Outcomes of patients with childhood B-cell precursor acute lymphoblastic leukaemia with late bone marrow relapses: long-term follow-up of the ALLR3 open-label randomised trial

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Summary

Background The ALLR3 trial investigated outcomes of children with B-cell precursor acute lymphoblastic leukaemia who had late bone marrow relapses. We analysed long-term follow-up outcomes of these patients.

Methods ALLR3 was an open-label randomised clinical trial that recruited children aged 1–18 years with B-cell precursor acute lymphoblastic leukaemia who had late bone marrow relapses. Eligible patients were recruited from centres in Australia, Ireland, the Netherlands, New Zealand, and the UK. Patients were randomly assigned from Jan 31, 2003, to Dec 31, 2007, and the trial closed to recruitment on Oct 31, 2013. Randomly assigned patients were allocated to receive either idarubicin or mitoxantrone in induction by stratified concealed randomisation; after randomisation stopped in Dec 31, 2007, all patients were allocated to receive mitoxantrone. After three blocks of therapy, patients with high minimal residual disease ($\geq 10^{-4}$ cells) at the end of induction were allocated to undergo allogeneic stem-cell transplantation and those with low minimal residual disease ($<10^{-4}$ cells) at the end of induction were allocated to receive chemotherapy. Minimal residual disease level was measured by real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements. The primary endpoint of the original ALLR3 clinical trial was progression-free survival of randomly assigned patients. The primary endpoint of this long-term follow-up analysis was progression-free survival of patients with late bone marrow relapses stratified by minimal residual disease level. Outcomes were correlated with age, site, time to recurrence, and genetic subtypes, and analysed by both intention to treat and actual treatment received. This trial is registered on the ISRCTN registry, number ISRCTN45724312, and on ClinicalTrials.gov, number NCT00967057.

Findings Between Feb 2, 2003, and Oct 28, 2013, 228 patients with B-cell precursor acute lymphoblastic leukaemia and late bone marrow relapses were treated. After a median follow-up of 84 months (IQR 48–109), progression-free survival of all randomly assigned patients was 60% (95% CI 54–70). 220 patients achieved second complete remission, and minimal residual disease was evaluable in 192 (87%). 110 patients with late bone marrow relapses and high minimal residual disease at the end of induction were allocated to undergo stem-cell transplantation, and 82 patients with low minimal residual disease at the end of induction were allocated to receive chemotherapy. In the patients allocated to undergo stem-cell transplantation, four relapses and three deaths were reported before the procedure, and 11 patients were not transplanted. Of the 92 patients transplanted, 58 (63%) remained in second complete remission, 13 (14%) died of complications, and 21 (23%) relapsed after stem-cell transplantation. In patients allocated to receive chemotherapy, one early treatment-related death was reported and 11 patients were transplanted. Of the 70 patients who continued on chemotherapy, 49 (70%) remained in second complete remission, two (3%) died of complications, and 19 (27%) relapsed. Progression-free survival at 5 years was 56% (95% CI 46–65) in those with high minimal residual disease and 72% (60–81) in patients with low minimal residual disease (p=0·0078). Treatment-related serious adverse events were not analysed in the long-term follow-up.

Interpretation Patients with B-cell precursor acute lymphoblastic leukaemia with late bone marrow relapses and low minimal residual disease at end of induction had favourable outcomes with chemotherapy without undergoing stem-cell transplantation. Patients with high minimal residual disease benefited from stem-cell transplantation, and targeted therapies might offer further improvements in outcomes for these patients.

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Research in context

Evidence before the study

We searched for articles published in PubMed before September, 2018, without date limitations, for outcomes of children with "B-cell precursor acute lymphoblastic leukaemia" and "bone marrow relapse"; the search was further refined to include only those patients with a relapse 36 months after the first diagnosis or more than 6 months after stopping front-line therapy and who were treated uniformly for relapse, with minimal residual disease measured after induction. Two relevant studies were identified. The Children's Oncology Group ALL 01P2 study treated 55 patients with B-cell precursor acute lymphoblastic leukaemia and late bone marrow relapses. 1-year event-free survival was 86% (SD 8) in patients with end-of-induction minimal residual disease comprising fewer than 10^{-4} cells and was 77% (SD 9; p=0·005) for those with minimal residual disease comprising 10^{-4} cells or more. Details of allogeneic stem-cell transplantation were not available. The BFM REZ 2002 study treated 236 patients with B-cell precursor acute lymphoblastic leukaemia and late bone marrow relapses. 8-year event-free survival was 70% (SD 5) for patients with end-of-induction minimal residual disease comprising fewer than 10^{-3} cells stratified for no stem-cell transplantation, and 64% (SD 5; p=0·29) in those with end-of-induction minimal residual disease comprising 10^{-3} cells or more, stratified for stem-cell transplantation. We next searched for studies describing outcomes of children with relapsed B-cell precursor acute lymphoblastic leukaemia in whom minimal residual disease assessment was done before stem-cell transplantation. We identified six articles. Five were retrospective, not stratified by the time to relapse, and identified a pre-stem-cell transplantation minimal residual disease of 10^{-4} cells or more as being predictive of relapse after stem-cell transplantation. The BFM REZ 96 and 2002 trials prospectively analysed minimal residual disease before stem-cell transplantation in 35 patients with B-cell precursor acute lymphoblastic leukaemia and late bone marrow relapses. 4-year event-free survival was 68% (SD 12) in patients with minimal residual disease of fewer than 10^{-4} cells before stem-cell transplantation and 20% (SD 12; p=0·02) in those with minimal residual disease of 10^{-4} cells or more. These studies suggest that a proportion of children with B-cell precursor acute lymphoblastic leukaemia and late bone marrow relapses do not require stem-cell transplantation to maintain long-term remission as ascertained by end-of-induction minimal residual disease. For those requiring a transplant, minimal residual disease of 10^{-4} cells or more before stem-cell transplantation appears to be associated with high recurrence rates after stem-cell transplantation.

Added value of this study

In this long-term follow-up of patients in the ALLR3 trial with B-cell precursor acute lymphoblastic leukaemia who had late bone marrow relapses, a high end-of-induction minimal residual disease level of 10^{-4} cells or more was used to stratify patients to stem-cell transplantation. Patients with low minimal residual disease (defined as $<10^{-4}$ cells) had significantly better progression-free survival and overall survival than did those with high minimal residual disease, suggesting that this is the preferred level to ascertain eligibility of patients for stem-cell transplantation. High-risk cytogenetic groups and *IKZF1*, *PAX5*, or *NR3C1* deletions or *NRAS* mutations were associated with higher levels of minimal residual disease and poor outcomes. Patients with high minimal residual disease ($\geq 10^{-4}$ cells) before stem-cell transplantation had reasonable outcomes after stem-cell transplantation.

Implications of all available evidence

An end-of-induction minimal residual disease of fewer than 10^{-4} cells in patients with B-cell precursor acute lymphoblastic leukaemia and late bone marrow relapses is associated with favourable outcomes with systemic chemotherapy and avoidance of cranial irradiation in those without CNS involvement. In this group, improvements in the sensitivity of techniques for detecting minimal residual disease might allow further refinement; second relapses are salvageable with stem-cell transplantation in patients who are in third complete remission, and experimental therapies will require careful evaluation. Although patients with end-of-induction minimal residual disease of 10^{-4} cells or more benefited from stem-cell transplantation in this study, transplant-related mortality and relapse rates after stem-cell transplantation remained problematic; this group might benefit from newer treatment modalities.

Introduction

Outcomes after isolated or combined bone marrow relapses in patients with childhood B-cell precursor acute lymphoblastic leukaemia are associated with the duration of first complete remission. Irrespective of treatment strategies, children relapsing more than 6 months after stopping therapy have better survival rates than those who relapse earlier (overall survival 45–73% for those who relapse >6 months later *vs* 22–38% for those who relapse earlier),[1–4] suggesting that recurring blasts remain chemosensitive in many patients. The BFM Study Group identified a subset of patients with late bone marrow relapses (isolated or combined) who had low minimal residual disease (defined as $<10^{-3}$ cells) at end of induction and could thus be maintained in second complete remission with chemotherapy and targeted radiotherapy.[5] Those with minimal residual disease comprising 10^{-3} cells or more, however, frequently had a second relapse. The Children's Oncology Group (USA), in a smaller cohort of patients, showed substantially better outcomes in patients with end-of-induction minimal residual disease comprising fewer than 10^{-4} cells than in those with minimal residual disease comprising 10^{-4} cells or more.[6] Consequently, the ALLREZ BFM 2002 clinical trial

assigned patients with end-of-induction minimal residual disease of 10^{-3} cells or more to allogeneic stem-cell transplantation, while patients with end-of-induction minimal residual disease comprising fewer than 10^{-3} cells were allocated to chemotherapy with cranial irradiation for isolated late bone marrow relapses and targeted radiotherapy for combined relapses.[3] The international collaborative ALLR3 clinical trial protocol recommended stem-cell transplantation for patients with B-cell precursor-acute lymphoblastic leukaemia and late bone marrow relapses with an end-of-induction minimal residual disease of 10^{-4} cells or more, whereas those with minimal residual disease of fewer than 10^{-4} cells were assigned to chemotherapy. The ALLR3 trial used a four-drug anthracycline-based induction, similarly to the COG trial,[6] which also used a minimal residual disease level of 10^{-4} cells, and in contrast to the anthracycline-free induction in the ALLREZ BFM trial,[5] which used a minimal residual disease level of 10^{-3} cells. In the ALLR3 trial, patients were randomly assigned to receive idarubicin versus mitoxantrone in induction. Mitoxantrone had a substantial effect on progression-free survival and overall survival compared with idarubicin, despite similar levels of end-of-induction minimal residual disease in the two groups.[7] This study did not report on the outcomes of minimal residual disease risk stratification.

Treatment and other biological factors affect outcomes of patients with relapsed acute lymphoblastic leukaemia, independently of end-of-induction minimal residual disease status; in particular, high-risk cytogenetics and deletions and mutations in *TP53* have been shown to be associated with poor outcomes in patients with late bone marrow relapses, irrespective of minimal residual disease following induction.[8,9] Here, we report long-term follow-up outcomes of the ALLR3 trial, in which patients with B-cell precursor acute lymphoblastic leukaemia who had late isolated and combined bone marrow relapses were stratified to continue chemotherapy or to have stem-cell transplantation on the basis of their end-of-induction minimal residual disease. We also investigated the influence of cytogenetics, previously identified prognostic somatic copy number alterations,[8] and recurrent gene mutations, including the recent characterisation of *IKZF*^{plus}.[10]

Methods

Study design and participants

ALLR3 was an open-label randomised clinical trial that recruited children aged 1–18 years with B-cell precursor acute lymphoblastic leukaemia who had isolated or combined late bone marrow relapses. Patients were recruited from children's centres in Australia, Ireland, the Netherlands, New Zealand, and the UK,[9] and were randomly assigned from Jan 31, 2003, to Dec 31, 2007; the trial closed to recruitment on Oct 31, 2013. The study was approved by the relevant ethics committees in each country and patients were recruited after written consent was obtained from parents or carers. Outcomes of patients with late isolated extramedullary relapses have been previously published and are excluded from these analyses.[11]

Details of randomisation, risk stratification, and results of the randomised study along with the ALLR3 protocol have been reported previously[7,8,11] and are summarised in the appendix (p 1). From Jan 31, 2003, to Dec 31, 2007, patients were randomly assigned to receive either mitoxantrone or idarubicin during induction. By Dec 31, 2007, randomisation was stopped because of differences in progression-free survival and overall survival between the two groups, with mitoxantrone conferring a significant benefit. After randomisation was stopped, patients continued to receive mitoxantrone until closure of the trial on Oct 31, 2013.

See Online for appendix

Late relapses were defined as those occurring more than 6 months after the end of front-line therapy. Isolated late bone marrow relapses were defined as more than 25% blasts in the bone marrow and combined late bone marrow relapses defined as more than 5% bone marrow blasts, along with at least one extramedullary disease site. CNS relapse was defined as pleocytosis (>5 blasts per μL) with blasts in the cerebrospinal fluid (CSF). Testicular disease was diagnosed clinically and confirmed by biopsy or ultrasonography. All patients with late bone marrow relapses were treated with three blocks of chemotherapy. A four-drug induction with pulsed dexamethasone, anthracycline, vincristine, and polyethylene glycol conjugated L-asparaginase was followed by a consolidation block with high-dose methotrexate, cyclophosphamide, and etoposide. The third intensification block consisted of high-dose cytarabine followed by high-dose methotrexate. Patients were defined as having achieved second complete remission when bone marrow blasts were less than 5% at the end of induction (with normal CSF findings if they had concomitant CNS disease). Patients were stratified to receive a matched donor stem-cell transplantation or chemotherapy on the basis of minimal residual disease levels at the end of induction. This was a predefined secondary outcome of the ALLR3 trial.

Procedures

Minimal residual disease was measured from bone marrow samples obtained at the end of induction (timepoint 1) and again either before stem-cell transplantation or at the end of block 3 chemotherapy (timepoint 2) by use of real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements.[7,12] At timepoint 1, high minimal residual disease, measured by at least one marker, was defined as 10^{-4} cells or more, whereas low minimal residual disease, measured by two sensitive markers, was defined as fewer than 10^{-4} cells. When minimal residual disease was not measured or markers had lower sensitivity, these data were reported as being not available. Patients with

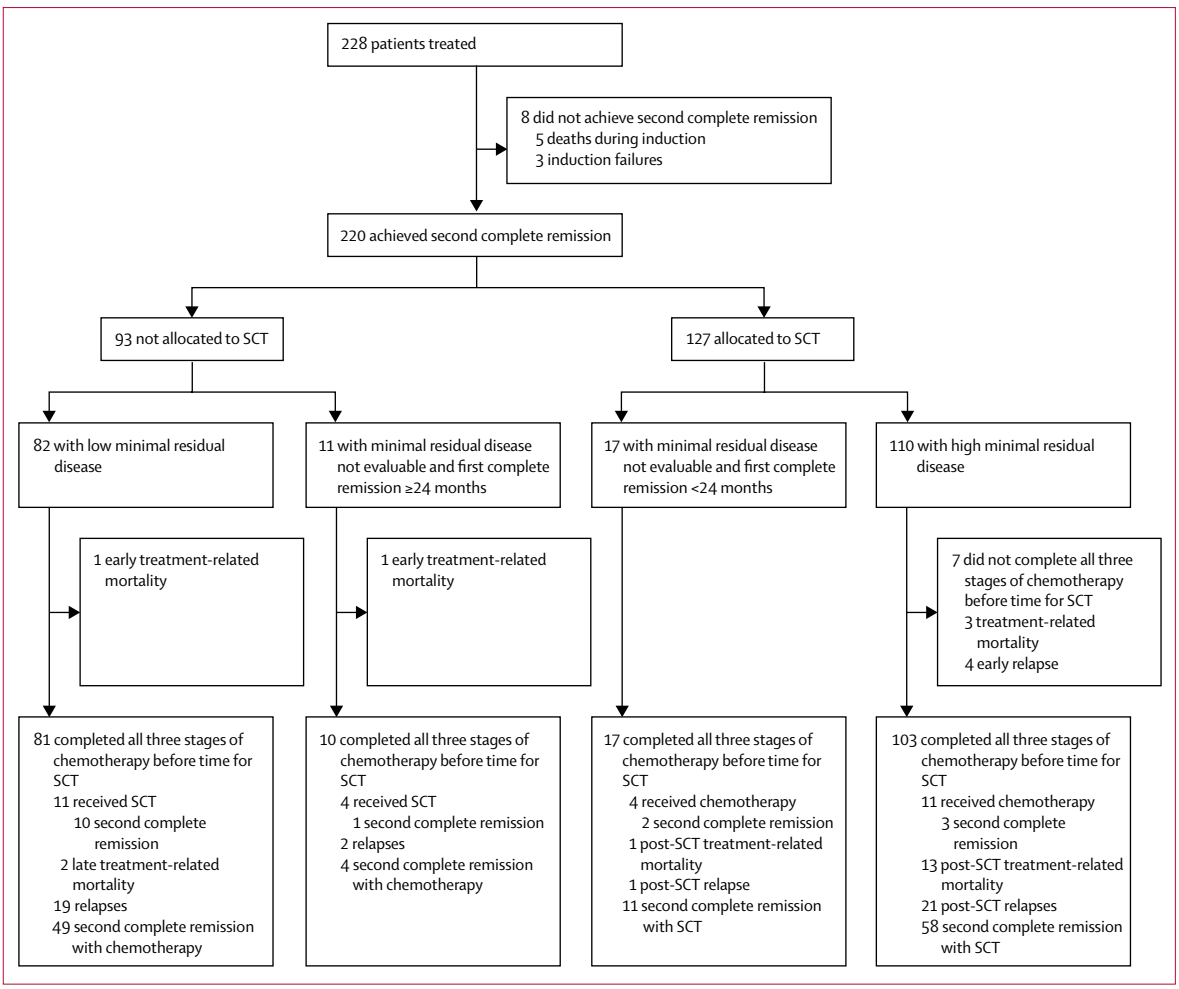

Figure 1: **Schematic showing outcome of patients with B-cell precursor-acute lymphoblastic leukaemia with late bone marrow relapses in the ALLR3 trial as per allocated treatment**

First complete remission refers to the time from completing front-line therapy. High minimal residual disease defined as ≥10^{-4} cells. Low minimal residual disease defined as <10^{-4} cells. SCT=stem-cell transplantation.

isolated or combined late bone marrow relapses and high minimal residual disease after induction were eligible for matched-donor stem-cell transplantation following completion of the third (or phase 3) chemotherapy treatment block. Those with low minimal residual disease continued with further chemotherapy alone (isolated late bone marrow relapses) or with site-directed radiotherapy (combined late bone marrow relapses). Where the minimal residual disease measurement was not available, patients with a relapse less than 24 months after stopping therapy were eligible for stem-cell transplantation.[1,7] In this report, stem-cell donors matched at 10/10 HLA loci were classified as matched donors and the rest as mismatched donors, including mismatched unrelated and haploidentical related donors. Cord refers to stem-cell transplantation done with donor cord blood stem cells.

Cytogenetic analysis was done locally and reviewed centrally by the Leukaemia Research Cytogenetics Group. Where information about cytogenetics not available for relapse, cytogenetics at first presentation was used. Integrated cytogenetics and molecular genetics were used to categorise patients into standard-risk, intermediate-risk, and high-risk genetic groups, as reported previously.[7] The copy number status of *IKZF1*, *CDKN2A/B*, *PAX5*, *EBF1*, *ETV6*, *BTG1*, *RB1*, *NR3C1*, and *PAR1* were ascertained with the SALSA Multiplex Ligation dependent Probe Amplification (MLPA) kit P335 (MRC Holland, Amsterdam, Netherlands). *TP53* deletions were assessed by a combination of cytogenetics and MLPA with the P056 kit. Key exons of *TP53*, *NRAS*, *KRAS*, *PTPN11*, *FLT3*, and *CBL* were assessed for mutations by denaturing high-performance liquid chromatography and Sanger or next-generation sequencing as previously described.[8,9] Patients were further classified according to the UKALL somatic copy number alteration classifier into good, intermediate, or poor-risk groups,[13] and according to *IKZF1*^{plus} profile

	Patients (%)	Log-rank analysis				Univariate Cox regression analysis			
		Progression-free survival (95% CI)	p value	Overall survival (95% CI)	p value	HR (95% CI) for progression-free survival	p value	HR (95% CI) for overall survival	p value
Total	228 (100%)	60 (54–70)	..	72 (65–78)	..	..		..	..
Age, years									
1–9	118 (52%)	61 (51–70)	0·72	72 (63–80)	0·72	1	..	1	..
10–14	77 (34%)	62 (50–72)	..	72 (60–81)	..	0·95 (0·59–1·52)	0·84	1·02 (0·58–1·78)	0·95
≥14	33 (14%)	55 (36–71)	..	70 (50–83)	..	1·22 (0·69–2·15)	0·50	1·31 (0·67–2·54)	0·43
Sex									
Women	102 (45%)	68 (58–77)	0·063	75 (65–83)	0·36	1	..	1	..
Men	126 (55%)	55 (45–63)	..	69 (60–77)	..	1·50 (0·97–2·30)	0·065	1·27 (0·77–2·09)	0·36
Site									
Isolated bone marrow	186 (82%)	62 (54–69)	0·60	75 (67–81)	0·24	1	..	1	..
Combined bone marrow*	42 (18%)	54 (37–68)	..	60 (43–74)	..	1·15 (0·69–1·93)	0·60	1·41 (0·79–2·51)	0·25
Minimal residual disease at timepoint 1†									
$<10^{-4}$ cells	82 (43%)	72 (60–81)	0·0078	87 (77–93)	0·0013	1	..	1	..
$\geq 10^{-4}$ cells	110 (57%)	56 (46–65)	..	64 (54–73)	..	1·94 (1·18–3·18)	0·009	2·77 (1·45–5·31)	0·0020
Intended treatment‡									
Chemotherapy	93 (42%)	68 (57–77)	0·078	84 (74–90)	0·020	1	..	1	..
Stem-cell transplantation	127 (58%)	59 (49–67)	..	66 (56–74)	..	1·50 (0·95–2·36)	0·080	1·94 (1·10–3·41)	0·022
Intended and received treatment§									
Chemotherapy	76 (42%)	70 (57–79)	0·39	86 (75–92)	0·046	1	..	1	..
Stem-cell transplantation	105 (58%)	67 (56–75)	..	71 (60–79)	..	1·26 (0·74–2·12)	0·39	1·96 (1·00–3·84)	0·050
Actual treatment¶									
Chemotherapy	91 (43%)	64 (53–73)	0·96	83 (73–90)	0·11	1	..	1	..
Stem-cell transplantation	120 (57%)	67 (57–75)	..	72 (63–80)	..	1·01 (0·64–1·59)	0·96	1·43 (0·82–2·48)	0·21
Genetic abnormalities									
Cytogenetic risk‖									
Standard	132 (60%)	66 (57–74)	0·0006	77 (68–84)	0·0005	0·64 (0·41–1·01)	0·054	0·61 (0·35–1·04)	0·070
Intermediate	69 (32%)	56 (43–67)	..	69 (56–78)	..	1	..	1	..
High	18 (8%)	30 (11–52)	..	39 (16–62)	..	2·09 (1·08–4·06)	0·030	2·35 (1·12–4·94)	0·024
Individual copy number alterations or mutations**									
IKZF1	37 (24%)	54 (37–68)	0·13	65 (47–78)	0·058	1·51 (0·88–2·59)	0·14	1·81 (0·97–3·39)	0·062
NR3C1	9 (7%)	42 (11–71)	0·40	63 (24–87)	0·96	1·48 (0·59–3·72)	0·41	1·03 (0·32–3·35)	0·96
PAX5	27 (17%)	65 (43–80)	0·77	77 (57–89)	0·96	0·90 (0·46–1·78)	0·77	1·02 (0·47–2·19)	0·96
IKZF1/PAX5/NR3C1	56 (34%)	49 (35–61)	0·012	63 (49–75)	0·014	1·85 (1·34–3·00)	0·013	2·01 (1·14–3·57)	0·016
NRAS	16 (11%)	38 (15–60)	0·030	52 (24–74)	0·12	2·10 (1·06–4·15)	0·034	1·88 (0·83–4·25)	0·13
IKZF1/PAX5/NR3C1/NRAS	63 (38%)	50 (37–62)	0·010	65 (52–76)	0·032	1·87 (1·15–3·02)	0·011	1·85 (1·04–3·28)	0·035

Progression-free survival and overall survival are shown as 5-year estimates with 95% CI. *Extramedullary sites: CNS (n=25), testes (n=16), skin (n=1). †Measured for those in second complete remission. ‡Censored at second complete remission. §Censored at time for stem-cell transplantation analysed as intention to treat. ¶Censored at time for stem-cell transplantation analysed as treated. ‖Patients with unknown cytogenetics excluded. High-risk group includes iAMP21 (n=11), *KMT2A* (n=4), *TCF3-PBX1* (n=1), low hypodiploid (n=1), and haploid (n=1). **Data shown are the presence of each copy number alteration or mutation and the comparison is for present versus not present. Full details are provided in the appendix (pp 2–6).

Table 1: **Frequency and outcome of patients with late bone marrow relapses, stratified by clinical characteristics, treatment, responses, and genetic features**

(*IKZF1* deletions with concomitant *CDKN2A/B*, *PAX5*, or *PAR1* deletions) as previously described,[10] with the exception of *ERG* deletions, which were assumed to be either absent or rare in this relapse cohort, given their association with good outcomes in patients with acute lymphoblastic leukaemia.[14,15]

Outcomes

In the original study,[7] the primary outcome was progression-free survival and the secondary outcome was overall survival. Progression-free survival was defined as the time from relapse, by the date of registration in the ALLR3 trial until induction failure, second relapse, second tumour or death, or censoring at last contact if no events had been observed. Overall survival was defined as the time from first relapse to death, censoring at last contact if no events had been observed. This long-term follow-up analysis was planned once the trial closed, although the follow-up period was not specified. The primary outcome of the long-term follow-up analysis, after closure of randomisation, was defined in the study protocol as progression-free survival of patients stratified

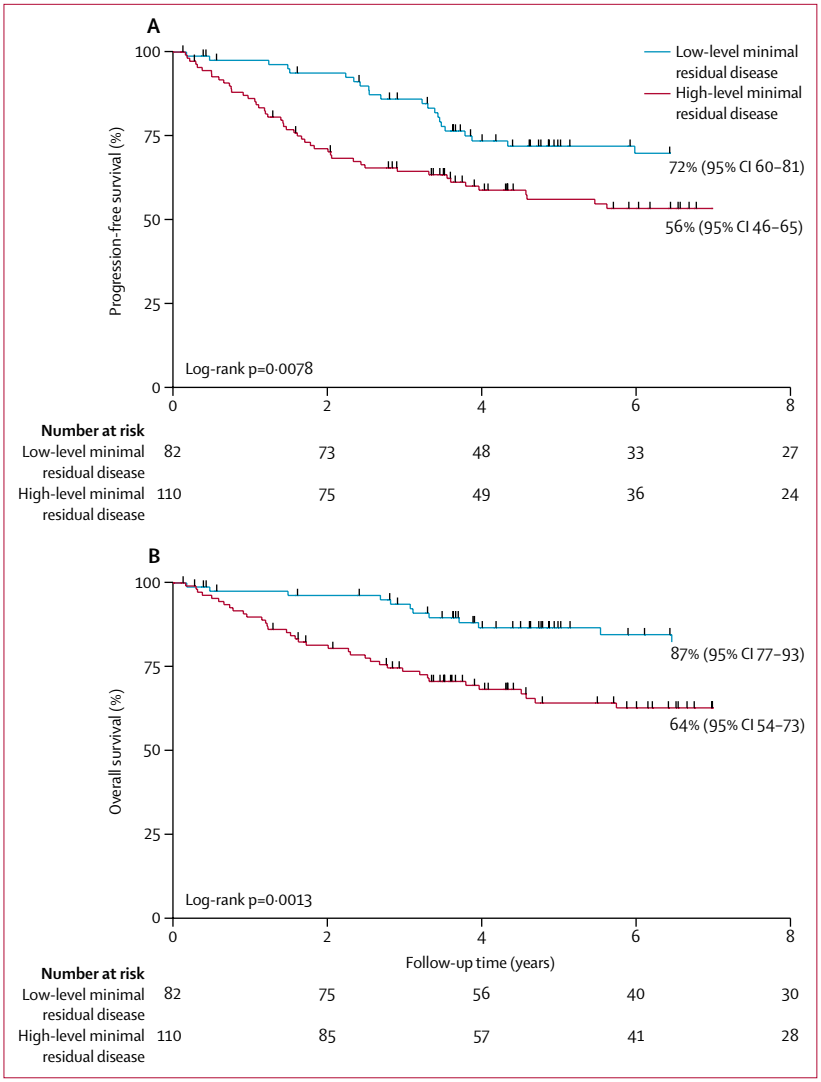

Figure 2: **5-year Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival in patients with high and low minimal residual disease**
High minimal residual disease defined as ≥10^{-4} cells. Low minimal residual disease defined as <10^{-4} cells.

by minimal residual disease level. Secondary outcomes of the long-term follow-up cohort included progression-free survival and overall survival within risk groups, by treatment given (ie, chemotherapy or stem-cell trans-plantation) and the proportion of patients with minimal residual disease levels available at timepoints 1 and 2.

Treatment-related serious adverse events were reported in the report of the original study[7] and were not analysed in the long-term follow-up.

Statistical analysis

Survival analyses were done with the Kaplan-Meier method and differences in progression-free survival and overall survival were assessed with the two-tailed log-rank test. Cumulative incidence of relapse was estimated, taking into account death as a competing risk and then compared with the Gray test. Other comparisons were done with the χ^2 test, Fisher's exact test, or Mann-Whitney *U* test, as appropriate. Univariate and multivariate analyses were done with Cox regression models. The prognostic effect of chemotherapy and stem-cell trans-plantation was analysed by both intention to treat and treatment received, and compared with the Mantel-Byar method.[15] Most prognostic factors had some missing data (appendix pp 2–5). For multivariate analysis, missing values were imputed with multiple imputation by chained equations.[13] 60 imputed datasets were created with simulated values for missing values from a set of imputation models constructed from all of the potential prognostic factors and outcome variables (Nelson-Aalen estimator for each of progression-free survival and overall survival). Distributions of imputed values were visually checked for comparability with the observed data. Cox regression analysis was done on each imputed dataset and the imputation-specific coefficients were combined with Rubin's rules.[14] The significance of each prognostic factor was assessed with the Wald test statistic. Only prognostic factors associated with the outcome ($p<0·1$) in univariate analyses were considered in multivariate analyses. The final multivariate model was built with progression-free survival and backwards selection was done on the pooled coefficients with p values less than 0·1 to remove variables. The final model was assessed for proportional hazards with Schoenfeld residuals,[16] assessed by the Harrell's c-index measure of discrimination, and applied to overall survival data. The c-index was estimated in each imputed dataset and then pooled across imputations with Rubin's rules. Sensitivity analyses were done only in cases with complete data about the risk factors in the final model. All analyses were done with Intercooled Stata, version 14, and R, version 3.4.3.

This trial is registered on the ISRCTN registry, number ISRCTN45724312, and on ClinicalTrials.gov, number NCT00967057, and is now completed.

Role of the funding source

The funders and sponsors of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication. VS, CP, SK, AVM, and LH had access to all the raw data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Feb 2, 2003, and Oct 28, 2013, 228 patients with B-cell precursor acute lymphoblastic leukaemia and late bone marrow relapses were treated. After a median follow-up of 84 months (IQR 48–109), progression-free survival was 60% (95% CI 54–70) and overall survival was 72% (65–78). There were three (1%) induction failures,

	Patients (%)	Log-rank analysis				Gray's test		Univariate Cox regression analysis (Mantel-Byar method)		Competing risk regression
		Progression-free survival (95% CI)	p value	Overall survival (95% CI)	p value	Cumulative incidence of relapse	p value	Progression-free survival, hazard ratio (95% CI)	Overall survival	Subdistribution hazard ratio
High minimal residual disease										
Chemotherapy	11 (11%)	31 (11–56)	0·13	54 (28–75)	0·37	50 (16–77)	0·034	1	1	1
Stem-cell transplantation	92 (89%)	54 (34–71)	..	59 (36–75)	..	22 (14–31)	..	0·57 (0·28–1·19), p=0·13	0·69 (0·31–1·56), p=0·38	0·36 (0·16–0·83), p=0·016
Low minimal residual disease										
Chemotherapy	70 (86%)	70 (57–79)	0·20	85 (74–92)	0·74	26 (16–38)	0·23	1	1	1
Stem-cell transplantation	11 (14%)	88 (39–98)	..	100	..	13 (5–44)	..	0·29 (0·04–2·15), p=0·23	0·71 (0·09–5·56), p=0·74	0·32 (0·04–2·36), p=0·27

High minimal residual disease defined as $\geq 10^{-4}$ cells. Low minimal residual disease defined as $<10^{-4}$ cells. To compare the prognostic effect of chemotherapy with stem-cell transplantation, the Mantel-Byar method was applied in which time starts at the moment of treatment initiation, and all patients begin in the non-transplantation group. Those who receive the transplant enter the transplantation group at the time of transplantation and remain there until death, second relapse, or censoring.

Table 2: **Cumulative incidence functions for competing events in patients receiving chemotherapy or stem-cell transplantation within the high and low minimal residual disease groups**

five (2%) induction deaths, and 220 (97%) patients achieved second complete remission. For the mitoxantrone treatment group (n=173), the median age was 10·1 years (IQR 7·33–12·9) and mean age was 10·5 years (SD 3·6); for the idarubicin treatment group (n=55) the median age was 9·6 years (6·9–13·2) and mean age was 10·1 years (3·7). Consistent with the previously published randomised analyses,[7] 5-year progression-free survival was significantly higher with mitoxantrone treatment than with idarubicin (66% [95% CI 58–73] *vs* 46% [33–59]; p=0·0098), although overall survival did not differ significantly between the two groups (75% [67–81] *vs* 63% [49–74]; p=0·10). As reported previously,[9] the proportion of patients with low minimal residual disease at timepoint 1 was similar with both drugs (63 [43%] of 147 with mitoxantrone *vs* 19 [41%] of 46 with idarubicin). Among patients with late relapses, neither the duration of first complete remission nor the time since stopping front-line treatment were significantly associated with the 5-year outcome (appendix pp 2–5).

Figure 1 shows outcomes after the second complete remission. Minimal residual disease at timepoint 1 was evaluable in 192 (87%) patients, 82 (43%) of whom had low minimal residual disease. Of the 28 patients with no minimal residual disease measurements available, 11 had relapsed 24 months or more after stopping front-line therapy and were allocated, along with patients who had low minimal residual disease, to the no stem-cell transplantation group. The remaining 17 patients with minimal residual disease measurements, who relapsed within 24 months of stopping front-line therapy, together with 110 patients with high minimal residual disease, were allocated to the stem-cell transplantation group. Thus, 93 (42%) patients were allocated to the no stem-cell transplantation group and 127 (58%) allocated to stem-cell transplantation. In the no stem-cell transplantation group, two treatment-related deaths were reported before completion of phase 3 of treatment. Of the 91 patients who completed all three phases of chemotherapy, 15 (16%) actually received stem-cell transplantation and 11 remained in second complete remission (with one treatment-related death and three relapses after stem-cell transplantation). Of the 76 patients who continued on chemotherapy as allocated, there were two (2%) treatment-related deaths at a late stage in maintenance therapy, 21 (28%) second relapses, and 53 (70%) patients remained in second complete remission. In the stem-cell transplantation group, three treatment-related deaths and four second relapses were reported before stem-cell transplantation. 15 patients did not undergo transplantation and instead continued with chemotherapy; five (33%) of these patients remained in second complete remission. Of the 105 transplanted patients, 22 (21%) relapsed again, 14 (13%) died of transplant-related complications, and 69 (66%) remained in second complete remission.

As stratification to stem-cell transplantation or chemotherapy was guided by minimal residual disease at timepoint 1 and duration of first complete remission was similar in both groups (appendix p 8), we separately examined outcomes of patients in whom minimal residual disease at timepoint 1 was measured. 70 patients with low-level minimal residual disease allocated to no stem-cell transplantation completed the first 12 weeks of chemotherapy and continued treatment. There were two (3%) treatment-related deaths, 19 (27%) second relapses (five late in maintenance and 14 after completing therapy), and 49 (70%) patients remained in second complete remission at final follow-up. 11 patients with low minimal residual disease were transplanted, of whom ten remained in second complete remission. 103 (94%) of 110 patients with high minimal residual disease allocated to stem-cell transplantation reached the timepoint for stem-cell transplantation. Of the 11 patients not transplanted, seven relapsed again, one died, and three remained in second complete remission. Of the

	Low minimal residual disease (n=82)	High minimal residual disease (n=110)	p value*
Age, years			
Mean (SD)	10·33 (3·16)	10·29 (3·60)	..
Median (IQR)	10·08 (6·58–12·17)	9·33 (7·25–13·08)	0·68
Sex			
Women	38 (46%)	48 (44%)	0·71
Men	44 (54%)	62 (56%)	..
Drug			
Mitoxantrone	63 (77%)	84 (76%)	0·94
Idarubicin	19 (23%)	26 (24%)	..
Single site			
Isolated bone marrow	66 (80%)	91 (83%)	0·59
Bone marrow with CNS	7 (9%)	12 (11%)	..
Bone marrow with testes or skin	9 (11%)	7 (6%)	..
Combined sites			
Isolated bone marrow	66 (80%)	91 (83%)	0·69
Combined bone marrow	16 (20%)	19 (17%)	..
Cytogenetic risk			
Standard	56 (71%)	59 (57%)	0·12
Intermediate	20 (25%)	36 (35%)	..
High	3 (4%)	9 (9%)	..
UKALL CNA profile			
Good risk	25 (48%)	30 (39%)	0·33
Intermediate or poor risk	27 (52%)	46 (61%)	..
Deletions or mutations			
IKZF1	9 (17%)	21 (28%)	0·18
NR3C1	1 (3%)	6 (8%)	0·18
PAX5	7 (13%)	17 (22%)	0·21
IKZF1/PAX5/NR3C1	13 (24%)	33 (41%)	0·039
CDKN2A/B	18 (35%)	25 (33%)	0·91
ETV6	11 (21%)	11 (14%)	0·33
TP53	5 (8%)	6 (7%)	0·74
P2RY8-CRLF2	4 (8%)	6 (8%)	1
NRAS	5 (9%)	7 (10%)	0·92
KRAS	7 (13%)	7 (10%)	0·58
IKZF1/PAX5/NR3C1/NRAS	16 (29%)	36 (44%)	0·060

Patients without minimal residual disease measured at timepoint 1 were excluded. High minimal residual disease defined as ≥10^{-4} cells. Low minimal residual disease defined as <10^{-4} cells. CNA=copy number alteration. *p value for χ^2, Fisher's exact test, or Mann-Whitney *U* test.

***Table 3*: Distribution of patients by minimal residual disease level measured at the end of induction stratified by clinical characteristics, treatment, and genetic features**

92 transplanted patients, 13 (14%) died of transplant-related complications, 21 (23%) had a second relapse, and 58 (63%) remained in second complete remission. Survival outcomes at 5 years were significantly higher in patients with low minimal residual disease than in those with high minimal residual disease (progression-free survival 72% [95% CI 60–81] *vs* 56% [46–65], p=0·0078; overall survival 87% [77–93] *vs* 64 [54–73], p=0·0013; table 1, figure 2). Patients allocated to the no stem-cell transplantation group had significantly better progression-free survival and overall survival than did those allocated to the stem-cell transplantation group. Although a difference in overall survival was observed when patients were censored at second complete remission (p=0·010), no differences in outcomes were observed in the two groups when analysed on the basis of actual treatment received (table 1, appendix p 2).

As minimal residual disease at timepoint 1 was used to allocate patients to stem-cell transplantation, we examined the effect of stem-cell transplantation versus chemotherapy stratified by minimal residual disease at timepoint 1 (appendix p 6). For patients with high minimal residual disease at timepoint 1, receiving stem-cell transplantation significantly reduced their relapse risk (hazard ratio [HR] 0·36 [95% CI 0·16–0·83], competing risk analysis Gray's test p=0·016) compared with chemotherapy alone. By contrast, for patients with low minimal residual disease at timepoint 1, no clear benefit was seen with stem-cell transplantation and all patients had an overall survival greater than 85% at 5 years (table 2).

We investigated the effect of minimal residual disease at timepoint 2 (before stem-cell transplantation) because high minimal residual disease before stem-cell transplantation has been associated with higher relapse rates.[16–19] The actual minimal residual disease and outcomes after stem-cell transplantation are shown in the appendix (pp 6–7). No patients with low minimal residual disease at timepoint 1 had high minimal residual disease at timepoint 2 and one patient with no minimal residual disease result at timepoint 1 was found to have high minimal residual disease at timepoint 2. In patients who underwent transplantation, progression-free survival was 68% (95% CI 53–79) and overall survival was 77% (62–87) in those with low minimal residual disease at timepoint 2, whereas progression-free survival was 58% (95% CI 36–75) and overall survival was 61% (38–77) in those with high minimal residual disease at timepoint 2. The differences in outcomes were not significant (HR 1·69 [95% CI 0·80–3·61], p=0·18, for progression-free survival; and 1·83 [0·78–4·27], p=0·16, for overall survival; appendix p 7), possibly reflecting the small numbers and the unexpected poor outcome of patients with a minimal residual disease level of 0. Although the trial recommended only matched-donor stem-cell transplantation, other donors were used and outcomes within the different donor groups were similar. In the six patients for whom the donor source was unknown, five had adverse events and one had a follow-up period of 2 years (appendix pp 2–5). Competing risk analyses showed relapse as the most frequent event after stem-cell transplantation, although transplant-related mortality also influenced outcomes (appendix p 9).

132 (60%) of 228 patients in this follow-up cohort had standard-risk cytogenetics (*ETV6-RUNX1* and high hyperdiploid), and had significantly improved progression-free survival and overall survival (table 1, appendix p 10). The distribution of patients by cytogenetic risk group did not differ significantly when they were

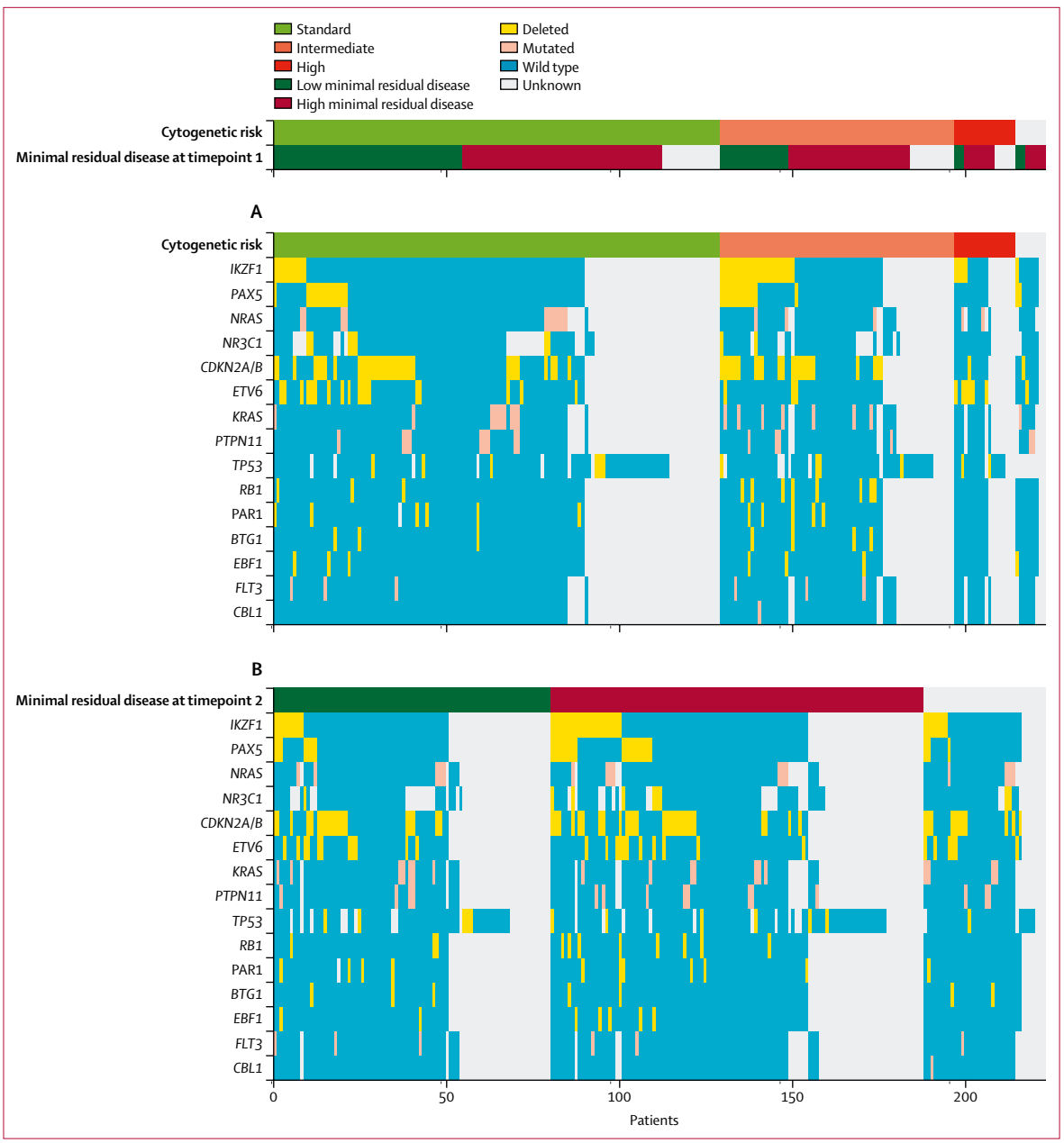

Figure 3: **Frequency of somatic copy number alterations and mutations according to (A) cytogenetic risk groups and (B) minimal residual disease at timepoint 1**

High minimal residual disease defined as ≥10^{-4} cells. Low minimal residual disease defined as <10^{-4} cells.

stratified by minimal residual disease risk group (table 3). Within the standard-risk cytogenetic group, outcomes of *ETV6-RUNX1* and high hyperdiploid patients did not differ: progression-free survival was 63% (95% CI 48–75) versus 68% (55–77) and overall survival was 77% (95% CI 63–87) versus 77% (65–85). Although patients with high-risk cytogenetics were rare, their outcome was poor and similar to that of patients with early bone marrow relapses, as previously reported.[8] 131 (57%) to 181 (79%) patients were screened at relapse for a range of somatic copy number alterations and mutations affecting 15 genes (appendix pp 3–5). The frequency of some of these deletions and mutations varied with the underlying cytogenetic risk group and with the likelihood of minimal residual disease positivity at the end of induction (figure 3). In particular, deletions affecting *IKZF1* were more prevalent in the intermediate-risk and high-risk cytogenetic groups than in the standard risk group. Similarly, these patients were more likely to have minimal residual disease positivity at end of induction (table 3).

	HR (95% CI)	p value	p value (joint test)
Imputation model (n=220)			
Progression-free survival			
Sex			
Men *vs* women	1·60 (1·00–2·56)	0·049	..
Drug			
Idarubicin *vs* mitoxantrone	1·54 (0·96–2·47)	0·072	..
Minimal residual disease*			
High *vs* low	1·71 (1·05–2·78)	0·031	..
Cytogenetic risk			
Standard *vs* intermediate	0·74 (0·46–1·20)	0·22	0·091
High *vs* intermediate	1·68 (0·76–3·73)	0·20	..
NRAS			
Mutated *vs* wild type	2·08 (1·06–4·13)	0·036	..
Model performance			
C-index=0·68	..	..	..
Overall survival			
Sex			
Men *vs* women	1·54 (0·88–2·70)	0·14	..
Drug			
Idarubicin *vs* mitoxantrone	1·36 (0·77–2·40)	0·29	..
Minimal residual disease*			
High *vs* low	2·42 (1·29–4·56)	0·006	..
Cytogenetic risk			
Standard *vs* intermediate	0·77 (0·43–1·38)	0·38	0·084
High *vs* intermediate	2·04 (0·83–5·02)	0·12	..
NRAS			
Mutated *vs* wild type	2·27 (1·03–5·02)	0·040	..
Model performance			
C-index=0·70	..	..	..

(Table 4 continues in next column)

(Continued from previous column)

	HR (95% CI)	p value	p value (joint test)
Available cases (n=118)			
Progression-free survival			
Sex			
Men *vs* women	1·68 (0·90–3·13)	0·10	..
Drug			
Idarubicin *vs* mitoxantrone	1·34 (0·72–2·48)	0·359	..
Minimal residual disease*			
High *vs* low	2·00 (1·05–3·79)	0·034	..
Cytogenetic risk			
Standard *vs* intermediate	0·91 (0·49–1·71)	0·77	0·63
High *vs* intermediate	1·55 (0·51–4·75)	0·44	..
NRAS			
Mutated *vs* wild type	1·27 (0·53–3·07)	0·59	..
Model performance			
C-index=0·64	..	..	..
Overall survival			
Sex			
Men *vs* women	1·87 (0·86–4·08)	0·11	..
Drug			
Idarubicin *vs* mitoxantrone	1·15 (0·54–2·49)	0·72	..
Minimal residual disease*			
High *vs* low	2·45 (1·08–5·56)	0·032	..
Cytogenetic risk			
Standard *vs* intermediate	1·03 (0·48–2·24)	0·94	0·70
High *vs* intermediate	1·75 (0·46–6·67)	0·41	..
NRAS			
Mutated *vs* wild type	1·70 (0·63–4·57)	0·30	..
Model performance			
C-index=0·65	..	..	..

Patients who did not have successful induction (n=8) were excluded from the modelling, as end of induction minimal residual disease was included in imputed missing data. *Minimal residual disease was measured at the end of induction. High minimal residual disease defined as $\geq 10^{-4}$ cells. Low minimal residual disease defined as $<10^{-4}$ cells.

Table 4: **Final multivariate Cox regression models for progression-free survival and overall survival for patients in the ALLR3 trial with late bone marrow relapses**

Patients with deletions in *IKZF1*, *NR3C1*, and *PAX5* showed inferior outcomes or a likelihood of minimal residual disease positivity at the end of induction, or a combination of both, but these findings were not significant (table 1, table 3); however, when grouped collectively, these outcomes were significant (p=0·014). Although mutations in *NRAS* were relatively rare (observed in 16 [11%] of 150 patients), patients with an *NRAS* mutation had significantly worse progression-free survival and overall survival (p=0·030; table 1) despite this mutation not being strongly associated with minimal residual disease at end of induction. Collectively, patients with a deletion or mutation in *IKZF1*, *PAX5*, *NR3C1*, or *NRAS* had a progression-free survival of approximately 50% (95% CI 37–62; table 1).

To ascertain whether these risk factors were independent of each other, we did multivariate Cox regression modelling. Somatic copy number alterations and mutations were considered individually as well as collectively (*IKZF1*, *PAX5*, or *NR3C1*; and *IKZF1*, *PAX5*, *NR3C1*, or *NRAS*). Only end-of-induction minimal residual disease and *NRAS* mutations remained significant independent risk factors for both progression-free survival and overall survival (table 4). The prognostic performance of the final models was 68% for progression-free survival and 70% for overall survival, as measured by Harrell's c-index (table 4). Adverse genetic features (data not shown) were not observed and other risk factors were not associated with relapse after stem-cell transplantation (appendix pp 2–6).

Discussion

The long-term follow-up analyses of the ALLR3 trial indicate favourable outcomes in patients with B-cell precursor-acute lymphoblastic leukaemia and late bone marrow relapses who were risk stratified by minimal residual disease at end of induction to not undergo stem-cell transplantation and to continue chemotherapy. There are various caveats to this observation. The median follow-up time of this cohort was around 8 years and although almost all events occurred within this time, a few late relapses are still expected. Information about minimal residual disease was not available in 28 (13%) of 220 patients at timepoint 1, and these patients were stratified for stem-cell transplantation according to duration of first complete remission. The data suggest that time to relapse is not predictive of end-of-induction minimal residual disease and patients in this category might not have been risk stratified appropriately. 30 (14%) of the 211 patients who completed the first three blocks of therapy were not treated according to the risk stratification. The findings from the ALLR3 trial and the ALL REZ BFM 2002 trial[3] indicate that in the real-world setting around 15% of patients are unable to follow the recommended treatment plan stratified by minimal residual disease level. We speculate that for patients in the low minimal residual disease group, the decision to transplant might reflect intolerance to therapy coupled with the availability of a matched donor. The results from the ALLR3 and ALL REZ BFM 2002 trials suggest that although this is a reasonable approach for such patients, fractionated total body irradiation is associated with long-term toxicities. Some patients with high minimal residual disease were possibly not transplanted because of the unavailability of a matched donor. For transplanted patients, pre-stem-cell transplantation minimal residual disease with fewer than 10^{-4} cells was associated with the best outcomes, although survival after stem-cell transplantation in patients with higher minimal residual disease levels at timepoint 2 was also better than previously reported.[19] The numbers are small in this group, and factors such as conditioning, immunosuppression, and graft versus host disease, which contribute to outcomes after stem-cell transplantation, were not analysed.

In patients in whom end-of-induction minimal residual disease was assessed, those with fewer than 10^{-4} cells had with significantly better outcomes than did those with 10^{-4} cells or more. Patients with combined and isolated late bone marrow relapses had similar survival, and we conclude that prophylactic cranial irradiation is not necessary in patients with isolated late bone marrow relapses. In the ALL REZ BFM 2002 study, similar outcomes were observed for patients with minimal residual disease of fewer than 10^{-3} cells with no stem-cell transplantation (overall survival 68%) and in those with minimal residual disease of 10^{-3} cells or more with stem-cell transplantation (overall survival 73%).[3] Although induction therapies were different in REZ2002 and ALLR3, by the time patients reach this stage of disease, they have been exposed to multiple modalities of therapy, both during front-line and relapse treatment. In this study, second relapses occurred in 28% of patients allocated to receive chemotherapy only on the basis of their end-of-induction minimal residual disease. These relapses occurred mostly after stopping therapy or late in maintenance and were often rescued with a stem-cell transplantation in third complete remission. This implies that these recurrent clones continue to be chemosensitive. Thus, a minimal residual disease level of fewer than 10^{-4} cells at end of induction appears to be more sensitive than that of fewer than 10^{-3} cells in identifying patients with late bone marrow relapses who do not require stem-cell transplantation. Newer, more sensitive minimal residual disease assays[20] might predict more clearly which patients within the low minimal residual disease group are likely to remain in second complete remission and not have a second relapse, but this hypothesis needs to be prospectively investigated. Patients with a minimal residual disease of 10^{-4} cells or more at timepoint 1 benefited from stem-cell transplantation. This observation suggests that high-risk disease can be defined as a minimal residual disease of 10^{-4} cells or more. Similar to findings of the ALLREZ BFM 2002 study, matched donor stem-cell transplantation appeared to be associated with better outcomes, although in both studies the choice of donor was not significant. Second relapse rates were high in patients with high-level minimal residual disease who were not transplanted. As a result of advances in transplantation, outcomes for patients with late bone marrow relapses who undergo stem-cell transplantation with a mismatched donor are similar to those of patients who undergo stem-cell transplantation with a matched donor.[21] This indicates that in patients with high minimal residual disease who have late bone marrow relapses, stem-cell transplantation with any donor should be the preferred option.

Latency of recurrence (duration of first complete remission) was not predictive for minimal residual disease, and differences in minimal residual disease kinetics are thought to reflect the different underlying biology of acute lymphoblastic leukaemia subtypes, and not exclusively drug sensitivity.[22] In the high minimal residual disease group, four patients relapsed before undergoing stem-cell transplantation, one had high-risk cytogenetics, and the other had an *IKZF1*^{plus} profile, supporting the contention that minimal residual disease reflects the biology of acute lymphoblastic leukaemia. *TP53* deletions and mutations did not influence outcomes in this cohort,[8,9] but only one patient harboured a point mutation and the other 11 *TP53* alterations were heterozygous deletions. Univariate analyses showed a deleterious effect of *IKZF1*, *PAX5*, or *NR3C1* mutations on outcome and these somatic copy number alterations were more frequent in patients with high minimal

residual disease. IKZF1, NR3C1, and PAX5 regulate glucose metabolism in B-cell precursor lymphoblasts and haploinsufficiency leads to increased glucose metabolism and promotes resistance to glucocorticoids,[17] establishing a plausible biological mechanism for residual disease after therapy. In murine models, targeting the metabolic processes regulated by these genes restored sensitivity to glucocorticoids, indicating potential targeted therapeutic alternatives.[23] The results of this study show that stem-cell transplantation offers reasonable outcomes for patients with high minimal residual disease and patients who relapsed after stem-cell transplantation did not have these somatic copy number alterations. Both recurrence and mortality after transplantation contributed to the worse outcomes in transplanted patients, and no predictive variables were identified for relapses after stem-cell transplantation in this study. Immune therapies target the expression of antigens on the surface of B-cell precursor acute lymphoblastic leukaemia, and reduce the minimal residual disease burden before transplantation.[24–28] These immune therapies could decrease recurrences after stem-cell transplantation in patients with high minimal residual disease at end of induction and might even serve as an alternative to stem-cell transplantation in the future. The frequency of the *NRAS* mutations was 11% overall but 19% among high hyperdiploid patients. Consistent with previous analysis of the entire ALLR3 cohort,[8] *NRAS* mutations were associated with an inferior outcome in patients with late bone marrow relapses. RAS pathway mutations confer sensitivity to MEK inhibition,[29] and a phase 1/2 trial is currently assessing selumetinib in combination with dexamethasone in treatment of patients with multiply relapsed childhood acute lymphoblastic leukaemia with *RAS* mutations.[30]

Overall, the long-term results of the ALLR3 clinical trial indicate that patients with B-cell precursor-acute lymphoblastic leukaemia who have late bone marrow relapses with end of induction minimal residual disease of fewer than 10^{-4} cells have satisfactory outcomes with available therapeutic modalities. Patients who have a higher minimal residual disease at end of induction and persistent minimal residual disease before stem-cell transplantation might benefit from enrolment into trials exploring experimental therapies.

Contributors

VS designed the study. CP managed the study. JAEI, RPK, JH, RS, and AVM did laboratory tests. VS, PH, and TR were trial coordinators. CP, SK, LH, AVM, and VS analysed data. SK, AVM, LH, and VS wrote the paper. VS had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors reviewed the draft of the paper submitted for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Individual participant data that underlie the results reported in this Article can be made available, after de-identification, beginning 9 months and ending 36 months after Article publication, for an individual participant data meta-analysis. After 36 months the data will be available in our university's data warehouse but without investigator support other than deposited metadata. Investigators who wish to use the data will require approval from an independent review committee (learned intermediary) identified for this purpose.

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