10/15	33.3 (9.4 to 57.2)	
Down syndrome			
Yes	3/8	62.5 (29.0 to 96.0)	.7
No	25/51	48.8 (34.5 to 63.1)	
Disease			
WCC at diagnosis			
< 20	10/27	59.9 (40.1 to 79.7)	.2*
20-	4/7	42.9 (6.2 to 79.6)	
50-	4/9	55.6 (23.1 to 88.1)	
100-	4/6	33.3 (0 to 70.9)	
NCI risk group			
Standard	4/13	65.9 (37.9 to 93.9)	.2
High	24/46	46.2 (31.3 to 61.1)	
Immunophenotype			
T cell	9/17	45.3 (21.0 to 69.6)	.6
B precursor	19/42	53.6 (38.1 to 69.1)	
Cytogenetic risk group (B-precursor ALL only)			
Good	2/9	76.2 (47.2 to 100.0)	.3*
Intermediate	11/21	47.6 (26.2 to 69.0)	
High	3/6	44.4 (0.9 to 87.9)	
Treatment			
Early response			
Rapid	11/26	56.8 (37.4 to 76.2)	.5
Slow	17/33	46.2 (28.6 to 63.8)	
Bone marrow status day 29			
M2	18/44	57.1 (41.8 to 72.4)	.1
M3	10/15	33.3 (9.4 to 57.2)	
Regimen administered			
A	0/4	100.0	.3*
B	5/9	44.4 (11.9 to 76.9)	
C	23/46	47.3 (32.0 to 62.6)	
MRD level day 29			
< 0.01%	0/6	100.0	.001*
0.01 to < 1%	2/9	76.2 (47.2 to 100.0)	
1 to < 5%	1/4	75.0 (32.5 to 100.0)	
≥ 5%	16/26	36.9 (17.7 to 56.1)	

Abbreviations: ALL, acute lymphoblastic leukemia; EFS, event-free survival; MRD, minimal residual disease; NCI, National Cancer Institute; WCC, white blood cell count.
*Trend P values are a test across ordered groups.

with M3 marrow (Appendix Table A2, online only). Seventeen patients with IF (10 M2, 7 M3) underwent hematopoietic stem cell transplantation in their first remission (6 matched related donor, 9 matched unrelated donor, 1 not known). The 5-year EFS in these patients was 41.2% (95% CI, 18.6 to 62.6) compared with 55.9% (95% CI, 39.3 to 29.6; $P = .627$) for those patients with M2 and M3 marrows who did not undergo hematopoietic stem cell transplantation in CR1.

IF was associated with several high-risk characteristics, including age, WCC, T-cell phenotype, and high-risk cytogenetic subgroups (Table 1). IF was particularly frequent in older patients; 6.6% of patients age ≥ 16 years experienced IF compared with 0.9% in those age < 10 years and 3.8% in those age 10 to 15 years ($P_{trend} < .0001$). In addition, patients with Down's syndrome were at greater risk of IF (5.7% v 1.8%; $P = .005$).

Several factors seem to influence outcome after IF (Table 2). Age was significantly associated with EFS; patients age < 10 years experienced superior outcome compared with those age 10 to 15 years and age ≥ 16 years (5-year EFS, 69.7% [95% CI, 49.3 to 90.1] v 45.1% [95% CI, 23.7 to 66.5] v 33.3% [95% CI, 9.4 to 57.2; $P = .007$). Other factors also showed a nonsignificant trend to worse outcome, including WCC and intermediate- or high-risk cytogenetics. A multivariable Cox proportional hazards regression analysis confirmed that age (hazard ratio [HR] per year of age, 1.10 [95% CI, 1.02 to 1.18]) and MRD (HR for MRD ≥ 5%, 4.46 [95% CI, 1.26 to 15.77]) were independently associated with EFS in morphologic IF.

MRD and IF

Because MRD is the strongest predictor of outcome in pediatric ALL and as MRD was the only treatment response factor

that was independently related to EFS in our patients with M2 and M3 marrows, we explored the role of MRD assessment in stratifying those with morphologic IF. MRD data were available in 45 (76%) of 59 cases; of cases without MRD, most were T-ALL (10 of 14). These data identified a minority of patients (n = 6; Fig 3) with M2 marrows but MRD < 0.01% who had a 5-year EFS of 100% (Table 2).

We next compared EOI MRD in patients with M2 and M3 marrows with patients in complete morphologic remission (M1 marrow). MRD levels were highly variable, including in a small

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group of patients (54 of 2,349; 2.3%) with M1 marrows and high MRD levels (≥ 5%), which suggested IF despite morphologic remission (Fig 3 and Appendix Tables A3 and A4, online only). As shown in Fig 4, increasing EOI MRD levels in patients with morphologic remission was strongly correlated with outcome. Of importance, those with MRD ≥ 5% had EFS of 47.0% at 5 years; 95% CI, 32.9 to 61.1 (OS, 61.8% [95% CI, 47.9 to 75.7]), which was comparable to patients with M2 and M3 marrows. Outcome was similar in those patients with no EOI morphologic marrow status recorded (n = 284) but EOI MRD ≥ 5% (n = 7; 5-year EFS, 38.1% [95% CI, 0 to 77.2]), and they were therefore also included in further analyses. Using MRD values > 5% was not further discriminatory (data not shown).

High MRD (≥ 5%) in the context of morphologic remission was particularly common in patients with T-ALL (8.0%; 22 of 276 patients with T-ALL with MRD available) compared with patients with B-precursor ALL (1.5%; 36 of 2,357; $P < .001$). As previously described, patients with EOI MRD ≥ 0.01% were randomly assigned to either continue their initial regimen (A/B) or move to the more intensive regimen C. Patients with MRD ≥ 5% who were treated with regimen C had a better outcome than did those who were treated with regimen A/B, which suggests that intensified treatment may be beneficial, although this difference was not significant (regimen A and B [n = 19] 5-year EFS 32.0% [95% CI,
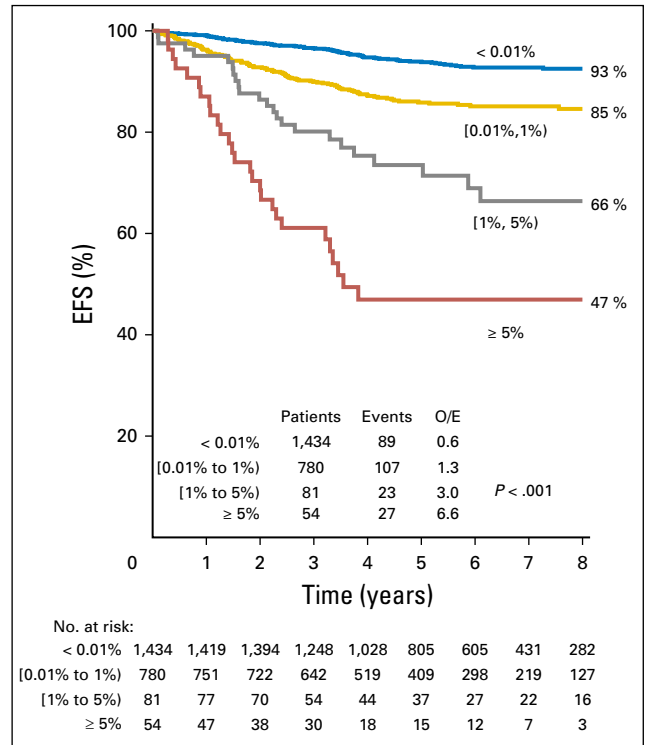
Fig 4. Comparison of event-free survival (EFS) in patients in morphologic remission (M1 marrow; < 5% blasts) stratified into four groups on the basis of end of induction minimal residual disease level (< 0.01%, 0.01% to 1%, 1% to 5%, > 5%). Data indicate 8-year EFS estimates. Numbers in each group are indicated within the at risk table beneath the graph. O/E, observed/expected.
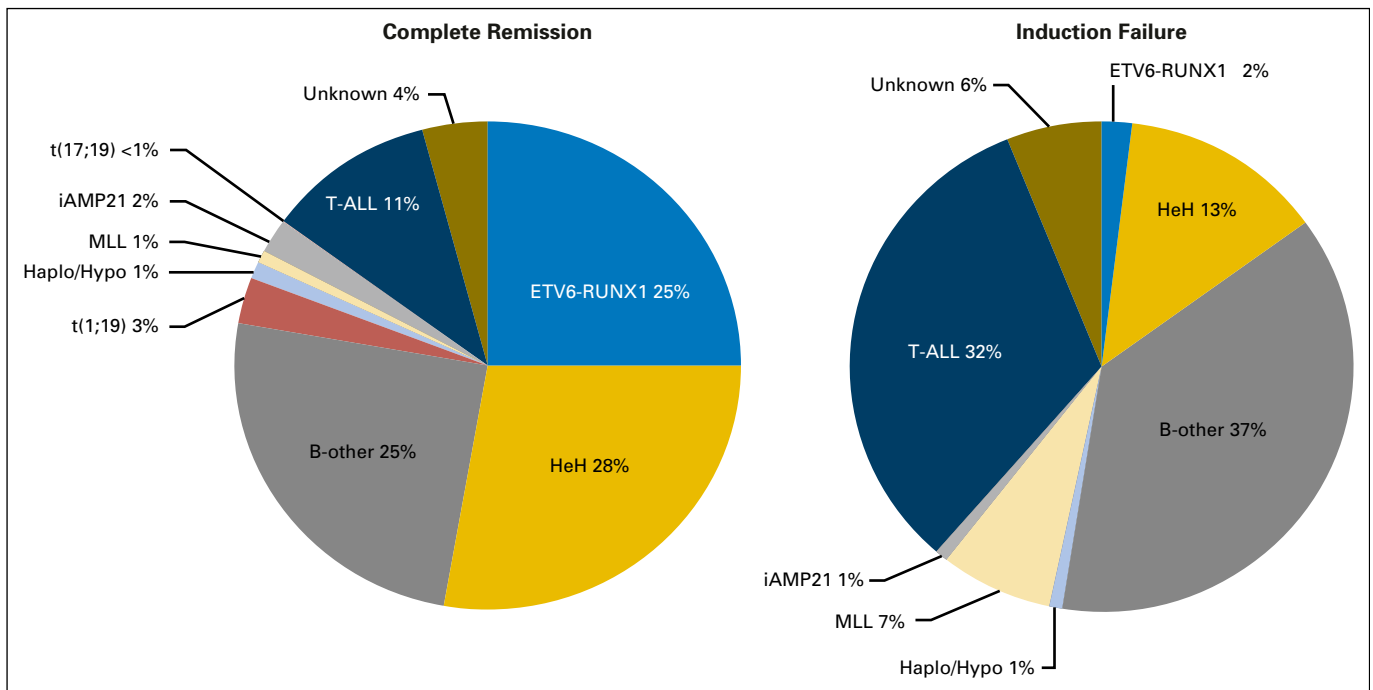
6.5 to 57.5] *v* regimen C [n = 42] 5-year EFS 51.1% [95% CI, 35.6 to 66.7]; $P = .7$).

If all patients with MRD ≥ 5% are classified as IF along with those with morphologic IF, 3.9% of trial entrants (120 to 3,113) would be so defined, including 10.1% (39 of 386) of patients with T-ALL and 5.6% of those with B-other ALL (43 of 772). This combined IF group had 5-year EFS of 48.0% (95% CI, 39.3 to 58.6; Appendix Fig A1, online only). The characteristics of this group are listed in Appendix Table A5 (online only) and outcome in Appendix Table A6 (online only). A multivariate Cox proportional hazards regression analysis demonstrated the independent significance of MRD (HR for MRD ≥ 5%, 4.78 [95% CI, 1.49 to 15.39] and National Cancer Institute risk group (HR for high risk, 2.33 [95% CI, 1.13 to 4.80]) on EFS in IF.

Genetics of IF Defined by MRD and Morphology

A full breakdown of cytogenetic subgroups in the 120 patients with IF (M2 and M3 marrow and/or MRD ≥ 5%) is shown in Fig 5. Compared with responders, a larger proportion of patients with IF have B-other or T-ALL. In contrast, IF is exceedingly rare in *ETV6-RUNX1*.

Outcome was significantly better for patients with good risk cytogenetics—almost all of whom had high hyperdiploidy—than for patients with intermediate- or high-risk cytogenetics (5-year EFS 70.8% [95% CI, 49.3 to 92.4] *v* 40.9 [95% CI, 24.8 to 57.0] *v* 53.0 [95% CI, 22.7 to 83.4] respectively, $P_{good\ v\ intermediate/high} = .03$). Outcomes did not differ between B-precursor ALL and T-ALL

Fig 3. Relationship between morphologic and molecular detection of minimal residual disease (MRD) at the end of induction therapy. End of induction MRD levels are plotted against end of induction (day 29) bone marrow status. Each point represents a single patient. Date indicate number of patients within each category.

Fig 5. Breakdown of cytogenetic subgroups in patients who achieved complete remission at the end of induction and those patients with induction failure. iAMP21, intrachromosomal amplification of chromosome 21; ALL, acute lymphoblastic leukemia; HeH, high hyperdiploidy; Haploid/Hypo, near haploidy/low hypodiploidy.

(5-year EFS 47.8% [95% CI, 36.0 to 59.6] *v* 47.3 [95% CI, 33.1 to 63.5]; *P* = .7).

Given the over-representation of B-other ALL within the IF group, we screened 44 of 52 cases with B-other ALL or unknown failed cytogenetic cases for one or more of ABL-class and/or JAK-STAT rearrangements that involve *ABL1, ABL2, PDGFRB, CSF1R, CRLF2,* and *JAK2.* These 44 patients were representative of the 52 patients with B-other ALL or failed cytogenetics in terms of sex, age, WCC, and survival.

We did not detect any patients with IF who harbored a gene fusion that involved *CSF1R* (0 of 36) or *JAK2* (0 of 35). Two patients had an *ABL1* (1 of 33) or *ABL2* (1 of 23) fusion; however, 11 (31%) of 36 patients with B-other with IF had a rearrangement that involved the *PDGFRB* gene, which was significantly more frequent than among the remaining B-other cases (3 of 224; 1.3%; *P* < .001). In 9 of 11 patients, rearrangement was confirmed to be *EBF1-PDGFRB* fusion,[12] whereas one case had *ATF7IP-PDGFRB.* The remaining case did not involve *EBF1,* and insufficient material was available for further analysis. Patients with *EBF1-PDGFRB* fusions have been shown to respond to targeted therapy with imatinib, which provides a potential treatment in these high-risk patients.[14,15]

Among 38 B-other patients with IF, a total of four (11%) harbored a *CRLF2* rearrangement (*PR2Y8-CRLF2,* n = 3; and *IGH-CRLF2,* n = 1). Frequencies of *PR2Y8-CRLF2* (approximately 10%) and *IGH-CRLF2* (approximately 3%) among patients with B-other ALL with IF are comparable to those observed in our previous studies.[12,16]

Because IF has previously been associated with the early T-precursor (ETP) ALL subtype,[17,18] we used data from a published United Kingdom analysis[19] to explore the role of this

phenotype in driving IF. Although this analysis had previously identified 35 cases of ETP-ALL, two cases have since been reclassified, which gives 33 ETP-ALL cases in total. Of 39 patients with T-ALL in the IF group, 16 had immunophenotyping available that was adequate to classify as ETP. Of these, four (25%) of 16 were consistent with ETP-ALL, which suggests that ETP-ALL could constitute approximately one quarter of IF seen in T-ALL. Furthermore, although MRD assessment was only possible in 17 of 33 ETP-ALL cases, four (23.5%) of 17 cases had MRD ≥ 5% compared with 6 (3.8%) of 158 non–ETP T-ALL cases (*P* = .005), which confirmed the increased prevalence of IF in ETP-ALL (Appendix Tables A4 and A7, online only). Of importance, this subgroup of ETP-ALL with MRD ≥ 5% had a 5-year EFS of 50.0% (95% CI, 1.0 to 99.0) compared with 92.3% (95% CI, 77.8 to 100.0) for patients with ETP-ALL with MRD < 5% (*P* = .05).

DISCUSSION

If outcomes in pediatric ALL are to further improve, stratification must accurately identify patients with high-risk disease who require intensification of therapy while sparing the majority of patients from potentially toxic treatment. Treatment response that uses morphology or MRD has formed an integral part of stratification in recent trials and has been shown to effectively risk-stratify patients, with those with morphologic IF among those with poorest outcome. Disappointingly, despite improvements in overall outcome in pediatric ALL, our study demonstrates that there has been little advance in the treatment of children with IF.

MRD has now been established as the most powerful factor in predicting outcome in pediatric ALL. Our study adds to this

evidence by demonstrating the value of MRD assessment in the context of morphologic IF. By using MRD assessment, we identified a small subgroup of patients within the IF group who had low MRD levels. Crucially, these patients had an excellent outcome, with 5-year EFS of 100%. Without central review, the accuracy of M2 status cannot be assured in these six cases and may explain the nonconcordance of morphologic and MRD levels. Although this only affects a small number of cases, integration of MRD results could potentially spare these patients the toxicity of more intensive therapy.

Furthermore, whereas the majority of studies use low MRD values to guide treatment stratification, our study shows that high MRD levels can be effectively used to identify patients with an extremely poor outcome. It is important to note that the real-time quantitative PCR method that was used for MRD measurement is designed to optimally detect disease between 0.01% and 1% and may therefore not give an exact measurement of disease at higher levels. However, our data clearly show that those patients with morphologic remission, but high EOI MRD ($\geq$ 5%), have outcomes similar to patients with morphologic IF. Overall, this suggests that MRD measurement provides a more reliable assessment of disease response than morphology, as previously described in pediatric acute myeloid leukemia.[5,6,20]

Do these data indicate that treatment response in ALL should be based solely on MRD assessment? High levels of MRD certainly select a poor risk population in the context of morphologic remission. Conversely, those with morphologic IF and MRD $<$ 5% have a relatively good outcome (Table 2), which suggests that remission status could be based solely on MRD level. However, given the relatively small number of cases and the fact that outcomes are likely dependent on treatment and MRD technique used, our data must be confirmed in other cohorts before we can abandon morphologic assessment altogether. This question will therefore be the subject of a forthcoming international analysis by the Ponte di Legno working group.

In light of our results, within the United Kingdom group, we have amended the current protocol to redefine IF as EOI MRD $\geq$ 5% and/or M3 marrow. Those patients with M2 marrow without an EOI MRD result will have morphology assessed by central review. Given the extremely poor outcome in this population, it is essential that these patients be accurately identified so that they can be considered for early treatment intensification and use of novel agents.

Results of the cytogenetic screening are therefore significant, identifying *EBF1-PDGFRB* rearrangements in approximately 10% of patients with IF. This result is particularly interesting given reports of the effectiveness of ABL tyrosine kinase inhibitors, such as imatinib, in the treatment of children with refractory ALL that harbors ABL-class fusions.[14,15] Early intervention in these patients led to CR and long-term survival, which highlights the importance of identifying targetable lesions early in therapy.

Despite making up only 12.5% of patients in the trial, 32% of IF occurred in T-ALL. Even so, our analysis suggests that approximately one quarter of these cases may be a result of ETP-ALL and that MRD $\geq$ 5% identifies a subgroup of ETP-ALL with a poor outcome, full immunophenotyping and MRD results were not available in all T-ALL patients and these data require confirmation in other cohorts. However, importantly, previous studies have shown that ETP-ALL is frequently associated with an absence of molecular markers for MRD assessment,[18,19] which means that PCR-based MRD detection may fail to identify high-risk patients. Flow-based MRD assessment should therefore be considered in all patients with ETP-ALL. Whereas ETP-ALL accounts for a subgroup of these patients, the other mechanisms that drive IF in T-ALL remain largely unknown and detailed genetic characterization of the T-ALL subgroup is urgently needed. Furthermore, as the complexity of genetic characterization of pediatric ALL increases, individual genetic subgroups will contain smaller patient numbers, underlining the need for collaborative international trials that are adequately powered to test new therapies in these high-risk patients.

Given the enrichment of *PDGFRB* fusions in IF and the potential for targeted therapy, the United Kingdom group has adopted a targeted molecular screening strategy directed at high-risk patients (Appendix Fig A2, online only). Therefore, any patient on the current UKALL 2011 trial with B-other or T-ALL with EOI MRD $>$ 1% will undergo screening for ABL-class fusions. Patients with targetable lesions will be considered for treatment with novel agents.

Overall, our results suggest that MRD provides a more objective measure of IF than morphology. Of importance, combining patients who fail to achieve a morphologic remission with those with MRD $\geq$ 5% selected almost 4% of trial entrants, which suggests that IF is more common than previously reported. This strategy allows identification of a subgroup of patients with extremely poor outcome with conventional treatment. As a result of this study, the United Kingdom trials group has revised the definition of IF and implemented a new screening algorithm to identify targetable lesions in an attempt to improve outcomes in this high-risk group.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at ascopubs.org/journal/jco.

AUTHOR CONTRIBUTIONS

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Affiliations

David O'Connor, **Ronald M.R. Tan**, **Jack Bartram**, and **Sujith Samarasinghe**, Great Ormond Street Hospital for Children NHS Foundation Trust; **Rachael Hough**, University College London Hospitals NHS Foundation Trust, London; **Anthony V. Moorman**, **Claire Schwab**, and **Christine J. Harrison**, Newcastle University, Newcastle Upon Tyne; **Rachel Wade**, University of Oxford, Oxford; **Jeremy Hancock**, North Bristol NHS Trust; **John Moppett**, Royal Hospital for Children, Bristol; **Ajay Vora** and **Katharine Patrick**, Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom; and **Nick Goulden**, Trapehade, Monferran Plaves, France.

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Use of Minimal Residual Disease Assessment to Redefine Induction Failure in Pediatric Acute Lymphoblastic Leukemia

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David O'Connor
No relationship to disclose

Anthony V. Moorman
No relationship to disclose

Rachel Wade
No relationship to disclose

Jeremy Hancock
No relationship to disclose

Ronald M.R. Tan
No relationship to disclose

Jack Bartram
Travel, Accommodations, Expenses: Jazz Pharmaceuticals

John Moppett
Consulting or Advisory Role: Jazz Pharmaceuticals
Travel, Accommodations, Expenses: Jazz Pharmaceuticals

Claire Schwab
No relationship to disclose

Katharine Patrick
Travel, Accommodations, Expenses: EUSA Pharma, Bayer, Medac

Christine J. Harrison
No relationship to disclose

Rachael Hough
No relationship to disclose

Nick Goulden
Travel, Accommodations, Expenses: EUSA Pharma

Ajay Vora
Honoraria: Jazz Pharmaceuticals, Pfizer, Amgen
Travel, Accommodations, Expenses: Jazz Pharmaceuticals, Medac

Sujith Samarasinghe
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Appendix

### Supplementary Methods, Participants, and Trial Protocol

The trial recruited children and young people with acute lymphoblastic leukemia (ALL) at 45 centers in the United Kingdom and Ireland between October 2003 and June 2011. Patients age < 1 year or with mature B-cell ALL or Philadelphia chromosome–positive ALL were not eligible. Initially, upper age limit was 18 years, but was increased to the 20th birthday in April 2006 and 24th birthday from September 2007.

Patients were initially stratified according to clinical risk of relapse on the basis of the National Cancer Institute (NCI) risk criteria (NCI standard risk: patients age < 10 years with white blood cell count $< 50 \times 10^9$/L; NCI high risk: patients age ≥ 10 years and/or white blood cell count $\geq 50 \times 10^9$/L) and cytogenetics (patients with *MLL/KMT2A* gene rearrangement, near haploidy [< 30 chromosomes], low hypodiploidy [< 40 chromosomes], t(17;19)(q22;p13)/*TCF3-HLF*, or intrachromosomal amplification of chromosome 21 [iAMP21] were classified as high risk). In the absence of high-risk cytogenetics, NCI standard-risk patients were allocated to the standard risk arm, regimen A, which included a three-drug induction (dexamethasone, vincristine, and asparaginase). NCI high-risk patients were allocated to the intermediate-risk arm, regimen B, and those with high-risk cytogenetics were allocated to the high-risk arm, regimen C; regimens B and C used a four-drug induction (dexamethasone, vincristine, asparaginase, and daunorubicin). Further details of chemotherapy regimens can be found in the tables below.

Further stratification was based on morphologic early response and minimal residual disease (MRD). Bone marrow morphology was assessed in patients age < 16 years; those with $> 25\%$ bone marrow blasts at day 8 (NCI high risk) or 15 (NCI standard risk) were allocated to the high-risk arm, regimen C, and were not eligible for MRD stratification. All patients age ≥ 16 years were treated as intermediate risk irrespective of days 8 and 15 response and were eligible for MRD stratification.

Patients with MRD $< 0.01\%$ at end of induction (day 29) were classified as low risk and remained on their starting regimen (A or B). Those with at least 0.01% end of induction MRD were classified as MRD high risk and randomly assigned between standard postremission therapy and regimen C. Clinical high-risk patients were not eligible for MRD stratification.

The protocol was approved by the Scottish Multi-Centre Research Ethics Committee. Patients were enrolled at individual treatment centers by principal investigators after written informed consent from caregivers or patients was obtained. The trial was monitored by an independent data monitoring committee.

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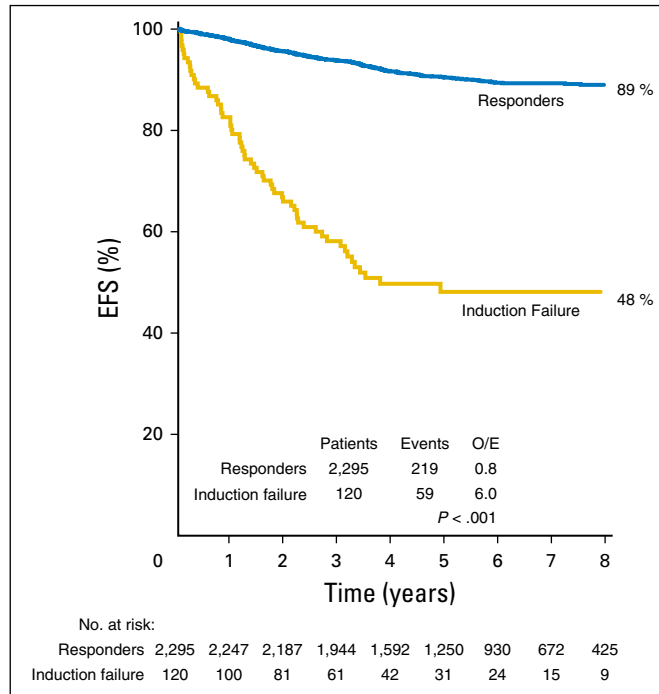
Fig A1. Event-free survival (EFS) in 120 patients with induction failure on the basis of new criteria (M2 and M3 marrow and/or end of induction minimal residual disease ≥ 5%) compared with those patients who achieved complete remission at the end of induction. Data indicate 8-year EFS estimates. Numbers within each group are indicated in the at-risk table beneath the graph. O/E, observed/expected.

UKALL2011 screening algorithm for ABL-class fusions

Criteria for testing
Poor responding patients who have:
  Induction failure or M2/M3 marrow at the end of induction
  MRD > 1% at day 28 or > 0.5% at week 14
AND
  B-other ALL* or T-ALL

Initial action by the local genetics lab
  FISH with *PDGFRB* dual color break apart probe
  Review *BCR-ABL1* FISH results for *ABL1* involvement†
  Review cytogenetic result for 1q25 *(ABL2)* involvement

Centralized screening by the LRCG
  If 1q25 is abnormal by cytogenetics, FISH with *ABL2* probe
  If *BCR-ABL1* FISH shows extra *ABL1* signal, FISH with *ABL1*
  break apart probe

What happens if the patient has an ABL-class fusion?
  Discuss result with UKALL2011 trial coordinator and consider
  treatment with a tyrosine kinase inhibitor

What research is performed by the LRCG?
  Collate all genetic results to assess outcome
  Determine the partner gene of all positive patients
  Explore the genomic landscape of all ABL-class patients

Fig A2. Current UK2011 trial screening algorithm for ABL-class fusions in acute lymphoblastic leukemia (ALL). *B-other ALL, B-cell precursor ALL lacking *ETV6-RUNX1*, high hyperdiploidy, *BCR-ABL1*, *KMT2A* (*MLL*) rearrangement, *TCF3-PBX1*, *TCF3-HLF*, near-haploidy, low hypodiploidy, iAMP21; †*ETV6-RUNX1* FISH results should also be reviewed to exclude rare cases of *ETV6-ABL1*. FISH, fluorescence in situ hybdridization; LRCG, Leukaemia Research Cytogenetics Group; MRD, minimal residual disease.

Table A1. Treatment Schedules

Drug	Dose	Day of Cycle
Regimen A		
3-Drug induction		
Dexamethasone	6 mg/m^2/d	1-28
Vincristine	1.5 mg/m^2	2, 9, 16, 23, 30
Pegylated L-asparaginase	1,000 IU/m^2	4, 18
Intrathecal methotrexate	< 2 years: 8 mg; 2 years: 10 mg; > 2 years: 12 mg	1, 8, 28
6-Mercaptopurine	75 mg/m^2/d	29-35
CNS-directed therapy		
Intrathecal methotrexate	As above	1, 8, 15
6-Mercaptopurine	75 mg/m^2/d	1-21
Interim maintenance blocks		
Dexamethasone	6 mg/m^2/d × 5 days	1-5, 29-33
Vincristine	1.5 mg/m^2	1, 29
6-Mercaptopurine	75 mg/m^2/d	1-49
Oral methotrexate	20 mg/m^2/wk	1, 8, 22, 29, 36, 43, 50
Intrathecal methotrexate	As above	15
Delayed intensification blocks		
Dexamethasone	10 mg/m^2/d × 7 days	2-8, 16-22
Vincristine	1.5 mg/m^2	2, 9, 16
Doxorubicin	25 mg/m^2	2, 9, 16
Pegylated L-asparaginase	1,000 IU/m^2	4
Intrathecal methotrexate	As above	1
Cyclophosphamide	1,000 mg/m^2	29
6-Mercaptopurine	60 mg/m^2/d	29-42
Cytarabine	75 mg/m^2/d × 4 days	30-33, 37-40
Intrathecal methotrexate	As above	29, 36
Maintenance cycles		
Dexamethasone	6 mg/m^2/d × 5 days	1-5, 29-33, 57-61
Vincristine	1.5 mg/m^2	1, 29, 57
6-Mercaptopurine	75 mg/m^2/d	Continuous
Oral methotrexate	20 mg/m^2/wk	1, 8, 15, 22, 29, 36, 43, 50
Intrathecal methotrexate	As above	15
Regimen B		
4-Drug induction		
Dexamethasone	6 mg/m^2/d	1-28
Vincristine	1.5 mg/m^2	2, 9, 16, 23, 30
Daunorubicin	25 mg/m^2	2, 9, 16, 23
Pegylated L-asparaginase	1,000 IU/m^2	4, 18
Intrathecal methotrexate	< 2 years: 8 mg; 2 years: 10 mg; > 2 years: 12 mg	1, 8, 28
6-Mercaptopurine	60 mg/m^2/d	29-35
BFM consolidation		
Cyclophosphamide	1,000 mg/m^2	1, 15
Cytarabine	75 mg/m^2/d × 4 days	2-5, 9-12, 16-19, 23-26
6-Mercaptopurine	60 mg/m^2/d	36-70
Intrathecal methotrexate	As above	1, 8, 15
Interim maintenance blocks		
Dexamethasone	6 mg/m^2/d × 5 days	2-6, 30-34
Vincristine	1.5 mg/m^2	2, 30
6-Mercaptopurine	75 mg/m^2/d	1-49, 50-56
Oral methotrexate	20 mg/m^2/wk	8, 15, 22, 36, 43, 50
Intrathecal methotrexate	As above	1, 29
Delayed intensification blocks		
Dexamethasone	10 mg/m^2/d × 7 days	2-8, 16-22
Vincristine	1.5 mg/m^2	2, 9, 16
Doxorubicin	25 mg/m^2	2, 9, 16
Pegylated L-asparaginase	1,000 IU/m^2	4
Intrathecal methotrexate	As above	1
Cyclophosphamide	1,000 mg/m^2	29
6-Mercaptopurine	60 mg/m^2/d	29-42
Cytarabine	75 mg/m^2/d × 4 days	30-33, 37-40
Intrathecal methotrexate	As above	29, 36
Maintenance cycles		
Dexamethasone	6 mg/m^2/d × 5 days	1-5, 29-33, 57-61
Vincristine	1.5 mg/m^2	1, 29, 57
6-Mercaptopurine	75 mg/m^2/d	Continuous
Oral methotrexate	20 mg/m^2/wk	1, 8, 15, 22, 29, 36, 43, 50
Intrathecal methotrexate	As above	15

(continued on following page)

Table A1. Treatment Schedules (continued)

Drug	Dose	Day of Cycle
Regimen C		
4-Drug induction		
Dexamethasone	6 mg/m^2/d	1-28
Vincristine	1.5 mg/m^2	2, 9, 16, 23, 30
Daunorubicin	45 mg/m^2	16, 23
Pegylated L-asparaginase	1,000 IU/m^2	18
Intrathecal methotrexate	< 2 years: 8 mg; 2 years: 10 mg; > 2 years: 12 mg	1, 8, 28
6-Mercaptopurine	60 mg/m^2/d	29-35
BFM consolidation		
Cyclophosphamide	1,000 mg/m^2	1, 29
Cytarabine	75 mg/m^2/d × 4 days	2-5, 9-12, 30-33, 37-40
6-Mercaptopurine	60 mg/m^2/d	1-21, 29-42
Vincristine	1.5 mg/m^2	16, 23, 44, 51
Pegylated L-asparaginase	1,000 IU/m^2	16, 44
Intrathecal methotrexate	As above	1, 8, 15
Capizzi maintenance		
Vincristine	1.5 mg/m^2	2, 12, 22, 32, 42
Intravenous methotrexate	Initial 100 mg/m^2, escalated by 50 mg/m^2 in each subsequent dose	2, 12, 22, 32, 42
Pegylated L-asparaginase	1,000 IU/m^2	3, 23
Intrathecal methotrexate	As above	1, 31
Delayed intensification blocks		
Dexamethasone	10 mg/m^2/d × 7 days	2-8, 16-22
Vincristine	1.5 mg/m^2	2, 9, 16
Doxorubicin	25 mg/m^2	2, 9, 16
Pegylated L-asparaginase	1,000 IU/m^2	4, 43
Intrathecal methotrexate	As above	1
Cyclophosphamide	1,000 mg/m^2	29
6-Mercaptopurine	60 mg/m^2/d	29-42
Cytarabine	75 mg/m^2/d × 4 days	30-33, 37-40
Intrathecal methotrexate	As above	29, 36
Maintenance cycles		
Dexamethasone	6 mg/m^2/d × 5 days	1-5, 29-33, 57-61
Vincristine	1.5 mg/m^2	1, 29, 57
6-Mercaptopurine	75 mg/m^2/d	Continuous
Oral methotrexate	20 mg/m^2/wk	Weekly
Intrathecal methotrexate	As above	15

Table A2. Subsequent Off-Protocol Treatment of 15 Patients With M3 Marrow

Patient	Subsequent Treatment	CR1 HSCT	Comments
1	ALL-R3 protocol	Yes	Died of adenovirus viremia
2	ALL-R3 protocol	Yes	Relapsed, CR2 HSCT, alive
3	FLA-Ida	No	Died of relapse
4	MidAC	No	Died of relapse
5	UKALL2003 regimen C	No	Alive
6	UKALL2003 regimen C	Yes	Alive
7	MidAC	Yes	Alive
8	Nelarabine, asparaginase	Yes	Died of GvHD
9	Not known	Yes	Alive
10	Not known	No	Died of relapse
11	UKALL2003 regimen C	Yes	Alive
12	Nelarabine, cyclophosphamide, etoposide	No	Died of progressive disease
13	FLA-Ida	Yes	Died of progressive disease
14	ALL-R3 protocol	No	Died of progressive disease
15	UKALL2003 regimen C	No	Down's syndrome, died of progressive disease

Abbreviations: CR1, first complete remission; FLA-Ida, fludarabine, cytarabine, idarubicin; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MidAC, mitoxantrone, cytarabine.

Table A3. Comparison of Morphologic Marrow Status and MRD Level at End of Induction in Patients With B-Precursor ALL

	B-Precursor ALL						
	MRD at End of Induction						
BM at Day 29	MRD < 0.01%	0.01% ≤ MRD < 0.1%	0.1% ≤ MRD < 1%	1% ≤ MRD < 5%	MRD > 5%	Indeterminate	Total
M1	1,331	448	226	66	33	270	2,374
M2	5	1	6	3	16	3	34
M3	0	0	1	0	6	1	8
NR	161	47	29	10	6	58	311
Total	1,497	496	262	79	61	332	2,727

NOTE. Data indicate No. of patients within each group.
Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; MRD, minimal residual disease; NR, not recorded.

Table A4. Comparison of Morphologic Marrow Status and MRD Level at End of Induction in Patients With T-ALL

	T-ALL						
	MRD at the End of Induction						
BM at Day 29	MRD < 0.01%	0.01% ≤ MRD < 0.1%	0.1% ≤ MRD < 1%	1% ≤ MRD < 5%	MRD > 5%	Indeterminate	Total
M1	103	52	54	15	21	74	319
M2	1	0	1	1	1	6	10
M3	0	0	0	0	3	4	7
NR	15	9	5	1	1	19	50
Total	119	61	60	17	26	103	386

NOTE. Data indicate No. of patients within each group.
Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; MRD, minimal residual disease; NR, not recorded.

Characteristic	Total (n = 3,113)	Indeterminate (n = 698)	Remission (n = 2,295)	Induction Failure (n = 120)	P*
Table A5. Characteristics of Patients With Induction Failure Defined by Morphology and MRD Level					
Patient					
Sex					
Male	1,767 (57%)	400 (57%)	1,296 (56%)	71 (59%)	.6
Female	1,346 (43%)	298 (43%)	999 (44%)	49 (41%)	
Age group, years					
< 10	2,278 (73%)	496 (71%)	1,737 (76%)	45 (38%)	< .001
10-15	608 (20%)	144 (21%)	420 (18%)	44 (37%)	
≥ 16	227 (7%)	58 (8%)	138 (6%)	31 (26%)	
Median (range)	5 (1-24)	5 (1-24)	4 (1-24)	13 (1-24)	< .001
Ethnicity					
Black	74 (2%)	17 (2%)	56 (2%)	1 (1%)	.4
White	2,525 (81%)	538 (77%)	1,882 (82%)	105 (88%)	(.1†)
Asian	232 (7%)	66 (9%)	161 (7%)	5 (4%)	
Other	151 (5%)	35 (5%)	111 (5%)	5 (4%)	
Unknown	131 (4%)	42 (6%)	85 (4%)	4 (3%)	
Down's syndrome					
No	3,026 (97%)	675 (97%)	2,236 (97%)	115 (96%)	.3
Yes	87 (3%)	23 (3%)	59 (3%)	5 (4%)	
Disease					
WBC at diagnosis, ×10^9/L					
< 20	1,904 (61%)	463 (66%)	1,385 (60%)	56 (47%)	< .001
20-	524 (17%)	93 (13%)	414 (18%)	17 (14%)	
50-	313 (10%)	63 (9%)	235 (10%)	15 (13%)	
100-	199 (6%)	40 (6%)	146 (6%)	13 (11%)	
≥ 200	173 (6%)	39 (6%)	115 (5%)	19 (16%)	
Median (range)	12 (0-881)	9 (0-783)	13 (1-881)	26 (1-800)	< .001
NCI risk group					
Standard	1,812 (58%)	399 (57%)	1,384 (60%)	29 (24%)	< .001
High	1,301 (42%)	299 (43%)	911 (40%)	91 (76%)	
Immunophenotype					
B-precursor	2,727 (88%)	575 (82%)	2,071 (90%)	81 (68%)	< .001
T-cell	386 (12%)	123 (18%)	224 (10%)	39 (33%)	
Cytogenetic risk group‡					
Good	1,586 (58%)	317 (55%)	1,251 (60%)	18 (22%)	< .001§
Intermediate	860 (32%)	182 (32%)	635 (31%)	43 (53%)	
High	121 (4%)	30 (5%)	80 (4%)	11 (14%)	
Unknown	160 (6%)	46 (8%)	105 (5%)	9 (11%)	
CNS disease at diagnosis					
No	3,061 (98%)	684 (98%)	2,260 (98%)	117 (98%)	.4
Yes	52 (2%)	14 (2%)	35 (2%)	3 (3%)	
Treatment					
Slow early response					
No	2,751 (88%)	621 (89%)	2,075 (90%)	55 (46%)	< .001
Yes	362 (12%)	77 (11%)	220 (10%)	65 (54%)	
Bone marrow status day 29					
M1	2,693 (87%)	344 (49%)	2,295 (100%)	54 (45%)	N/A
M2	44 (1%)	0 (0%)	0 (0%)	44 (37%)	
M3	15 (0%)	0 (0%)	0 (0%)	15 (13%)	
Unknown	361 (12%)	354 (51%)	0 (0%)	7 (6%)	
MRD level day 29					
< 0.01%	1,616 (52%)	176 (25%)	1,434 (62%)	6 (5%)	< .001
0.01%-1%	879 (28%)	90 (13%)	780 (34%)	9 (8%)	
> 1%	183 (6%)	11 (2%)	81 (4%)	91 (76%)	
Unknown	435 (14%)	421 (60%)	0 (0%)	14 (12%)	
Regimen administered					
A	1,537 (49%)	362 (52%)	1,162 (51%)	13 (11%)	< .001
B	842 (27%)	214 (31%)	609 (27%)	19 (16%)	
C	734 (24%)	122 (17%)	524 (23%)	88 (73%)	

Abbreviations: MRD, minimal residual disease; N/A, not applicable; NCI, National Cancer Institute; WBC, white blood cell.

*P value for trend for ordered groups: age group, WBC, cytogenetic risk group, MRD level; otherwise, P value for heterogeneity. 'Unknown' category excluded.

†White v black, Asian, and other.

‡Risk groups defined for B-precursor acute lymphoblastic leukemia. Good risk cytogenetics = ETV6-RUNX1, high hyperdiploidy; high risk = near haploidy, low hypodiploidy, KMT2A(MLL), iAMP21, t(17;19); intermediate = all others.

§B-precursor ALL only: good v intermediate v high.

Table A6. Univariate Analysis of Factors Influencing EFS in Induction Failure Defined by Morphology and MRD

Characteristic	Events/Patients	5-Year EFS (95% CI)	P (log rank)
Patient			
Sex			
Male	37/71	43.8 (31.2 to 56.5)	.5
Female	22/49	53.9 (39.6 to 68.1)	
Age, years			
< 10	17/45	59.1 (45.5 to 76.7)	.003*
10-15	23/44	45.7 (32.7 to 63.9)	
≥ 16	19/31	35.9 (21.5 to 60.0)	
Down's syndrome			
Yes	4/5	40.0 (0 to 82.9)	.01
No	55/115	49.4 (39.7 to 59.1)	
Disease			
WCC at diagnosis			
< 20	12/36	64.8 (48.5 to 81.0)	.003*
20-	10/20	36.8 (9.0 to 64.7)	
50-	8/17	50.3 (25.4 to 75.2)	
100-	7/15	53.3 (28.1 to 78.6)	
200-	22/32	30.6 (14.3 to 46.8)	
NCI risk group			
Standard	10/29	62.0 (42.9 to 81.1)	.03
High	49/91	44.1 (33.4 to 54.7)	
Immunophenotype			
T cell	20/39	47.3 (31.1 to 63.5)	.7
B precursor	38/76	47.8 (36.0 to 59.6)	
Cytogenetic risk group (B-precursor ALL only)			
Good	5/18	70.8 (49.3 to 92.4)	.03†
Intermediate	24/43	40.9 (24.8 to 57.0)	
High	5/11	53.0 (22.7 to 83.4)	
Treatment			
Early response			
Rapid	25/55	51.3 (37.0 to 65.5)	.4
Slow	34/65	45.4 (32.8 to 58.1)	
Bone marrow status day 29			
M1	27/54	47.0 (32.9 to 61.1)	.2
M2	18/44	57.1 (41.8 to 72.4)	(.4*)
M3	10/15	33.3 (9.5 to 57.2)	
Regimen administered			
A	4/13	63.9 (35.0 to 92.8)	.4*
B	12/19	33.8 (11.3 to 56.4)	
C	43/88	49.3 (38.5 to 60.2)	
MRD level day 29			
< 0.01%	0/6	100.0	.006*
0.01 to < 1%	2/9	76.2 (47.2 to 100.0)	
1 to < 5%	1/4	75.0 (32.6 to 100.0)	
≥ 5%	47/87	43.2 (32.2 to 54.3)	

Abbreviations: ALL, acute lymphoblastic leukemia; EFS, event-free survival; MRD, minimal residual disease; NCI, National Cancer Institute; WCC, white blood cell count.
*Trend P values are a test across ordered groups.
†Good v intermediate/high.

Table A7. Comparison of Morphological Marrow Status and MRD Level at the End of Induction in Patients with Early T-Precursor ALL

| | Early T-Precursor ALL | | | | | | |
| | MRD at End of Induction | | | | | | |
BM at Day 29	MRD < 0.01%	0.01% ≤ MRD < 0.1%	0.1% ≤ MRD < 1%	1% ≤ MRD < 5%	MRD > 5%	Indeterminate	Total
M1	3	1	5	3	3	12	27
M2	0	0	0	0	0	0	0
M3	0	0	0	0	1	0	1
NR	0	1	0	0	0	4	5
Total	3	2	5	3	4	16	33

NOTE. Data indicate No. of patients within each group.
Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; MRD, minimal residual disease; NR, not recorded.