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Original Research

Risk factors and outcomes in children with high-risk B-cell precursor and T-cell relapsed acute lymphoblastic leukaemia: combined analysis of ALLR3 and ALL-REZ BFM 2002 clinical trials

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KEYWORDS
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Abstract ***Aim:*** Outcomes of children with high-risk (HR) relapsed acute lymphoblastic leukaemia (ALL) (N = 393), recruited to ALLR3 and ALL-REZ BFM 2002 trials, were analysed. Minimal residual disease (MRD) was assessed after induction and at predetermined time points until haematopoietic stem cell transplantation (SCT).
Methods: Genetic analyses included karyotype, copy-number alterations and mutation analyses. Ten-year survivals were analysed using Kaplan-Meier and Cox models for multivariable analyses.
Results: Outcomes of patients were comparable in ALLR3 and ALL-REZ BFM 2002. The event-free survival of B-cell precursor (BCP) and T-cell ALL (T-ALL) was 22.6% and 26.2% ($P = 0.94$), respectively, and the overall survival (OS) was 32.6% and 28.2% ($P = 0.11$), respectively. Induction failures (38%) were associated with deletions of *NR3C1* ($P = 0.002$) and *BTG1* ($P = 0.03$) in BCP-ALL. The disease-free survival (DFS) and OS in patients with good vs poor MRD responses were 57.4% *vs* 22.6% ($P < 0.0001$) and 57.8% *vs* 32.0% ($P = 0.0004$), respectively. For BCP- and T-ALL, the post-SCT DFS and OS were 42.1% and 56.8% ($P = 0.26$) and 51.6% and 55.4% ($P = 0.67$), respectively. The cumulative incidences of post-SCT relapse for BCP- and T-ALL were 36.9% and 17.8% ($P = 0.012$) and of death were 10.7% and 25.5% ($P = 0.013$), respectively. Determinants of outcomes after SCT were acute graft versus host disease, pre-SCT MRD ($\geq 10^{-3}$), HR cytogenetics and *TP53* alterations in BCP-ALL.
Conclusion: Improvements in outcomes for HR ALL relapses require novel compounds in induction therapy to improve remission rates and immune targeted therapy after induction to maintain remission after SCT.
Trial registration: ALLR3: NCT00967057; ALL REZ-BFM 2002: NCT00114348

1. Introduction

Duration of first complete remission and immunophenotypes predict outcomes in patients with relapsed acute lymphoblastic leukaemia (ALL) [1–3]. B-cell precursor (BCP) ALL with bone marrow relapses occurring later than 6 months after stopping therapy has survival rates of more than 80% [4,5]. In contrast, patients with high-risk (HR) relapses defined as BCP-ALL relapses within 18 months of first diagnosis or with isolated medullary relapses occurring within 6 months of stopping therapy, and T-cell isolated or combined medullary relapses at any time, have survival rates between 15% and 30% [3,6,7] even after allogeneic haematopoietic stem cell transplantation (SCT) [2,3,8–10]. Second complete remission (CR2) rates in HR patients range between 68% and 88% [3,9,10]. More recently, HR relapsed patients are receiving immune-directed therapies. For patients with HR BCP-ALL, CD19- and CD22-targeted therapies with the CD19-directed bispecific T-cell engager blinatumomab, the CD22-directed toxin-conjugated monoclonal antibody inotuzumab ozogamicin [11–16] or chimeric antigen receptor T-cells (CAR-Ts) [17,18] offer superior remission rates, although the long-term outcomes of these novel approaches are awaited.

In this context, we have analysed the long-term outcomes of HR patients treated in the ALLR3 and ALL-REZ BFM 2002 clinical trials for relapsed ALL. Both ALLR3 and ALL-REZ BFM 2002 used a common risk stratification method and assessed minimal residual disease (MRD) after induction and before SCT, and all HR patients were eligible for SCT after 12 weeks of chemotherapy. This permitted combining the trial data sets for patients with HR bone marrow ALL relapses treated in both trials to evaluate different chemotherapy approaches for induction remission in HR relapsed ALL and the relationship of MRD at different time points with survival, along with additional genetic analyses.

2. Patients and methods

2.1. Study population

This analysis reports on patients with HR (Table 1) relapsed ALL without a previous SCT, treated in ALLR3 (NCT00967057, N = 136) or ALL-REZ BFM 2002 (NCT00114348, N = 257) trials. Early isolated extramedullary relapses were excluded. Patients at the age between 1 and 18 years at relapse diagnosis were included in the analyses. Trials were approved by relevant institutional ethical committees, and patients were recruited after written consent between 28th December 2001 and 18th June 2011 (ALL-REZ BFM 2002) and between 31st January 2003 and 31stOctober 2013 (ALLR3).

2.2. Therapy

Chemotherapy for ALL-REZ BFM 2002 [8] and ALLR3 [19] has been previously reported and is briefly described in the supplemental section (Supplemental Table S1). ALLR3 randomised patients to idarubicin or mitoxantrone in induction, and early closure of the

Table 1
Risk stratification of relapsed ALL in ALLR3 and ALL-REZ BFM 2002.

Immunophenotype	B-cell precursor			T-cell ALL		
Site of relapse Time point of relapse	Isolated extramedullary	Combined bone marrow	Isolated bone marrow	Isolated extramedullary	Combined bone marrow	Isolated bone marrow
Very early	HR/IR*	**HR**	**HR**	HR/IR*	**HR**	**HR**
Early	IR	IR	**HR**	IR	**HR**	**HR**
Late	SR	IR	IR	SR	**HR**	**HR**

HR, high risk; IR, intermediate risk; SR, standard risk; ALL, acute lymphoblastic leukaemia.
HR/IR* were HR in *ALLR3* and IR in *ALL-REZ BFM 2002* and excluded from this study. Patients classified as HR categories in bold are included in this analysis.

randomisation in favour of mitoxantrone [19] allowed the evaluation of clofarabine, cyclophosphamide and etoposide (CCE) in induction.

2.3. Assessment of response

CR2 was defined as <5% blasts in the marrow and no blasts in the cerebrospinal fluid (CSF) at the end of induction (EOI)—otherwise defined as induction failure. MRD was measured after induction and in some patients after each block of therapy before SCT, with the validated and standardised real-time quantitative polymerase chain reaction assays for clonal gene rearrangements in immunoglobulin/T-cell receptor loci [8,19]. Two postinduction MRD response groups were defined. The good response (GR) group was that with an MRD of $<10^{-4}$ at the EOI or between $\geq 10^{-4}$ and $<10^{-3}$ at the EOI, with a subsequent MRD value of $<10^{-4}$ after induction. All others were defined as poor response (PR).

2.4. Cytogenetics and molecular genetics

Fusion genes (*ETV6-RUNX1*, *TCF3-PBX1*, *KMT2A-AFF1* and *KMT2A-MLLT1*) or aneuploidies (high hyperdiploid, low hypodiploid, near-haploidy) were detected as described previously [20–23]. Patients were grouped into standard risk (SR), HR and B-other groups by integrating cytogenetic and genetic data as reported for relapsed ALL. Copy number status of *IKZF1*, *CDKN2A/B*, *PAX5*, *EBF1*, *ETV6*, *BTG1*, *RB1*, *NR3C1* and *PAR1* was determined using the SALSA multiplex ligation-dependent probe amplification kit P335; *TP53* deletions by the P007 or P056 kit (MRC Holland, The Netherlands). Key exons of *TP53*, *NRAS*, *KRAS*, *PTPN11*, *FLT3* and *CBL* genes were assessed for mutations by denaturing high-performance liquid chromatography and/or Sanger sequencing [21,22].

2.5. Statistical analysis

Survival analysis considered five main end-points. Event-free survival (EFS) was calculated as the time from first relapse, using the date of relapse, until the first event (induction death, induction failure, second relapse, death in CR2 and second malignancy) or last follow-up. Disease-free survival (DFS) included only patients who achieved CR2 and was calculated as the time from first relapse until the first event (second relapse, death, second malignancy) or last follow-up. For outcomes after SCT, the date of SCT was used as the starting point for DFS. Overall survival (OS) was defined as time from first relapse to death censoring at the last contact. Cumulative incidence of subsequent relapses (CIR) or cumulative incidence of death (CID) was calculated as the time from first relapse until second relapse or death in CR2, taking other DFS events into account as competing events. EFS, DFS and OS probabilities and cumulative incidence functions for competing events are given at 10 years in the following Results and Discussion sections. Further details are in Supplemental section.

3. Results

3.1. Comparison of patients treated uisng the different regimens

Twenty-five, 60 and 51 HR ALLR3 patients received idarubicin, mitoxantrone, CCE arms; 257 patients ALL-REZ BFM 2002 respectively (Supplemental Results). CR2 rates and EFS were comparable within the different treatment protocols for both BCP-ALL and T-cell ALL (T-ALL) (Fig. 1, Table 2). As the EFS of the different therapeutic groups was comparable, data from all 393 patients were combined to further investigate the determinants of outcomes in HR relapsed ALL.

3.2. Overall outcomes of the combined cohort

Two hundred seventy-eight (71%) patients had BCP-ALL, and 115 (29%) had T-ALL. Progression through treatment for the patients with BCP- and T-ALL is shown in Fig. 1 and for the whole cohort is shown in Supplementary Fig. S1. The proportion of patients attaining CR2 and reaching SCT was comparable between the two groups. The EFS and OS were 22.6% (95% confidence interval [CI]: 17–29) and 26.2% (18–35) as

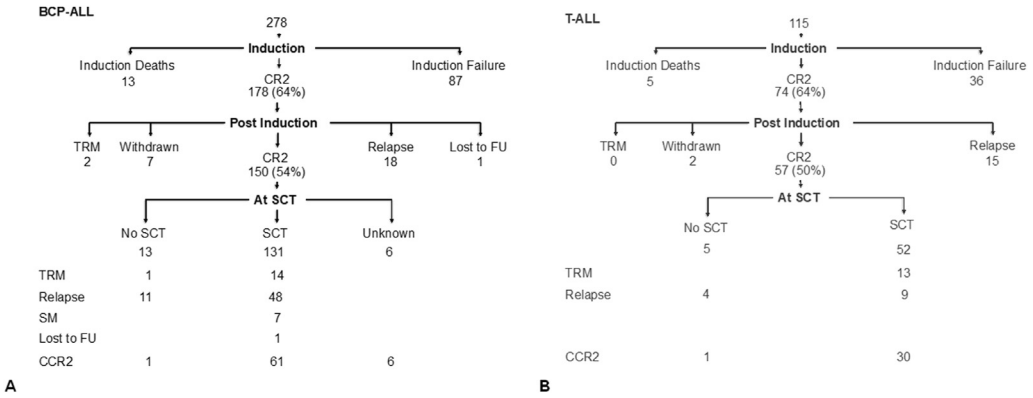


Fig. 1. CONSORT diagram—schematic of outcomes of patients with high-risk relapsed ALL with (A) B-cell precursor ALL and (B) T-cell ALL. ALL, acute lymphoblastic leukaemia; BCP, B-cell precursor, T, T-cell; CR2, second complete remission; TRM, therapy-related mortality; FU, follow-up; SCT, stem cell transplantation; SM, secondary malignancy; CCR2, second continuous complete remission.

well as 32.6% (27–39) and 28.2% (20–37), for BCP- and T-ALL, respectively (Fig. 2). Although outcomes of patients with BCP-ALL and T-ALL relapse were comparable, T-ALL relapses at any time point are classified as HR. In this cohort, 25% of T-ALL were late relapses, and these patients were more likely to achieve CR2 (Table 3).

3.3. Determinants of induction failure

Overall, for both BCP- and T-ALL, 4% of patients died in induction and 34% of patients failed induction (Fig. 1). Induction failures were more frequent in those relapsing within 18 months from first diagnosis in both BCP- and T-ALL ($P = 0.020$and $P = 0.017$, respectively), in older children with BCP-ALL ($P = 0.012$) and in BCP-ALL with isolated medullary relapse ($P = 0.040$). None of these factors appeared to influence EFS in those who achieved CR2 (Table 3). Induction failures were more frequent in patients with cytogenetic HR BCP-ALL (48%) than in cytogenetic SR (24%) and B-other patients (31%, $P = 0.032$), especially those with hypodiploidy (64%, $P = 0.017$) and associated with deletions of *BTG1* and *NR3C1* ($P = 0.031$, $P < 0.001$; Table 4). Eighteen (15%) of 123 patients with induction failures subsequently achieved remission, 9 with continuing protocol treatment and 9 with alternative treatment strategies.

3.4. Postinduction MRD response and correlation to outcomes

One hundred seventy-eight patients with BCP-ALL and 74 patients with T-ALL achieved CR2 (Fig. 1). A second relapse before SCT occurred in 18 of 140 patients (13%)

Table 2
Outcomes of patients with high-risk relapsed ALL treated with the ALLR3 and ALL-REZ BFM 2002 protocols.

Outcome/response rates	Protocol					*P* (trials)	*P*[a] (inductions)
	ALLR3				ALL-REZ BFM 2002		
	CCE	Idarubicin	Mitoxantrone	Total			
N	51	25	60	136	257		
EFS, % (95% CI)							
All patients							
Total	22.0 (12–35)	16.0 (5–33)	28.0 (17–40)	21.3 (14–30)	25.1 (20–31)	0.44	0.60
BCP-ALL	17.7 (6–34)	15.8 (4–35)	27.8 (15–42)	19.3 (11–30)	24.2 (18–32)	0.51	0.57
T-ALL	27.8 (11–48)	16.7 (1–52)	28.6 (12–48)	25.2 (14–39)	26.9 (17–38)	0.93	0.83
Excluding induction failures							
Total	32.1 (17–48)	20.0 (6–39)	39.9 (25–54)	29.8 (20–41)	47.7 (33–50)	0.0067	0.67
OS, % (95% CI)							
All patients							
Total	21.2 (11–34)	16.0 (5–33)	31.0 (20–43)	22.3 (15–31)	35.9 (30–42)	0.0001	0.30
BCP-ALL	16.8 (6–33)	15.8 (4–35)	30.1 (17–45)	20.3 (12–31)	38.0 (31–45)	0.0002	0.28
T-ALL	27.2 (10–47)	16.7 (1–52)	33.3 (15–53)	27.3 (15–41)	30.0 (19–41)	0.51	0.42
Excluding induction failures							
Total	31.4 (16–48)	20.0 (6–39)	39.8 (25–54)	29.4 (19–40)	49.2 (41–57)	0.0001	0.22

ALL, acute lymphoblastic leukaemia; CCE, clofarabine, cyclophosphamide and etoposide; EFS, event-free survival; OS, overall survival; CI, confidence interval; T, T-cell.

[a] Compares outcomes between mitoxantrone (ALLR3) and REZ 2002; p values are calculated using the log-rank test.

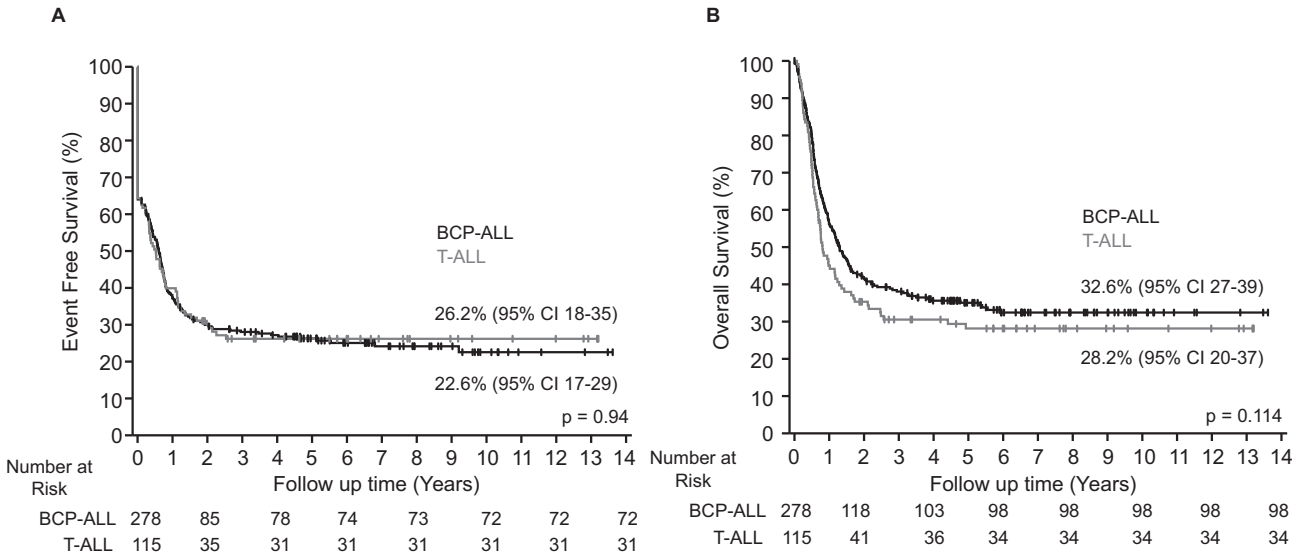


Fig. 2. Comparable outcomes of patients with high-risk relapse of BCP-ALL and T-ALL. Kaplan-Meier analyses of event-free (A) and overall (B) survival in BCP- and T-ALL high-risk relapsed ALL. ALL, acute lymphoblastic leukaemia; BCP, B-cell precursor; T, T-cell; CI, confidence interval.

with BCP-ALL and 15 of 55 patients (27%) with T-ALL ($P = 0.0157$, Fig. 1). The EOI MRD thresholds for the ALL-REZ BFM 2002 [8] and ALLR3 [19] trials were 10^{-3} and 10^{-4}, respectively. DFS and OS were significantly better in those with an EOI MRD of $<10^{-3}$ in both trials, but in ALLR3 only, these were better in those with an EOI MRD of $<10^{-4}$ (Table 5). Potentially, this discordance in the relationship of EOI MRD with survival relates to the use of anthracyclines in induction for ALLR3. ALL-REZ BFM 2002 uses

Table 3
Determinants of induction failure in patients with high-risk relapsed ALL.

Risk factors	Determinants	All patients excluding induction deaths				Patients reached CR2		
		N[a]	CR2, N (%)	Induction failure, N (%)	P[b]	DFS, %	95% CI	P[c]
BCP-ALL		265	178 (67)	87 (33)				
Sex	Male	156	104 (67)	52 (33)	0.84	38.1	29–48	0.96
	Female	109	74 (68)	35 (32)		32.5	19–47	
Age at relapse	Median, 95% CI (years)	6.7 (4.5–13.1)	6.5 (6.0–7.6)	10 (6–12)	**0.012**			
	<10 years	167	124 (70)	43 (49)	**0.004**	35.1	26–45	0.74
	≥10–14 years	49	25 (14)	24 (28)		35.1	13–59	
	≥15 years	49	29 (16)	20 (23)		39.4	22–57	
Time to relapse	Very early	113	67 (59)	46 (41)	**0.020**	41.4	29–54	0.84
	Early	151	110 (73)	41 (27)		31.7	21–49	
Site of relapse	Bone marrow isolated	251	165 (66)	86 (34)	**0.040**	34.2	26–43	0.44
	Bone marrow combined	14	13 (93)	1 (7)		53.9	25–76	
T-ALL		110	74 (76)	36 (24)				
Sex	Male	81	56 (69)	25 (31)	0.49	42.9	29–55	0.78
	Female	29	18 (62)	11 (38)		36.4	15–58	
Age at relapse	Median, 95% CI (years)	9.9 (6.8–14.8)	9.5 (9–11)	11.5 (7–14)	0.65			
	<10 years	55	40 (54)	15 (42)	0.45	40.7	25–56	0.27
	≥10–14 years	30	18 (24)	12 (33)		55.0	29–75	
	≥15 years	25	16 (22)	9 (25)		25.0	8–47	
Time to relapse	Very early	55	32 (58)	23 (42)	**0.017**	36.5	20–53	0.58
	Early	27	18 (67)	9 (33)		37.0	16–59	
	Late	27	24 (89)	3 (11)		48.4	37–67	
Site of relapse	Bone marrow isolated	76	50 (66)	26 (34)	0.62	45.2	31–58	0.37
	Bone marrow combined	34	24 (71)	10 (29)		31.1	14–50	

ALL, acute lymphoblastic leukaemia; CR2, second complete remission; BCP, B-cell precursor; DFS, disease-free survival; CI, confidence interval; T, T-cell.

P values in bold indicate those <0.05.

[a] Induction deaths are excluded.

[b] p values are calculated using the chi-square test.

[c] p values are calculated using the log-rank test.

Table 4
Genetic determinants of induction failure: BCP-ALL.

BCP-ALL genetic groups		N	%[a]	CR2, N (%)	Induction failure, N (%)	*P*[b]
		265		178	87	
Cytogenetic alterations						
ETV6-RUNX1 fusion	Yes	12	5	10 (83)	2 (17)	0.35
	No	229	95	151 (66)	78 (34)	
	NA	24		17	7	
High-hyperdiploid	Yes	26	11	19 (73)	7 (27)	0.47
	No	215	89	142 (66)	73 (34)	
	NA	24		17	7	
iAMP21	Yes	3	1	3 (100)	0	0.55
	No	238	99	158 (66)	80 (34)	
	NA	24		17	7	
KTM2A fusions	Yes	21	9	13 (62)	8 (38)	0.62
	No	220	91	148 (67)	72 (33)	
	NA	24		17	7	
TCF3-HLF, TCF3-PBX1	Yes	12	5	5 (42)	7 (58)	0.058
	No	229	95	156 (68)	73 (32)	
	NA	24		17	7	
Hypodiploid (<40 Chr)	Yes	14	6	5 (36)	9 (64)	**0.017**
	No	227	94	156 (69)	71 (31)	
	NA	24		17	7	
Cytogenetic risk groups						
	B-other	153	63	106 (69)	47 (31)	**0.032**
	Cyto-SR	38	16	29 (76)	9 (24)	
	Cyto-HR	50	21	26 (52)	24 (48)	
	NA	24		17	7	
Genetic alterations						
$IKZF1^{del}$	Yes	54	30	30 (56)	24 (44)	0.16
	No	129	70	86 (54)	43 (46)	
	NA	82		62	20	
$CDKN2A/B^{del}$	Yes	85	46	56 (66)	29 (34)	0.51
	No	98	54	60 (61)	38 (29)	
	NA	82		62	20	
$ETV6^{del}$	Yes	25	14	12 (48)	13 (52)	0.078
	No	157	86	104 (66)	53 (34)	
	NA	83		62	21	
$PAX5^{del}$	Yes	44	24	27 (61)	17 (39)	0.72
	No	138	76	89 (65)	49 (36)	
	NA	83		62	21	
$BTG1^{del}$	Yes	12	7	4 (33)	8 (67)	**0.031**
	No	170	93	112 (66)	58 (34)	
	NA	83		62	21	
$RB1^{del}$	Yes	4	2	2 (50)	2 (50)	0.62
	No	178	98	114 (64)	64 (36)	
	NA	83		62	21	
$EBF1^{del}$	Yes	6	3	4 (67)	2 (23)	1.00
	No	176	97	112 (64)	64 (36)	
	NA	83		62	21	
$NR3C1^{del}$	Yes	7	14	2 (29)	5 (71)	**0.002**
	No	44	86	39 (89)	5 (11)	
	NA	214		137	77	
$PAR1^{del}$	Yes	16	9	10 (63)	6 (27)	0.89
	No	165	91	106 (64)	59 (36)	
	NA	84		62	22	
TP53 alteration	Yes	34	20	19 (56)	15 (44)	0.32
	No	171	80	111 (65)	60 (35)	
	NA	60		48	12	
$NRAS^{mut}$	Yes	33	18	21 (64)	12 (36)	0.98
	No	152	82	97 (64)	55 (36)	
	NA	80		60	20	
$KRAS^{mut}$	Yes	30	16	16 (53)	14 (47)	0.22
	No	155	84	102 (66)	53 (34)	
	NA	80		60	20	
IKZF1/NR3C1/BTG1	Yes	73	55	39 (53)	34 (47)	**< 0.001**

Table 4 (*continued*)

BCP-ALL genetic groups		N	%[a]	CR2, N (%)	Induction failure, N (%)	*P*[b]
	No	60	45	50 (83)	10 (17)	
	NA	132		78	43	
IKZF1^{plus}[c]	Yes	67	37	23 (54)	20 (47)	0.123
	No	116	63	93 (66)	47 (34)	
	NA	82		62	20	

ALL, acute lymphoblastic leukaemia; BCP, B-cell precursor; CR2, second complete remission; cyto-SR, cytogenetic standard risk (ETV6-RUNX1, high-hyperdiploid); cyto-HR, cytogenetic high risk (<40 Chr., KTM2A, TCF3-HLF, TCF3-PBX1, iAMP21); NA, not analysed (data were excluded from the analyses); del, deletion; mut, mutation; NA, not available.
P values in bold indicate those <0.05.
[a] Percentage of numbers available for analyses.
[b] p values were estimated using the chi-square test or Fisher's exact test (n < 5).
[c] ERG deletions not assessed.

Table 5
Disease-free survival and overall survival based on end of induction MRD levels.

MRD level		10^{-4}			10^{-3}		
		<	≥	P	<	≥	P
ALLR3	N	23	43		30	21	
	DFS, %	55.9 (30–76)	14.9 (5–29)	**0.0011**	45.8 (26–64)	32.2 (19–46)	**0.0018**
	OS, %	55.7 (30–75)	14.4 (5–29)	**0.001**	44.5 (24–63)	7.1 (1–26)	**0.0019**
ALL-REZ BFM 2002	N	28	90		51	66	
	DFS, %	57.1 (37–73)	36.0 (24–48)	0.1	58.6 (44–71)	27.0 (13–43)	**0.0037**
	OS, %	56.0 (35–72)	46.7 (36–57)	0.26	59.9 (45–72)	41.1 (29–53)	**0.018**

MRD, minimal residual disease; DFS, disease-free survival; OS, overall survival.
Discrepancies of patient numbers between the 10^{-4} and 10^{-3} cut-off groups are due to sensitivity of MRD markers (only 10^{-3} or 5×10^{-4}, but not 10^{-4}) or the non-available quantitative MRD values to assign the MRD results to one of the positive MRD groups, either $\geq 10^{-3}$ or $<10^{-3}$–$\geq 10^{-4}$.
P values in bold indicate those <0.05.

Table 6
Outcomes of patients with high-risk relapsed ALL based on MRD response.

MRD	N[a]	DFS (%)	95% CI	*P*[b]	CIR (%)	95% CI	*P*[c]	OS (%)	95% CI	*P*[b]
BCP-ALL										
EOI $<10^{-4}$	38	58.9	14–73	**0.012**	23.9	12–39	**0.012**	58.2	40–73	**0.053**
EOI $\geq 10^{-4}$	100	28.9	18–41		48.0	38–58		39.5	30–49	
EOI $<10^{-3}$	60	58.4	44–70	**0.0005**	27.1	16–39	**0.0026**	59.9	46–71	**0.0066**
EOI $\geq 10^{-3}$	67	20.8	8–37		52.2	40–63		34.5	23–47	
GR	48	60.0	44–73	**0.0005**	23.6	13–37	**0.0018**	62.0	46–75	**0.004**
PR	75	23.4	11–38		50.7	39–61		35.5	24–47	
T-ALL										
EOI $<10^{-4}$	13	52.8	23–76	0.087	31.9	9–58	0.31	49.2	19–74	0.075
EOI $\geq 10^{-4}$	33	29.6	15–46		45.5	28–62		27.6	13–44	
EOI $<10^{-3}$	21	42.3	21–62	0.12	38.6	18–59	0.45	39.6	18–60	0.074
EOI $\geq 10^{-3}$	20	25.0	9–45		45.0	22–65		25.0	9–45	
GR	16	49.2	24–71	**0.016**	25.8	7–50	**0.036**	46.3	20–69	**0.019**
PR	24	20.8	8–39		54.1	32–72		20.8	8–35	
BCP- and T-ALL										
EOI $<10^{-4}$	51	57.4	42–69	**0.0025**	25.8	15–39	**0.0070**	55.4	40–69	**0.011**
EOI $\geq 10^{-4}$	133	28.9	19–39		47.5	39–56		36.7	28–45	
EOI $<10^{-3}$	81	54.3	43–64	**0.00024**	30.0	20–40	**0.0029**	54.3	42–65	**0.0019**
EOI $\geq 10^{-3}$	87	21.7	11–35		50.6	40–61		32.7	23–43	
GR[d]	64	57.4	44–69	**<0.0001**	24.1	14–35	**0.0002**	57.8	44–70	**0.0004**
PR[d]	99	22.6	13–35		51.5	41–61		32.0	23–42	

ALL, acute lymphoblastic leukaemia; CI, confidence interval; DFS, disease-free survival; CIR, cumulative incidence of relapses; OS, overall survival; MRD, minimal residual disease; BCP, B-cell precursor; EOI, end of induction; GR, good response; PR poor response; T, T-cell.
P values in bold indicate those <0.05.
[a] Total numbers between the three MRD categories (EOI </≥10^{-4}, EOI </≥10^{-3}, GR/PR) vary because of the sensitivity of MRD markers or missing MRD time points after induction.
[b] p values calculated using the log-rank test.
[c] p values calculated using the Gray test.
[d] MRD good response was defined as either $<10^{-4}$ at the end of induction or $<10^{-3}$ at the end of induction and subsequent MRD values during consolidation/before SCT $<10^{-4}$. The MRD poor response group included all other responses, $\geq 10^{-4}$ at the end of induction and one or more subsequent MRD values during consolidation/before SCT $\geq 10^{-4}$.

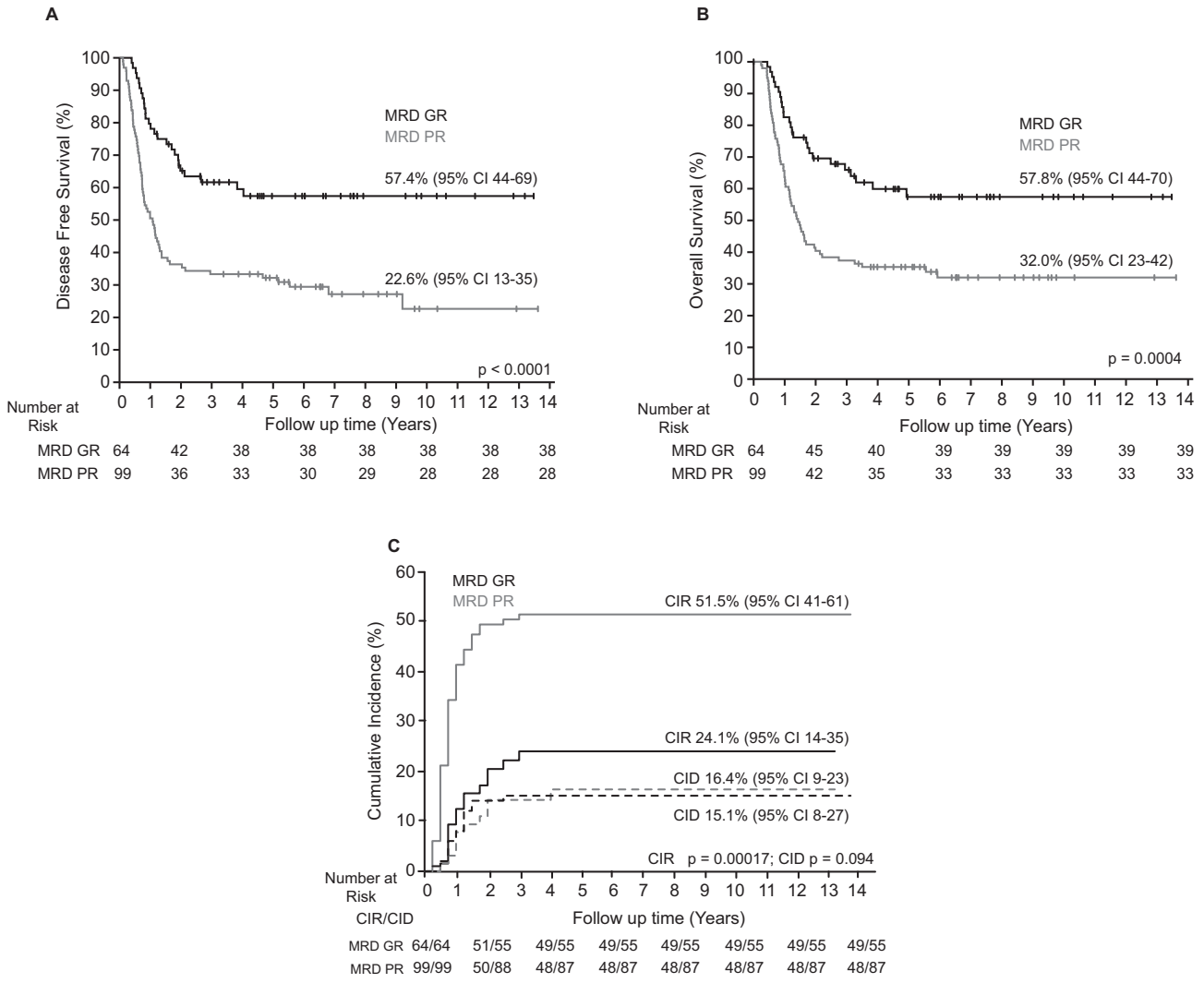


Fig. 3. Different disease-free survival, overall survival and cumulative incidence of subsequent relapses in patients with MRD good *vs* MRD poor response after induction treatment. Kaplan-Meier analyses of disease-free (A) and overall (B) survival and cumulative incidence of subsequent relapses and death (C) in patients who were in CR and MRD until SCT was measured. CR, complete remission; CI, confidence interval; CIR, cumulative incidence of relapses; CID, cumulative incidence of death; MRD, minimal residual disease; BCP, B-cell precursor; ALL, acute lymphoblastic leukaemia; GR, good response; PR poor response.

anthracycline after induction. As EFS in both trials was comparable, we analysed EOI and post-EOI MRD values as MRD-GR and MRD-PR. Grouped as MRD-GR and MRD-PR, DFS, CIR and OS were significantly better for patients with MRD-GR for both BCP- and T-ALL (Table 6 and Fig. 3). In BCP- or T-ALL, clinical risk parameters (Supplemental Table S3) or cytogenetic/genetic parameters (Supplemental Table S4) in BCP-ALL did not distinguish between MRD-GR and MRD-PR, although numbers in each group were small. The EOI MRD results were available in 18 of the 33 patients relapsing before SCT. Sixteen (89%) had an EOI MRD of $\geq 10^{-3}$, 6 had an EOI MRD of $\geq 10^{-2}$ and one had an EOI MRD of $<10^{-4}$. MRD was not available for 89 (35%) of 252 patients in CR2 (Table 6) and is detailed in Supplemental Table S5.

Table 7
MRD before SCT and disease-free/overall survival in all patients.

MRD before SCT	N	DFS in % (95% CI)	*P*	OS in % (95% CI)	*P*
$<10^{-4}$	76	47.0 (35–59)	**0.046**	49.2 (37–61)	**0.02**
10^{-3}–10^{-4}	11	45.5 (17–71)		45.5 (17–71)	
$\geq 10^{-3}$	11	18.2 (3–44)		18.3 (3–44)	

CI, confidence interval; SCT, stem cell transplantation; MRD, minimal residual disease; DFS, disease-free survival; OS, overall survival. P values in bold indicate those <0.05.

3.5. Determinants of outcomes after SCT

Of the 207 patients who reached the SCT time point, 183 (88%) received an SCT, 18 did not receive an SCT and SCT status was unknown for 6 patients (Fig. 1). The DFS and OS of patients who received an SCT and who did not

Table 8
Multivariable Cox regression analysis for disease-free survival and overall survival after SCT.

Parameter		N	Hazard ratio	95% CI	*P*
Disease-free survival					
Univariate					
MRD after induction	GR	56	1.00		
	PR	73	1.80	1.09–2.97	**0.022**
MRD before SCT	$<10^{-3}$	88	1.00		
	$\geq 10^{-3}$	14	2.56	1.12–5.76	**0.026**
Cytogenetic risk group	B-other	81	1.00		
	Cyto-SR	22	0.86	0.44–1.66	0.65
	Cyto-HR	18	1.77	0.93–3.36	0.082
TP53 alteration	No	85	1.00		
	Yes	12	2.63	1.31–5.27	**0.006**
aGVHD	Yes	56	1.00		
	No	20	2.17	1.13–4.14	**0.020**
Multivariable					
MRD before SCT	$<10^{-3}$	63	1.00		
	$\geq 10^{-3}$	8	2.79	1.19–6.53	**0.018**
Cytogenetic risk group	B-other	45	1.00		
	Cyto-SR	14	1.15	0.51–2.57	0.72
	Cyto-HR	12	2.58	1.15–5.77	**0.021**
Overall survival					
Univariate					
MRD after induction	GR	56	1.00		
	PR	73	1.55	0.92–2.60	0.098
MRD before SCT	$<10^{-3}$	88	1.00		
	$\geq 10^{-3}$	14	2.71	1.18–6.19	**0.018**
Cytogenetic risk group	B-other	81	1.00		
	Cyto-SR	22	0.98	0.49–1.96	0.95
	Cyto-HR	18	2.18	1.13–4.19	**0.020**
TP53 alteration	No	85	1.00		
	Yes	12	2.8	1.38–5.66	**0.004**
aGVHD	Yes	56	1.00		
	No	20	2.48	1.23–5.00	**0.012**
Multivariable					
MRD before SCT	$<10^{-3}$	63	1.00		
	$\geq 10^{-3}$	8	3.11	1.31–7.37	**0.010**
Cytogenetic risk group	B-other	45	1.00		
	Cyto-SR	14	1.14	0.49–2.68	0.72
	Cyto-HR	12	3.03	1.34–6.83	**0.008**

CI, confidence interval; MRD, minimal residual disease; GR, good response; PR, poor response; SCT, stem cell transplantation; cyto-SR, cytogenetic standard risk (*ETV6-RUNX1*, high-hyperdiploid); cyto-HR, cytogenetic high-risk (<40 Chr., *KTM2A, TCF3-HLF, TCF3-PBX1*, iAMP21); aGVHD, acute graft-versus-host disease.
Cox regression analysis includes all significant univariate variables: MRD after induction and before SCT, cytogenetic risk group, *TP53* alterations and aGVHD. Overlap of cytogenetic HR and aGVHD is small here (n = 1), and a model with cytogenetic stepwise forward testing, comparison of models using a log-likelihood ratio test risk group and aGVHD, is not possible.
P values in bold indicate those <0.05.

receive an SCT were 46.4% (95% CI: 38–55) and 6.7% (0.5–26) as well as 52.7% (45–60) and 13.3% (2–34), respectively. CR2 after SCT was maintained in 61 (47%) of 131 patients with a BCP-ALL relapse and 30 (58%) of 52 patients with a T-ALL relapse, with a DFS and OS of patients after SCT of 42.1% (32–52) and 56.8% (42–69) as well as 51.6% (42–60) and 55.4% (40–68), respectively.

For patients with BCP-ALL only, univariable analyses of variables affecting post-SCT survival identified acute graft-versus-host disease (GVHD), DFS 64% (60–75) with *vs* 25% (9–45) without acute GVHD (aGVHD) and OS 51% (33–67) and 30% (12–50), respectively, and a pre-SCT MRD $<10^{-3}$ (DFS: 56% (43–67)) *vs* MRD $\geq 10^{-3}$ (DFS: 13% (7–42)) and OS: 47% (33–59) *vs* 12.5% (7–42), respectively. Survival was concordant between the pre-SCT MRD groups of $<10^{-3}$–$\geq 10^{-4}$ and $<10^{-4}$ (DFS: 46% (17–71), 47% (35–59) and OS: 46% (17–71), 49% (37–61)) (Table 7). In patients without aGVHD, 14 of 15 events were a second relapse. Among patients with a BCP-ALL relapse, DFS after SCT was lower in patients with hypodiploidy ($P = 0.0002$), a *TCF3* rearrangement ($P < 0.0001$) or a $TP53^{del/mut}$ ($P = 0.002$), although numbers in each group were small (Supplemental Table S6). Multivariable analyses for DFS and OS confirmed a pre-SCT MRD of $\geq 10^{-3}$ and no aGVHD as independent predictors of poor outcomes after SCT in BCP-ALL (Table 8). For patients with

Table 9
Determinants of outcomes after SCT.

Risk factors		BCP-ALL							T-ALL						
		N	DFS	95% CI	*P*	OS	95% CI	*P*	N	PFS	95% CI	*P*	OS	95% CI	*P*
Sex	Male	78	50.6	39–61	0.78	50.1	39–62	0.72	38	60.1	43–74	0.37	58.7	41–73	0.33
	Female	53	54.2	40–67		36.6	19–54		14	47.1	20–71		46.8	20–70	
Age at relapse (years)	<10	89	50.0	74–99	0.40	45.1	33–56	0.71	27	61.4	40–77	0.38	55.6	35–72	0.71
	≥10–15	24	50.0	29–68		32.1	8–61		15	60.0	32–80		64.3	34–83	
	≥15	18	65.5	4–8		58.2	32–78		10	40.0	12–67		44.0	14–72	
Time to relapse	Very early	47	57.2	42–70	0.45	56.4	39–70	0.16	20	54.2	30–73	0.99	51.7	27–72	0.89
	Early	84	49.4	38–60		37.7	24–52		11	57.1	25–80		62.3	28–84	
	Late	–				–			21	55.5	32–74		55.3	32–74	
Site of relapse	Isolated	121	50.7	41–59	0.32	42.1	31–53	0.14	37	57.2	40–71	0.60	57.2	39–72	0.60
	Combined	10	70.0	33–89		80.0	41–95		15	50.9	24–73		50.3	23–72	
Donor type	MMD	26	38.5	20–56	0.29	38.5	20–56	0.28	13	60.6	29–81	0.95	60.1	29–81	0.95
	MRD	19	46.8	24–67		33.7	8–63		12	54.0	22–78		48.6	15–76	
	MUD	79	57.8	46–68		51.1	38–63		23	56.5	34–74		54.8	32–73	
SC source	BM	66	57.3	44–68	0.13	42.6	26–58	0.073	24	61.1	38–78	0.63[a]	59.3	36–77	0.64[a]
	PBSC	37	39.9	24–55		37.5	22–53		20	54.6	31–73		53	29–72	
	CB	12	58.3	27–80		66.7	34–86		3	0.0			0.0		
TBI	Yes	113	52.6	43–61	0.83	43.8	32–55	0.45	45	54.4	39–68	0.76	52.6	36–67	0.80
	No	15	52.5	25–74		59.3	31–79		5	60.0	13–88		60.0	13–88	
aGVHD	Yes	56	64.2	50–75	**0.0042**	51.4	33–67	**0.027**	23	56.5	34–74	0.32	55.9	36–73	0.35
	No	20	25.0	9–45		30.0	12–50		10	40.0	12–67		40.0	12–67	
cGVHD	Yes	10	90.0	47–99	0.11	45.0	1–80	0.49	11	45.5	17–71	0.94	45.5	17–71	0.90
	No	48	62.4	47–74		59.9	44–73		17	52.9	28–73		52.9	28–73	
MRD pre-SCT															
	$<10^{-3}$	68	55.6	43–67	**0.035**	46.5	33–59	**0.036**	20	43.8	22–64	0.16	41.9	20–63	0.96
	$\geq 10^{-3}$	8	12.5	7–42		12.5	1–42		6	16.7	8–52		16.7	8–52	
	$<10^{-4}$	58	58.4	40–74	0.085	47.4	33–61	0.12	18	42.9	20–64	0.30	40.5	17–63	0.30
	$>10^{-4}$	17	29.4	11–51		29.4	11–51		8	25.0	4–56		25.0	4–56	
MRD post-induction															
	$<10^{-3}$	49	61.3	46–74	0.0703	61.3	46–74	0.07	12	57.1	25–80	0.22	53.3	12–78	0.24
	$\geq 10^{-3}$	50	28.1	11–48		28.1	11–48		23	38.7	19–58		36.6	17–56	
	$<10^{-4}$	33	58.8	40–74	0.29	58.8	40–74	0.29	19	46.8	24–67	0.47	43.8	20–65	0.31
	$\geq 10^{-4}$	76	36.1	22–51		36.1	22–51		14	35.7	13–59		35.7	13–59	
	GR[b]	40	64.1	47–77	0.073	59.7	42–74	0.099	16	49.2	24–71	0.30	46.3	20–69	0.26
	PR[b]	58	46.6	33–59		30.6	15–48		15	33.3	12–56		33.3	12–56	

ALL, acute lymphoblastic leukaemia; CI, confidence interval; SCT, stem cell transplantation; T, T-cell; BCP, B-cell precursor; MMD, mismatched donor; MRD, matched related donor; MUD, matched unrelated donor; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; TBI, total body irradiation; aGVHD, acute graft-versus-host disease; cGHVD, chronic graft-versus-host disease; MRD pre-SCT/MRD post-induction, minimal residual disease before SCT/minimal residual disease after induction treatment; SC, stem cell.
P values in bold indicate those <0.05.

[a] The p value excludes CB; p values calculated using the log-rank test.

[b] MRD good response was defined as either $<10^{-4}$ at the end of induction or $<10^{-3}$ at the end of induction and subsequent MRD values during consolidation/before SCT $<10^{-4}$. The MRD poor response group included all other responses, $\geq 10^{-4}$ at the end of induction and one or more subsequent MRD values during consolidation/before SCT $\geq 10^{-4}$.

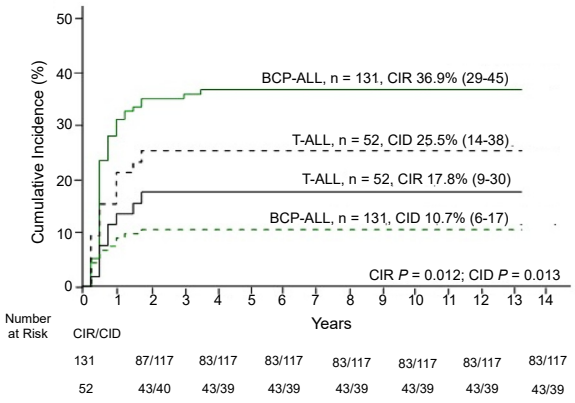


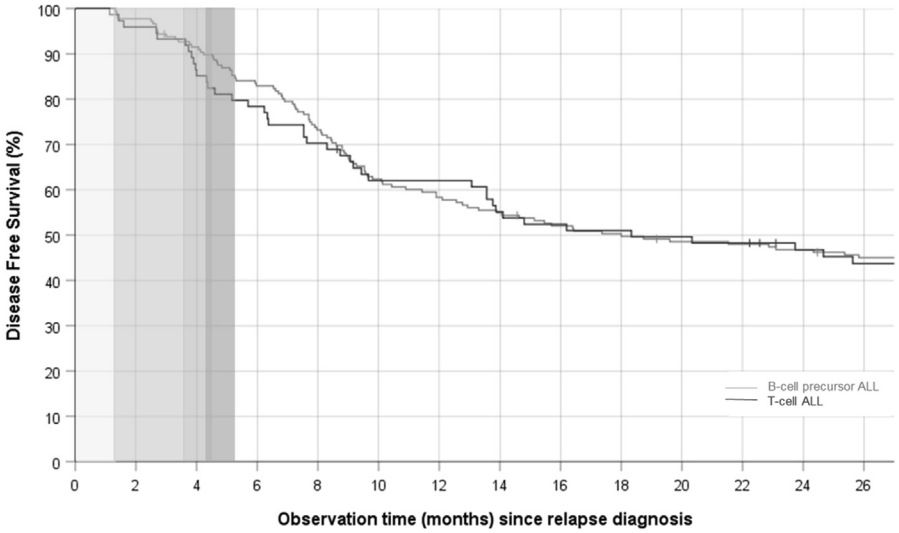
Fig. 4. Higher cumulative incidence of subsequent relapses in patients with high-risk BCP-ALL, but of death in patients with T-ALL after SCT. Cumulative incidence of subsequent relapses and death in patients with BCP- and T-ALL who underwent SCT. CIR, cumulative incidence of relapses; CID, cumulative incidence of death; BCP, B-cell precursor; T, T-cell; ALL, acute lymphoblastic leukaemia; SCT, stem cell transplantation.

T-ALL relapses, no risk factors correlated with DFS or OS after SCT (Table 9).

Of 183 patients, who received an SCT, transplant-related mortality occurred in 27 (15%) and relapse after SCT occurred in 57 (31%) patients. The CIR and CID after SCT were 31.6 (25–38) and 14.9 (10–21), respectively. The CIR and CID for BCP- vs T-ALL were 36.9% (29–45) and 17.8% (9–30) as well as 10.7% (6–17) and 25.5% (14–38, Fig. 4), respectively, suggesting an increase in post-SCT relapses in BCP-ALL and transplant-related mortality in T-ALL.

4. Discussion

The outcomes of HR relapsed ALL treated in the two trials were similar, allowing observations to be made on a combined analysis of prospectively treated and uniformly defined HR relapsed ALL. Nevertheless, the data need to be interpreted cautiously as a number of patients were withdrawn because of toxicity with idarubicin and CCE, the study population was heterogeneous, numbers in subgroup analyses were small and the median follow-up time is variable in the different groups, although most events occurred within 36 months. The trials also accrued patients between 2001 and 2013. Although the data are mature, during the current era, there have been improvements in transplant outcomes and the availability of immunotherapies. The results show comparable outcomes for both BCP- and T-ALL HR relapse with a benefit for SCT. The results of this paper confirm previous observations of poor outcomes in older patients [24], relapses within 18 months of diagnosis, with isolated medullary relapse [2] and with HR cytogenetics [22]. Neither the donor nor the stem cell source influenced outcomes [25].



B-cell precursor

Number of events	0	4	16	15	17	19	11	4	3	3	3	1	2	3
Number at risk	178	174	158	143	126	107	96	92	89	86	83	82	80	77

Number of events	0	3	8	5	6	6	0	5	2	1	1	1	4	2
Number at risk	74	71	63	58	52	46	46	41	39	38	37	36	32	30

Fig. 5. Heterogeneous patterns of event occurrence before and after SCT in patients with high-risk relapses in complete remission at the end of induction treatment. Disease-free survival of patients with B-cell precursor ALL and T-cell ALL in second complete remission. The different phases of treatment are highlighted in different grey scales and numbered (1 = induction; 2 = consolidation; 3 = intensification; 4 = SCT). ALL, acute lymphoblastic leukaemia; SCT, stem cell transplantation.

Although the trials were associated with differences in EOI MRD responses, the survival rates were similar. Those with an EOI MRD of $<10^{-4}$ or those with an EOI MRD of $\geq 10^{-4}$–$<10^{-3}$ with sequential decrease in MRD had the best outcomes, possibly reflecting sensitivity of residual cells to concurrent therapy. Thus, in HR relapses, serial assessment of MRD may better guide subsequent therapeutic interventions. Pre-SCT MRD levels of $\geq 10^{-3}$ [26,27] and $\geq 10^{-4}$ [5,28] have been reported to be associated with poor outcomes after SCT. In this cohort, a pre-SCT MRD of $<10^{-4}$ and $\geq 10^{-4}$–$<10^{-3}$ was associated with comparable outcomes, and only those with an MRD of $\geq 10^{-3}$ showed a significantly poorer outcome. Methodologically newer MRD assays (next-generation sequencing) may further increase the sensitivity of detecting lower levels of MRD [29]. Nevertheless, patients with low or absence of MRD before SCT relapsed after SCT in our cohort. MRD kinetics vary with different ALL subtypes, with MRD being less predictive of relapses in HR cytogenetic subtypes and T-ALL [30]. We speculate that in T-ALL, there may also be extramedullary reservoirs of disease [31] not readily assessed using marrow-based MRD assays only.

aGVHD appeared to be associated with a graft-versus-leukaemia effect in BCP-ALL but not in T-ALL in this cohort. Patients with T-ALL also had a higher post-SCT therapy–related mortality. A 3-year therapy-related mortality of 30% with an OS of 48% has been reported for 229 patients with T-ALL who underwent transplantation in CR2, with no impact of acute or chronic GVHD on the outcome [32]. Our study does not have the required data to provide a satisfactory explanation for the apparent benefit of aGVHD in BCP-ALL or the increased transplant-related toxicity in T-ALL, and this requires further prospective evaluation.

Achieving a second remission remains a major problem. Addition of the proteasomal inhibitor bortezomib to induction therapy in relapsed ALL has been reported to achieve CR2 rates of 63–72%, benefitting both BCP- and T-ALL, and was well tolerated [33,34]. Of those reaching SCT, other than high MRD, recurrence was seen more frequently in those with a *TCF3* rearrangement, $TP53^{del/mut}$ or hypodiploidy [35]. Preclinical data suggest that *TCF3*-rearranged and hypodiploid ALL may be susceptible to the BCL-2 inhibitor venetoclax [36,37] and that *TP53*-rearranged ALL may be susceptible to a combination of APR-246 and doxorubicin [38].

Given the genetic heterogeneity of ALL, targeting surface epitopes with immunotherapy offers a more uniform strategy [39]. Recent studies [12,13,16] reported impressive MRD responses and outcomes with minimal toxicities after CD19-directed therapy using blinatumomab or CAR-T therapy. In this study, for patients with BCP-ALL in CR2, there were 86 (49%) events in 174 patients at 18 months (Fig. 5). For T-ALL, venetoclax [40], navitoclax [41] and the anti-CD38 monoclonal daratumumab have shown promise [42,43].

Newer-generation CAR-Ts with enhanced expansion and long-term persistence may prevent post-SCT relapse or even replace the need for SCT. Nevertheless, disease recurrence in ALL is associated with a long latency, and careful long-term follow-up will be required. This study provides the background comparative data required to evaluate the benefit of the new drugs. A caveat is the high cost of immunotherapy and its non-availability to patients in low- and middle-income countries where most cases of ALL occur. For these newer therapies to make a significant impact to global outcomes of childhood ALL, this is a gap that needs to be bridged.

Author contribution statement

Cornelia Eckert, Writing – original draft, review & editing & final approval, Funding acquisition, Data curation, Methodology, Investigation, Formal analysis.

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Julie AE Irving, Writing – review & editing & final approval, Funding acquisition, Data curation.

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Stefanie Groeneveld-Krentz, Writing – review & editing & final approval, Methodology, Visualisation.

Tamas Révész, Writing – review & editing & final approval, Data curation, Funding acquisition.

Peter Hoogerbrugge, Writing – review & editing & final approval, Data curation, Funding acquisition.

Jeremy Hancock, Writing – review & editing & final approval, Data curation, Methodology.

Rosemary Sutton, Writing – review & editing & final approval, Funding acquisition, Data curation, Formal analysis.

Guenter Henze, Writing – review & editing & final approval, first chair of ALL-REZ BFM 2002, Supervision, Funding acquisition.

Christiane Chen-Santel, Writing – review & editing & final approval, Data curation, Formal analysis.

Andishe Attarbaschi, Writing – review & editing & final approval, Data curation, Funding acquisition.

Jean-Pierre Bourquin, Writing – review & editing & final approval, Data curation, Funding acquisition.

Lucie Sramkova, Writing – review & editing & final approval, Data curation, Funding acquisition.

Martin Zimmermann, Writing – review & editing & final approval, Supervision, Formal analysis.

Shekhar Krishnan, Writing – review & editing & final approval, Data curation, Formal analysis.

Arend von Stackelberg, Writing – review & editing & final approval, chair of ALL-REZ BFM 2002 (after GH), Conceptualisation, Investigation, Funding acquisition, Supervision.

Vaskar Saha, Writing – original draft, review & editing & final approval, chair of ALLR3, Conceptualisation, Funding, Data curation, Investigation, Formal analysis.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A. Supplementary data

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References

[1] Freyer DR, Devidas M, La M, Carroll WL, Gaynon PS, Hunger SP, et al. Postrelapse survival in childhood acute lymphoblastic leukemia is independent of initial treatment intensity: a report from the Children's Oncology Group. Blood 2011 Mar 17;117(11):3010–5. https://doi.org/10.1182/blood-2010-07-294678. Cited in: Pubmed; PMID 21193696.

[2] Tallen G, Ratei R, Mann G, Kaspers G, Niggli F, Karachunsky A, et al. Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. J Clin Oncol 2010 May 10;28(14):2339–47. https://doi.org/10.1200/JCO.2009.25.1983. Cited in: Pubmed; PMID 20385996.

[3] Roy A, Cargill A, Love S, Moorman AV, Stoneham S, Lim A, et al. Outcome after first relapse in childhood acute lymphoblastic leukaemia - lessons from the United Kingdom R2 trial. Epub 2005/06/29 Br J Haematol 2005 Jul;130(1):67–75. https://doi.org/10.1111/j.1365-2141.2005.05572.x. Cited in: Pubmed; PMID 15982346.

[4] Eckert C, Groeneveld-Krentz S, Kirschner-Schwabe R, Hagedorn N, Chen-Santel C, Bader P, et al. Improving stratification for children with late bone marrow B-cell acute lymphoblastic leukemia relapses with refined response classification and integration of genetics. Epub 2019/10/24 J Clin Oncol 2019 Dec 20;37(36):3493–506. https://doi.org/10.1200/JCO.19.01694. Cited in: Pubmed; PMID 31644328.

[5] Parker C, Krishnan S, Hamadeh L, Irving JAE, Kuiper RP, Revesz T, et al. 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. Epub 2019/03/04 Lancet Haematol 2019 Apr;6(4):e204–16. https://doi.org/10.1016/S2352-3026(19)30003-1. Cited in: Pubmed; PMID 30826273.

[6] Borgmann A, von Stackelberg A, Hartmann R, Ebell W, Klingebiel T, Peters C, et al. Unrelated donor stem cell transplantation compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission: a matched-pair analysis. Blood 2003 May 15;101(10):3835–9. https://doi.org/10.1182/-blood.V101.10.3835. Cited in: Pubmed; PMID 12732501.

[7] Nguyen K, Devidas M, Cheng SC, La M, Raetz EA, Carroll WL, et al. Children's Oncology G. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. Leukemia 2008 Dec;22(12):2142–50. https://doi.org/10.1038/leu.2008.251. Cited in: Pubmed; PMID 18818707.

[8] Eckert C, Hagedorn N, Sramkova L, Mann G, Panzer-Grumayer R, Peters C, et al. Monitoring minimal residual disease in children with high-risk relapses of acute lymphoblastic leukemia: prognostic relevance of early and late assessment. Leukemia 2015 Aug;29(8):1648–55. https://doi.org/10.1038/leu.2015.59. Cited in: Pubmed; PMID 25748682.

[9] Henze G, Fengler R, Hartmann R, Kornhuber B, Janka-Schaub G, Niethammer D, et al. Six-year experience with a comprehensive approach to the treatment of recurrent childhood acute lymphoblastic leukemia (ALL-REZ BFM 85). A relapse study of the BFM group. Blood 1991 Sep 01;78(5):1166–72. Epub 1991/09/01. Cited in: Pubmed; PMID 1878583.

[10] Raetz EA, Borowitz MJ, Devidas M, Linda SB, Hunger SP, Winick NJ, et al. Reinduction platform for children with first marrow relapse of acute lymphoblastic leukemia: a Children's Oncology Group Study[corrected]. J Clin Oncol 2008 Aug 20;26(24):3971–8. https://doi.org/10.1200/JCO.2008.16.1414. Cited in: Pubmed; PMID 18711187.

[11] Brivio E, Locatelli F, Lopez-Yurda M, Malone A, Diaz de Heredia C, Bielorai B, et al. A Phase I study of inotuzumab

ozogamicin in pediatric relapsed/refractory acute lymphoblastic leukemia (ITCC-059 study). Epub 2020/10/18 Blood 2020 Oct 16. https://doi.org/10.1182/blood.2020007848. Cited in: Pubmed; PMID 33067614.

[12] Brown PA, Ji L, Xu X, Devidas M, Hogan LE, Borowitz MJ, et al. Effect of Postreinduction Therapy Consolidation With Blinatumomab vs Chemotherapy on Disease-Free Survival in Children, Adolescents, and Young Adults With First Relapse of B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA 2021;325:833–42.

[13] Locatelli F, Zugmaier G, Rizzari C, Morris JD, Gruhn B, Klingebiel T, et al. Effect of blinatumomab vs chemotherapy on event-free survival among children with high-risk first-relapse B-cell acute lymphoblastic leukemia: a randomized clinical trial. JAMA 2021;325:843–54.

[14] Gore L, Locatelli F, Zugmaier G, Handgretinger R, O'Brien MM, Bader P, et al. Survival after blinatumomab treatment in pediatric patients with relapsed/refractory B-cell precursor acute lymphoblastic leukemia. Epub 2018/09/08 Blood Canc J 2018 Aug 22; 8(9):80. https://doi.org/10.1038/s41408-018-0117-0. Cited in: Pubmed; PMID 30190453.

[15] Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. Epub 2016/06/14 N Engl J Med 2016 Aug 25;375(8):740–53. https://doi.org/10.1056/-NEJMoa1509277. Cited in: Pubmed; PMID 27292104.

[16] Locatelli F, Whitlock JA, Peters C, Chen-Santel C, Chia V, Dennis RM, et al. Blinatumomab versus historical standard therapy in pediatric patients with relapsed/refractory Ph-negative B-cell precursor acute lymphoblastic leukemia. Epub 2020/02/26 Leukemia 2020 Feb 24;34(9):2473–8. https://doi.org/10.1038/s41375-020-0770-8. Cited in: Pubmed; PMID 32094465.

[17] Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. Epub 2018/02/01 N Engl J Med 2018 Feb 1;378(5):439–48. https://doi.org/10.1056/NEJMoa1709866. Cited in: Pubmed; PMID 29385370.

[18] Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Epub 2014/10/17 Lancet 2015 Feb 07;385(9967):517–28. https://doi.org/10.1016/S0140-6736(14)61403-3. Cited in: Pubmed; PMID 25319501.

[19] Parker C, Waters R, Leighton C, Hancock J, Sutton R, Moorman AV, et al. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. Lancet 2010 Dec 11; 376(9757):2009–17. https://doi.org/10.1016/S0140-6736(10)62002-8. Cited in: Pubmed; PMID 21131038.

[20] Groeneveld-Krentz S, Schroeder MP, Reiter M, Pogodzinski MJ, Pimentel-Gutierrez HJ, Vagkopoulou R, et al. Aneuploidy in children with relapsed B-cell precursor acute lymphoblastic leukaemia: clinical importance of detecting a hypodiploid origin of relapse. eng. Epub 2019/02/05 Br J Haematol 2019 Apr;185(2): 266–83. https://doi.org/10.1111/bjh.15770. Cited in: Pubmed; PMID 30714092.

[21] Hof J, Krentz S, van Schewick C, Korner G, Shalapour S, Rhein P, et al. Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. J Clin Oncol 2011 Aug 10;29(23):3185–93. https://doi.org/10.1200/JCO.2011.34.8144. Cited in: Pubmed; PMID 21747090.

[22] Irving JA, Enshaei A, Parker CA, Sutton R, Kuiper RP, Erhorn A, et al. Integration of genetic and clinical risk factors improves prognostication in relapsed childhood B-cell precursor acute lymphoblastic leukemia. Blood 2016 Aug 18;128(7):911–22. https://doi.org/10.1182/blood-2016-03-704973. Cited in: Pubmed; PMID 27229005.

[23] Krentz S, Hof J, Mendioroz A, Vaggopoulou R, Dorge P, Lottaz C, et al. Prognostic value of genetic alterations in children with first bone marrow relapse of childhood B-cell precursor acute lymphoblastic leukemia. Leukemia 2013 Feb;27(2):295–304. https://doi.org/10.1038/leu.2012.155. Cited in: Pubmed; PMID 22699455.

[24] Masurekar AN, Parker CA, Shanyinde M, Moorman AV, Hancock JP, Sutton R, et al. Outcome of central nervous system relapses in childhood acute lymphoblastic leukaemia–prospective open cohort analyses of the ALLR3 trial. PloS One 2014;9(10): e108107. https://doi.org/10.1371/journal.pone.0108107. Cited in: Pubmed; PMID 25279465.

[25] Shimosato Y, Tanoshima R, Tsujimoto SI, Takeuchi M, Shiba N, Kobayashi T, et al. Allogeneic bone marrow transplantation versus peripheral blood stem cell transplantation for hematologic malignancies in children: a systematic review and meta-analysis. Epub 2019/08/09 Biol Blood Marrow Transplant 2019 Aug 5. https://doi.org/10.1016/j.bbmt.2019.07.025. Cited in: Pubmed; PMID 31394270.

[26] Krejci O, van der Velden VH, Bader P, Kreyenberg H, Goulden N, Hancock J, et al. Level of minimal residual disease prior to haematopoietic stem cell transplantation predicts prognosis in paediatric patients with acute lymphoblastic leukaemia: a report of the Pre-BMT MRD Study Group. Epub 2003/10/02 Bone Marrow Transplant 2003 Oct;32(8):849–51. https://doi.org/10.1038/sj.bmt.1704241. Cited in: Pubmed; PMID 14520434.

[27] Bader P, Hancock J, Kreyenberg H, Goulden NJ, Niethammer D, Oakhill A, et al. Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post-transplant outcome in children with ALL. Epub 2002/08/30 Leukemia 2002 Sep;16(9):1668–72. https://doi.org/10.1038/sj.-leu.2402552. Cited in: Pubmed; PMID 12200679.

[28] Bader P, Kreyenberg H, Henze GH, Eckert C, Reising M, Willasch A, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. Epub 2008/12/10 J Clin Oncol 2009 Jan 20; 27(3):377–84. https://doi.org/10.1200/JCO.2008.17.6065. Cited in: Pubmed; PMID 19064980.

[29] Wood B, Wu D, Crossley B, Dai Y, Williamson D, Gawad C, et al. Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. Epub 2017/12/30 Blood 2018 Mar 22;131(12):1350–9. https://doi.org/10.1182/blood-2017-09-806521. Cited in: Pubmed; PMID 29284596.

[30] O'Connor D, Enshaei A, Bartram J, Hancock J, Harrison CJ, Hough R, et al. Genotype-specific minimal residual disease interpretation improves stratification in pediatric acute lymphoblastic leukemia. Epub 2017/11/14 J Clin Oncol 2018 Jan 1;36(1): 34–43. https://doi.org/10.1200/JCO.2017.74.0449. Cited in: Pubmed; PMID 29131699.

[31] Ma S, Shi Y, Pang Y, Dong F, Cheng H, Hao S, et al. Notch1-induced T cell leukemia can be potentiated by microenvironmental cues in the spleen. Epub 2014/11/05 J Hematol Oncol 2014 Nov 4;7:71. https://doi.org/10.1186/s13045-014-0071-7. Cited in: Pubmed; PMID 25366136.

[32] Burke MJ, Verneris MR, Le Rademacher J, He W, Abdel-Azim H, Abraham AA, et al. Transplant outcomes for children with T cell acute lymphoblastic leukemia in second remission: a report from the center for international blood and marrow transplant research. Epub 2015/09/04 Biol Blood Marrow Transplant 2015 Dec;21(12):2154–9. https://doi.org/10.1016/j.bbmt.2015.08.023. Cited in: Pubmed; PMID 26327632.

[33] Horton TM, Whitlock JA, Lu X, O'Brien MM, Borowitz MJ, Devidas M, et al. Bortezomib reinduction chemotherapy in high-

risk ALL in first relapse: a report from the Children's Oncology Group. Epub 2019/04/09 Br J Haematol 2019 Apr 7. https://doi.org/10.1111/bjh.15919. doi:10.1111/bjh.15919. Cited in: Pubmed; PMID 30957229.

[34] Roy P, Islam R, Saha D, Gogoi M, Kumar Mishra D, Arora N, et al. Efficacy and safety of a bortezomib and reduced-intensity cytarabine-based protocol, TMC ALLR1, for relapsed childhood ALL in India. Epub 2019/06/07 Br J Haematol 2019 Sep; 186(6):861–5. https://doi.org/10.1111/bjh.16005. Cited in: Pubmed; PMID 31168836.

[35] Holmfeldt L, Wei L, Diaz-Flores E, Walsh M, Zhang J, Ding L, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Epub 2013/01/22 Nat Genet 2013 Mar;45(3):242–52. https://doi.org/10.1038/ng.2532. Cited in: Pubmed; PMID 23334668.

[36] Fischer U, Forster M, Rinaldi A, Risch T, Sungalee S, Warnatz HJ, et al. Genomics and drug profiling of fatal TCF3-HLF-positive acute lymphoblastic leukemia identifies recurrent mutation patterns and therapeutic options. Epub 2015/07/28 Nat Genet 2015 Sep;47(9):1020–9. https://doi.org/10.1038/ng.3362. Cited in: Pubmed; PMID 26214592.

[37] Diaz-Flores E, Comeaux EQ, Kim KL, Melnik E, Beckman K, Davis KL, et al. Bcl-2 is a therapeutic target for hypodiploid B-lineage acute lymphoblastic leukemia. Epub 2019/03/14 Can Res 2019 May 1;79(9):2339–51. https://doi.org/10.1158/0008-5472.CAN-18-0236. Cited in: Pubmed; PMID 30862722.

[38] Demir S, Boldrin E, Sun Q, Hampp S, Tausch E, Eckert C, et al. Therapeutic targeting of mutant p53 in pediatric acute lymphoblastic leukemia. Epub 2019/05/11 Haematologica 2020 Jan; 105(1):170–81. https://doi.org/10.3324/haematol.2018.199364. Cited in: Pubmed; PMID 31073076.

[39] Mouttet B, Vinti L, Ancliff P, Bodmer N, Brethon B, Cario G, et al. Durable remissions in TCF3-HLF positive acute lymphoblastic leukemia with blinatumomab and stem cell transplantation. Epub 2019/02/16 Haematologica 2019 Jun;104(6): e244–7. https://doi.org/10.3324/haematol.2018.210104. Cited in: Pubmed; PMID 30765470.

[40] Richard-Carpentier G, Jabbour E, Short NJ, Rausch CR, Savoy JM, Bose P, et al. Clinical experience with venetoclax combined with chemotherapy for relapsed or refractory T-cell acute lymphoblastic leukemia. Epub 2020/02/10 Clin Lymphoma Myeloma Leuk 2020 Apr;20(4):212–8. https://doi.org/10.1016/j.clml.2019.09.608. Cited in: Pubmed; PMID 32035785.

[41] Lacayo NJ, Pullarkat VA, Stock W, Jabbour E, Bajel A, Rubnitz J, et al. Safety and efficacy of venetoclax in combination with navitoclax in adult and pediatric relapsed/refractory acute lymphoblastic leukemia and lymphoblastic lymphoma. Blood 2019;134(Supplement_1). https://doi.org/10.1182/blood-2019-126977. 285-285.

[42] Bride KL, Vincent TL, Im SY, Aplenc R, Barrett DM, Carroll WL, et al. Preclinical efficacy of daratumumab in T-cell acute lymphoblastic leukemia. Epub 2018/01/07 Blood 2018 Mar 1;131(9):995–9. https://doi.org/10.1182/blood-2017-07-794214. Cited in: Pubmed; PMID 29305553.

[43] Vogiatzi F, Winterberg D, Lenk L, Buchmann S, Cario G, Schrappe M, et al. Daratumumab eradicates minimal residual disease in a preclinical model of pediatric T-cell acute lymphoblastic leukemia. Epub 2019/07/18 Blood 2019 Aug 22;134(8): 713–6. https://doi.org/10.1182/blood.2019000904. Cited in: Pubmed; PMID 31311816.