



## RESEARCH PAPER

# Clinical outcomes of second relapsed and refractory first relapsed paediatric AML: A retrospective study within the NOPHO-DB SHIP consortium

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## Summary

As treatments for second relapsed and refractory first relapsed paediatric AML transition from purely palliative to more commonly curative in nature, comparative data is necessary for evaluating the effectiveness of emerging treatment options. Furthermore, little is known about predictors of prognosis following third-line therapy. From 2004 until 2019, 277 of the 869 patients enrolled in NOPHO-DB SHIP consortium trials experienced a first relapse and, of these patients, 98 experienced refractory first relapse and 59 a second relapse. Data on patient and disease characteristics within this cohort of 157 patients was analysed to determine probability of overall survival (pOS) and to identify factors influencing survival. Data on early treatment response and complete remission were not available. One and 5-year pOS were  $22 \pm 3\%$  and  $14 \pm 3\%$ , respectively. There was no statistically significant difference in survival between refractory first relapsed and second relapsed AML. Factors influencing prognosis included: late relapse, type of third-line treatment, *FLT3* mutational status, and original treatment protocol. These data provide a baseline for evaluating the effectiveness of emerging therapies for the treatment of children with refractory first relapsed and second relapsed paediatric AML and evidence that select patients receiving third-line therapy can be cured.

## KEY WORDS

paediatric acute myeloid leukaemia, refractory disease, relapse, survival, therapy

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## INTRODUCTION

Despite significant improvements in the prognosis of paediatric acute myeloid leukaemia (AML), 5-year overall survival has plateaued at approximately 75%.<sup>1-3</sup> Mortality is caused by early death due to leukaemia, refractory disease, treatment related complications, and relapse.<sup>4</sup> Despite attempts to intensify therapy, relapse remains the most important cause of treatment failure<sup>1</sup> and occurs in approximately 30% of patients who achieve a complete remission (CR) following initial diagnosis.<sup>5-7</sup> Prognosis following first relapse is well studied and current estimates of CR and 5-year probability of overall survival (pOS) following relapse are 64%–77% and 34%–40%, respectively.<sup>5-9</sup>

A considerable number of patients will have a refractory first relapse or experience a second relapse. In one randomized trial on Liposomal Daunorubicin in first relapsed paediatric AML, 32% of the patients registered had refractory disease and the 4-year estimated cumulative incidence of second relapse was  $24 \pm 2\%$ .<sup>6</sup> While these groups may have previously only been considered eligible for palliative care, patients, parents and their doctors now more often aim for curative treatment options following a second relapse or refractory first relapse. Despite this development, little is known about the prognosis for these children receiving third-line treatments. Estimates based on relatively old data suggest that the CR rate following third-line treatment is around 25%<sup>10</sup> and population based data on survival in this group is limited to the recently published study of Rasche *et al.*<sup>11</sup> who found a 5-year pOS of  $15 \pm 4\%$  in their cohort of 73 patients with a second relapse. Other estimates of survival in this group come from clinical trials in which patients are selected based on fitness to receive further therapy and are therefore not representative of all patients suffering a refractory first relapse or second relapse.

Data on prognosis following third-line treatment is essential for two reasons. Firstly, the lack of a standard third-line treatment for patients with a refractory first or second relapse makes them prime candidates for clinical trials with novel therapies. As paediatric AML is a relatively rare illness affecting approximately seven in every million children,<sup>12</sup> design of randomized controlled trials is a challenge.<sup>13</sup> To overcome this obstacle, historical data on survival of children with a refractory first relapse or second relapse is essential for evaluating the effectiveness of experimental therapies. Secondly, as possibly curative options are more frequently offered following a refractory first relapse or second relapse, it is important to know the prognosis for these patients and factors influencing prognosis in order to provide patients with the best possible care. The aim of this study was therefore to assess prognosis following third-line treatment in a historical cohort of patients with refractory first relapsed or second relapse paediatric AML.

## METHODS

Between January 2004 and January 2019, 869 patients from participating countries with de novo AML were treated within one of three protocols: DB-AML 01,<sup>3</sup> NOPHO-AML 2004,<sup>14</sup> or NOPHO-AML 2012.<sup>15</sup> Parents and patients included in these clinical trials consented to the use of their data for research purposes and medical ethics committee consent was obtained for all studies in which these patients originally participated. Patients with acute promyelocytic leukaemia and patients with myeloid proliferations related to Down Syndrome or inherited bone marrow (BW) failure syndromes were not included in these studies. Data was collected on patients during and following treatment, and entered in an anonymized study database.

### Definitions

Bone marrow relapse was defined as the presence of  $\geq 5\%$  leukaemic cells in BM following a previous remission and central nervous system (CNS) relapse was defined as the presence of leukaemic cells in the cerebrospinal fluid or identification of a mass in the CNS. Refractory disease was defined as the presence of  $\geq 5\%$  leukaemic cells in BM after the second course of induction. CR was achieved if the patient had  $< 5\%$  blast cells in BM, evidence of haematological regeneration of BM and no evidence of leukaemia elsewhere in the body. Early death was considered any death within 42 days following diagnosis of the relapse and early relapse was considered any relapse occurring within 12 months of diagnosis (initial AML diagnosis for patients with a refractory first relapse and diagnosis of the first relapse for patients with a second relapse).

### Statistical methods

Statistical analyses were performed using IBM SPSS Statistics (Mac Version 24.0). Differences in patient and disease characteristics between those with a refractory first relapse and second relapse were compared using Fisher's exact test (for  $2 \times 2$  tables) and Pearson's chi-squared test (for higher order tables). The Kaplan–Meier method was utilized to estimate survival rates. Survival times were calculated as the time between the date of diagnosis of relapse and the date of death. Censoring occurred if patients were lost to follow-up or were alive at the time of last follow up. Differences in survival between subgroups were analysed using the two-sided log-rank test. Multivariable analysis was performed using the Cox proportional hazards method. Factors included in the multivariable model included those which are known to be relevant for prognosis following first- and second-line treatments.<sup>5-8,16</sup> Unfortunately, correction for immortal time bias was not possible as transplantation dates were not available for all patients. For all analyses, a two-sided  $p < 0.05$  was considered significant.

## RESULTS

### Patient characteristics

Of the 869 patients treated according to the three protocols, 277 experienced a first relapse and of these, 98 had refractory first relapse and 59 patients a second relapse. Of this group of 157 patients, 110 (70.1%) relapsed within 1 year of diagnosis. Patients with a refractory first relapse had a median time to relapse of 9.2 months and patients with a second relapse had a median time to second relapse of 9.7 months. Median follow-up time for surviving patients was 29 months (range: 0.2–119 months). Patient characteristics are presented in [Table 1](#).

Patient characteristics were similar between refractory first relapse and second relapse, with the exception of original treatment protocol, cytogenetic profile, type of third-line treatment received, and frequency of early death. The proportion of patients with a second relapse was higher in NOPHO-AML 2004 and DB-AML 01 protocols whereas the proportion of patients with a refractory first relapse was higher in the NOPHO-AML 2012 protocol. This relative increase in refractory disease may reflect the increased intensity of the NOPHO-AML 2012 protocol. While sample sizes were too small to meaningfully analyse differences in specific cytogenetic alterations, grouping alterations based on prognostic value (see for example [Conneely et al.](#),<sup>17</sup> [Table 2](#)) revealed that patients with a second relapse more frequently had cytogenetic alterations with a good prognosis compared with patients with a refractory first relapse (second relapse = 19%, refractory first relapse = 5%;  $p = 0.04$ , [Table 1](#)). Finally, patients with a second relapse were more likely to suffer from early death ( $p = 0.005$ , [Table 1](#)) and were more often treated palliatively than patients with refractory first relapse ( $p = 0.001$ , [Table 1](#)).

### Third-line treatment

#### Refractory first relapse

Of the 73 out of 98 patients for whom treatment information was available, 63 patients were treated, at least initially, with curative intent. Forty-two patients were treated with chemotherapy alone and 19 patients were treated with chemotherapy followed by stem cell transplantation (SCT). Two patients were treated with donor leukocyte infusions (DLI). For an overview of the therapeutic agents, see [Table S1](#).

#### Second relapse

Of the 43 out of 59 patients for whom treatment information was available, 30 were treated with curative intent. For an overview of the therapeutic agents, see [Table S1](#). Fourteen patients were treated with chemotherapy alone, eight patients were treated with chemotherapy followed by SCT.

Three patients received DLI, four with a combination of chemotherapy and DLI, and one patient was treated with sorafenib and SCT. Thirteen patients who suffered a second relapse received palliative care.

### Overall survival

At the time of last follow-up, 29 of the 157 patients were alive (median follow-up = 29 months, range: 0.2–119 months; [Figure 1](#)). Overall probability of survival at 1- and 5-years for the entire cohort was  $22 \pm 3\%$  and  $14 \pm 3\%$ , respectively. In patients treated with curative intent ( $n = 93$ ), 1-year pOS was  $27 \pm 5\%$  and 5-year pOS was  $17 \pm 4\%$ . One-year pOS was  $25 \pm 5\%$  for patients with a refractory first relapse and  $18 \pm 5\%$  for patients with a second relapse. Log-rank tests did not reveal a statistically significant difference in survival between patients with a refractory first relapse and those with a second relapse ( $p = 0.14$ , [Table 3](#)). The comparable survival rates and similar distribution of patient characteristics between these two groups allowed us to combine these groups for multivariable survival analysis.

### Factors influencing survival

Factors included in the multivariable model were: original treatment protocol, age at initial diagnosis, risk group during original treatment, the prognostic value of main cytogenetic mutations at initial diagnosis, *FLT3* mutation status at initial diagnosis, WBC at initial diagnosis, timing of relapse, and the type of third-line treatment received (palliative care, chemotherapy, chemotherapy and SCT, other or unknown).

### Original treatment protocol

The 1-year pOS of patients treated initially according to NOPHO-AML 2012 was  $29 \pm 6\%$  compared to a 1-year pOS of  $17 \pm 4\%$  and  $24 \pm 9\%$  for NOPHO-AML 2004 and DB-AML 01, respectively ([Table 3](#)). While these differences were not statistically significant using log-rank tests ( $p = 0.40$ ), multivariable Cox regression revealed significantly worse hazard ratios (HR) for patients who were originally treated according to DB-AML 01 (HR = 2.19 [1.15–4.16]) and NOPHO-AML 2004 (HR = 1.79 [1.12–2.86]) ( $p = 0.02$ , [Table 4](#)).

### FLT3 status

A significant survival advantage was identified for patients who either had no *FLT3* mutation, a *FLT3-ALM* mutation or a *FLT3-ITD* mutation in the presence of a concurrent *NPM1* mutation relative to patients with a *FLT3-ITD* mutation either alone or in combination with a *WT1* mutation. No

**TABLE 1** Patient and disease characteristics according to event (refractory first or second relapsed paediatric AML). All patient and disease characteristics were gathered at time of initial diagnosis with the exception of: timing of relapse, type of third-line treatment, relapse site, and occurrence of early death which were determined at or following relapse

	Total	Refractory first relapse, N (%)	Second relapse, N (%)	p-value
All patients	157	98	59	—
<b>Protocol</b>				
DB-AML 01	21	10 (10.2)	11 (18.6)	0.01
NOPHO-AML 2004	76	42 (42.9)	34 (57.6)	
NOPHO-AML 2012	60	46 (46.9)	14 (23.7)	
<b>Sex</b>				
Male	81	47 (48.0)	34 (57.6)	0.25
Female	76	51 (52.0)	25 (42.4)	
<b>Age (years)</b>				
<2	38	24 (24.5)	14 (23.7)	0.99
2–9	56	35 (35.7)	21 (35.6)	
>10	63	39 (39.8)	24 (40.7)	
<b>WBC at initial diagnosis</b>				
<100 × 10 <sup>9</sup> /L	126	79 (80.6)	47 (79.7)	1.00
>100 × 10 <sup>9</sup> /L	31	19 (19.4)	12 (20.3)	
<b>Risk group (according to protocol)</b>				
Standard-risk	125	75 (78.5)	48 (81.4)	0.69
High-risk	32	21 (21.4)	11 (18.6)	
<b>FAB type</b>				
M0	10	5 (5.1)	5 (8.5)	—
M0/M1	3	2 (2.0)	1 (1.7)	
M1	19	9 (9.2)	10 (16.9)	
M1/M2	3	3 (3.1)	0	
M2	23	13 (13.3)	10 (16.9)	
M4	10	4 (4.1)	6 (10.2)	
M5	45	31 (31.6)	14 (23.7)	
M6	2	2 (2.0)	0	
M7	21	18 (18.4)	3 (5.1)	
Mixed phenotype	2	2 (2.0)	0	
Unknown	19	9 (9.2)	10 (16.9)	
<b>Cytogenetics at diagnosis</b>				
KMT2A	39	25 (25.5)	14 (23.7)	0.02 <sup>a</sup>
t(8;21)	9	2 (2.0)	7 (11.9)	
Monosomy 7	8	5 (5.1)	3 (5.1)	
Inv (16)	4	2 (2.0)	2 (3.4)	
Trisomy 8	5	3 (3.1)	2 (3.4)	
Complex	14	11 (11.2)	3 (5.1)	
Normal	37	24 (24.5)	13 (22.0)	
Other	38	24 (24.5)	14 (23.7)	
Unknown	3	2 (2.0)	1 (1.7)	
<b>FLT3 status</b>				
Normal	110	70 (71.4)	40 (67.8)	0.91
ITD+	11	4 (4.1)	7 (11.9)	
ITD+ & NPM1+	3	1 (1.0)	2 (3.4)	
ITD+ & WT1+	8	7 (7.1)	1 (1.7)	
FLT3 ALM+	3	2 (2.0)	1 (1.7)	
Unknown	22	14 (14.3)	8 (13.6)	

TABLE 1 (Continued)

	Total	Refractory first relapse, N (%)	Second relapse, N (%)	p-value
Early or late relapse (year)				
<1	110	70 (71.4)	40 (67.8)	0.72
>1	47	19 (19.4)	12 (20.3)	
SCT				
Never	80	67 (68.4)	14 (23.7)	
CR1	17	13 (13.3)	4 (6.8)	
Following first relapse	48	17 (17.3)	30 (50.8)	
Following second relapse	2	—	2 (3.4)	
Multiple timepoints	10	1 (1.0)	9 (15.3)	
Third-line treatment				
Palliative	23	10 (10.2)	13 (22.0)	0.001
Chemo	56	44 (44.9)	14 (23.7)	
Chemo + SCT	27	17 (17.3)	8 (13.6)	
Immunotherapy/DLI	10	2 (2.0)	8 (13.6)	
Unknown	41	25 (25.5)	16 (27.1)	
Relapse site				
BM	134	90 (91.8)	44 (74.6)	—
CNS	1	1 (1.0)	0	
BM + CNS	4	1 (1.0)	3 (5.1)	
BM + Other	3	1 (1.0)	2 (3.4)	
Extramedullary	14	4 (4.1)	10 (16.9)	
Missing	1	1 (1.0)	0	
Early death				
No	134	90 (91.8)	44 (74.6)	0.005
Yes	23	8 (8.2)	15 (25.4)	
Status last follow-up				
Alive	29	21 (21.4)	8 (13.6)	0.29
Dead	128	77 (78.6)	51 (86.4)	

Abbreviations: AML, acute myeloid leukaemia; ALM, activation loop mutation; BM, bone marrow; Chemo, chemotherapy; CNS, central nervous system; CR1, first complete remission; DB-AML 01, Dutch–Belgian paediatric AML protocol; DLI, donor leukocyte infusions; FAB, French–American–British classification; ITD, internal tandem duplication; *KMT2A*, histone-lysine N-methyltransferase 2A; NOPHO, Nordic Society for Paediatric Haematology and Oncology; NPM1, nucleophosmin 1; SCT, stem cell transplantation; WBC, white blood cell count; WT1, Wilm's tumour gene.

<sup>a</sup>Chi-squared analysis was performed after grouping cytogenetic mutations based on prognosis.

TABLE 2 Cytogenetic alterations appearing in patient cohort categorized by prognostic value in accordance with current literature on the genetics of AML

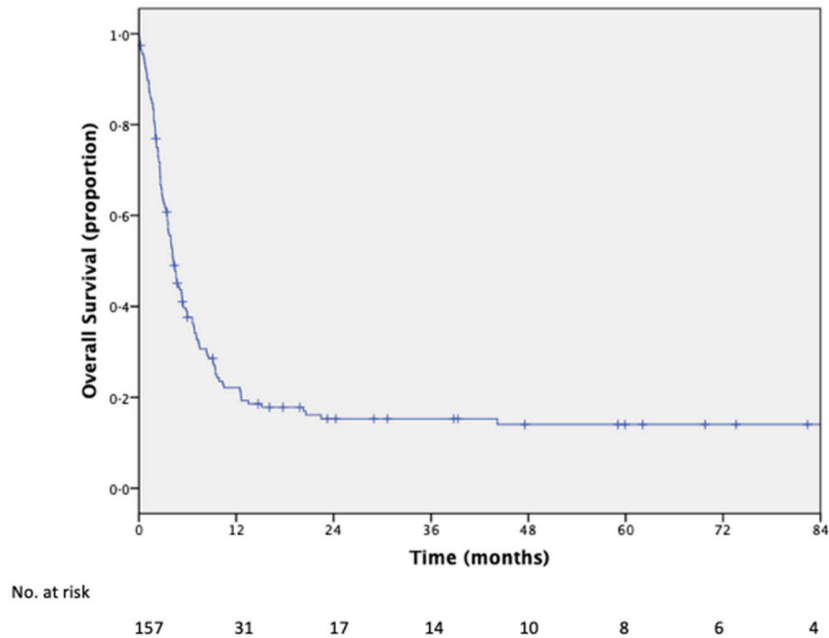
Poor prognosis	Intermediate prognosis	Good prognosis
<ul style="list-style-type: none"> <li>• Monosomy 5/del(5q)</li> <li>• Monosomy 7</li> <li>• t(4;11)(q21;q23)</li> <li>• t(6;11)(q27;q23)</li> <li>• t(10;11)(p11-p14;q23)</li> <li>• t(7;12)(q36;p13)</li> <li>• t(5;11)(q35;p15.5)</li> </ul>	<ul style="list-style-type: none"> <li>• t(9;11)(p21;q23)</li> <li>• t(11;19)(q23;p13.3)</li> <li>• All other <i>KMT2A</i> abnormalities without known poor prognosis</li> <li>• Complex karyotypes</li> <li>• Other abnormalities for which</li> <li>• Prognostic value is not known</li> </ul>	<ul style="list-style-type: none"> <li>• t(8;21)(q22;q22)</li> <li>• inv (16)(p13q22)</li> <li>• <i>NPM1</i> mutation (without <i>FLT3-ITD</i>)</li> <li>• <i>CEBPα</i></li> </ul>

Abbreviations: *CEBPα*, CCAAT/enhancer binding protein α; *KMT2A*, histone-lysine N-methyltransferase 2A; NPM1, nucleophosmin 1.

patients with a *FLT3-ITD* mutation with or without a *WT1* mutation survived to 1 year in contrast to a 1-year survival of  $26 \pm 4\%$  in those with either no or a relatively favourable mutation in *FLT3* ( $p = 0.023$ , Table 3, Figure 2A) (HR = 0.48 [0.27–0.86],  $p = 0.048$ , Table 4).

### Early versus late relapse

One-year survival was  $13 \pm 3\%$  for patients who had an early relapse and  $45 \pm 8\%$  for patients with a late relapse ( $p < 0.001$ , Table 3, Figure 2B). The HR for patients with a late relapse



**FIGURE 1** Overall survival of 157 patients with a refractory first relapse or second relapse from the NOPHO-DB-SHIP consortium. Median follow-up time for censored patients was 29 months (range: 0.2–119 months) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 3** Results from Kaplan Meier analysis: 1- and 5-year pOS for total cohort of AML patients suffering from either a refractory first relapse or a second relapse. All patient and disease characteristics were gathered at time of initial diagnosis with the exception of: timing of relapse, type of third-line treatment, relapse site, and occurrence of early death which were determined at or following relapse

	N	1-year OS, % ± SE (n)	5-year OS, % ± SE (n)	p-value
All patients	157	22 ± 3 (31)	14 ± 3 (8)	—
Treated with curative intent	93	27 ± 5 (23)	17 ± 4 (8)	—
Protocol				
NOPHO-AML 2004	76	17 ± 4 (13)	11 ± 4 (6)	0.40
NOPHO-AML 2012	60	29 ± 6 (13)	— (0)	
DB-AML 01	21	23 ± 9 (5)	19 ± 9 (2)	
Event				
Refractory first relapse	98	25 ± 5 (21)	15 ± 4 (6)	0.14
Second relapse	59	18 ± 5 (10)	12 ± 4 (2)	
Sex				
Male	81	23 ± 5 (17)	15 ± 5 (4)	0.75
Female	76	21 ± 5 (14)	13 ± 4 (5)	
Age (years)				
<2	38	21 ± 7 (5)	— (0)	0.17
2–9	56	25 ± 6 (14)	16 ± 5 (6)	
>10	63	21 ± 5 (12)	17 ± 5 (2)	
WBC at first diagnosis				
<100 × 10 <sup>9</sup> /L	126	22 ± 4 (26)	14 ± 4 (5)	0.50
>100 × 10 <sup>9</sup> /L	31	24 ± 8 (7)	20 ± 8 (4)	
Risk group				
Standard-risk	123	24 ± 4 (26)	15 ± 4 (5)	0.35
High-risk	32	16 ± 6 (5)	12 ± 6 (3)	

TABLE 3 (Continued)

	N	1-year OS, % ± SE (n)	5-year OS, % ± SE (n)	p-value
FAB type				
M0	10	20 ± 13 (2)	— (0)	0.87
M1	19	26 ± 10 (5)	26 ± 10 (3)	
M2	23	13 ± 7 (3)	13 ± 7 (1)	
M4	10	40 ± 16 (4)	27 ± 15 (1)	
M5	45	20 ± 7 (7)	17 ± 6 (2)	
M7	21	26 ± 10 (5)	0 (0)	
Other	10	20 ± 13 (2)	20 ± 13 (2)	
Unknown	19	18 ± 9 (3)	— (0)	
Cytogenetics				
Poor	26	22 ± 9 (5)	— (0)	0.64
Intermediate	112	22 ± 4 (22)	13 ± 4 (8)	
Good	16	25 ± 11 (4)	— (0)	
CBF-AML				
No	141	23 ± 4 (28)	14 ± 3 (8)	0.35
Yes	13	23 ± 12 (3)	— (0)	
FLT3 status				
Favourable <sup>a</sup>	116	26 ± 4 (26)	18 ± 4 (6)	0.02
ITD+/ITD+ & WT1+	19	0 (0)	0 (0)	
Unknown	22	23 ± 9 (5)	9 ± 6 (2)	
Early or late relapse (year)				
<1	110	13 ± 3 (12)	7 ± 3 (3)	<0.001
>1	47	45 ± 8 (19)	32 ± 7 (5)	
Third-line treatment				
Palliative	23	4 ± 4 (1)	0 (0)	<0.001
Chemo	56	7 ± 4 (3)	4 ± 3 (1)	
Chemo + SCT	27	59 ± 10 (16)	40 ± 10 (7)	
Immunotherapy/DLI	10	40 ± 16 (4)	— (0)	
Unknown	41	22 ± 7 (7)	— (0)	

Abbreviations: AML, acute myeloid leukaemia; ALM, activation loop mutation; Chemo, chemotherapy; DB-AML 01, Dutch–Belgian paediatric AML protocol; FAB, French-American-British classification; ITD, internal tandem duplication; n, number of patients within group; NOPHO, Nordic Society for Paediatric Haematology and Oncology; NPM1, nucleophosmin 1; SCT, stem cell transplantation; WBC, white blood cell count; *WT1*, Wilm's tumour gene.

<sup>a</sup>Favourable FLT3 status included patients with no FLT3 mutation, patients with FLT3-ALM and patients with FLT3-ITD in combination with a mutation in NPM1.

was 0.40 (0.26–0.62) relative to patients who relapsed within 1 year of diagnosis, indicating a significant survival advantage for patients who relapse after 1 year ( $p < 0.001$ , Table 4).

### Third line treatment

Only one patient receiving palliative care survived to 1 year (median survival = 2.6 months), while one-year overall pOS was 7 ± 4% for patients receiving chemotherapy alone (median survival = 4.0 months) and 59 ± 10% for those patients who were treated with chemotherapy and SCT (median survival = 13.5 months). Patients who received either immunotherapy or DLI had a one-year pOS of 40 ± 16% (median survival = 9.6 months) (Table S2) and those for whom no treatment information was available had a 1-year pOS of

22 ± 7% (median survival = 3.4 months) ( $p < 0.001$ , Table 3, Figure 2C). Multivariable Cox regression confirmed the finding that patients receiving palliative care had the worst outcomes and that patients who received SCT in addition to chemotherapy have the best outcomes (HR = 0.09 [0.04–0.17],  $p < 0.001$ , Table 4). The superior survival seen in patients receiving chemotherapy and SCT remained after repeating the analysis with only curatively treated patients. Three patients receiving only chemotherapy survived past 1 year and one of these patients survived past 5 years (Table S3).

### Stem cell transplantation

To examine the prognostic effect of prior SCT (SCT in CR1 for refractory first relapse and SCT in CR1 and/or CR2 for

**TABLE 4** Multivariable Cox regression analysis of factors influencing survival in overall cohort of patients with either a refractory first relapse or a second relapse ( $n = 157$ ). All patient and disease characteristics were gathered at time of initial diagnosis with the exception of: timing of relapse, type of third-line treatment, relapse site, and occurrence of early death which were determined at or following relapse

	Hazard ratio (95% CI)	<i>p</i> -value
<b>Protocol</b>		
AML2012	1	0.02
AML2004	1.79 (1.12, 2.86)	
DB-AML 01	2.19 (1.15, 4.16)	
<b>Age at initial diagnosis (years)</b>		
<2	1	0.28
2–10	0.75 (0.45, 1.24)	
>10	0.66 (0.39, 1.10)	
<b>Risk group</b>		
Standard risk	1	0.25
High risk	1.35 (0.81, 2.25)	
<b>WBC at diagnosis</b>		
<100 × 10 <sup>9</sup> /L	1	0.12
>100 × 10 <sup>9</sup> /L	0.67 (0.41, 1.11)	
<b>Cytogenetics at diagnosis</b>		
Poor prognosis	1	0.03
Intermediate prognosis	1.52 (0.88, 2.63)	
Good prognosis	0.90 (0.42, 1.94)	
Unknown	5.54 (1.48, 20.83)	
<b>FLT3 status</b>		
ITD/ ITD & WT1	1	0.048
Normal/FLT3-ALM/ITD & NPM1	0.48 (0.27, 0.86)	
Unknown	0.54 (0.27, 1.08)	
<b>Early versus late relapse</b>		
Early (<1 year)	1	<0.001
Late (>1 year)	0.40 (0.26, 0.62)	
<b>Third-line treatment</b>		
Palliative	1	<0.001
Chemotherapy	0.45 (0.26, 0.81)	
Chemotherapy + SCT	0.09 (0.04, 0.17)	
Immunotherapy/DLI	0.22 (0.09, 0.54)	
Unknown	0.58 (0.32, 1.06)	

A hazard ratio of 1 indicates that that particular factor was the reference category. Abbreviations: AML, acute myeloid leukaemia; ALM, activation loop mutation; Chemo, chemotherapy; DB-AML 01, Dutch–Belgian paediatric AML protocol; ITD, internal tandem duplication; NOPHO, Nordic Society for Paediatric Haematology and Oncology; NPM1, nucleophosmin 1; SCT, stem cell transplantation; WBC, white blood cell count; *WT1*, Wilm's tumour gene.

second relapse), patients with a refractory first relapse and second relapse were analysed separately using multivariable Cox regression. Due to the smaller number of events when analysing the groups separately, factors included in the multivariable models were limited to those which had

the greatest effects in the whole cohort. For both groups, the occurrence of one or more SCTs prior to relapse did not appear to be a significant predictor of survival (refractory first relapse: HR = 1.23 [0.53–2.89],  $p = 0.63$ , Table 5) (second relapse: SCT in CR1, HR = 2.01 [0.52–7.78]; SCT following first relapse, HR = 0.98 [0.29–3.26]; SCT in CR1 & following first relapse, HR = 0.88 [0.19–4.20];  $p = 0.64$ , Table 6).

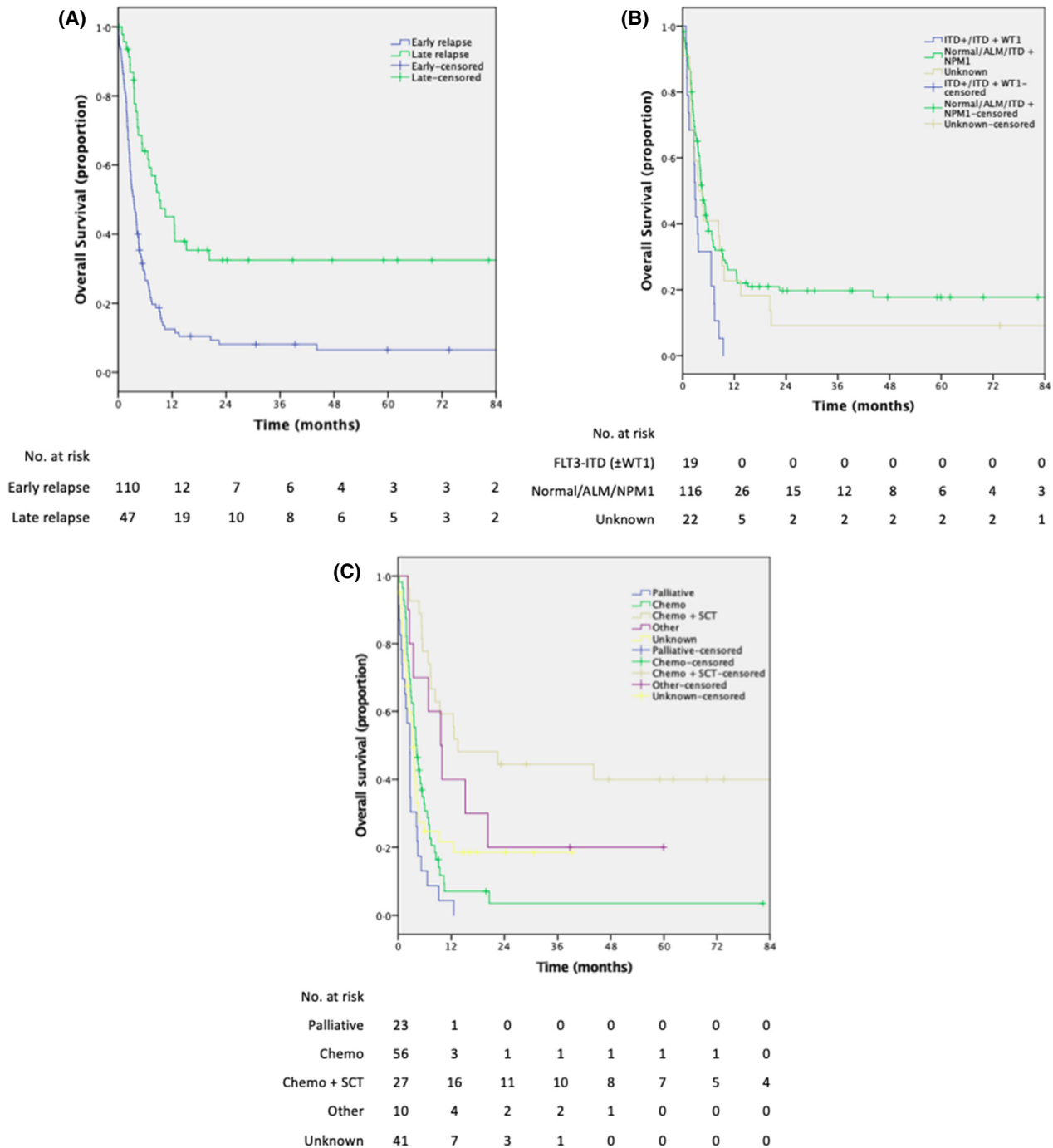
## DISCUSSION

The current study demonstrates that an estimated 14 ± 3% of the 157 patients suffering a refractory first relapse or second relapse survived past 5 years using current third-line treatment strategies. For patients treated curatively, these numbers were 27 ± 5% and 17 ± 4%, respectively. This is in line with recently published findings of Rasche *et al.*<sup>11</sup> who found that 5-year pOS was 15 ± 4% in their cohort of 73 patients with a second relapse. These findings are also in line with work of Zwaan *et al.* on the effectiveness of Mylotarg for treating refractory first or second relapsed paediatric AML. They reported that three of the 30 patients included in the trial survived with a median follow-up time of 3.5 years.<sup>18</sup> The three-year pOS of approximately 10% in this small cohort of patients is consistent with what was observed in our cohort of 157 patients whose estimated 3-year pOS was 15 ± 3%. In a similar study investigating the effectiveness of clofarabine as a replacement for fludarabine in the treatment of relapsed paediatric AML, van Eijkelenburg *et al.*<sup>19</sup> found a 2-year pOS of 32 ± 8% in their selected patient cohort. While this is higher than both the 1- and 3-year pOS observed in our patient cohort (22 ± 3% and 15 ± 3% respectively), the superior survival observed in their study may have resulted from the inclusion of patients with a first relapse who were not refractory, as well as from the selection of patients fit for further intensive therapy.

Of the patients for whom information on third-line treatment was available, 93/116 patients (80%) were treated with curative intent. The aforementioned study of Rasche *et al.*<sup>11</sup> had similar findings with 77% of their patient group receiving curative care following second relapse. This evidences a shifting perspective regarding the possibilities following a refractory first relapse or second relapse—namely that palliative care is no longer the only option for this group of patients. Our analysis supports this approach as evidenced by the 5-year pOS of 40% for the 27 patients (17%) who received chemotherapy and SCT as third-line therapy. Unfortunately, we had no information on third-line treatment response (achievement of CR), limiting conclusions that can be drawn about whether this superior survival arises from SCT itself or from the selection of patients responding well to re-induction chemotherapy, who also were fit enough to receive SCT. It is clear from this pOS that a select group of children suffering a refractory first relapse or second relapse may still be cured.

With this shift in perspective, it is essential to understand which patient and disease characteristics are predictive of prognosis. Similar to what has been identified for





**FIGURE 2** (A) Overall survival of patients with early ( $n = 110$ ) versus late ( $n = 47$ ) relapse.  $p < 0.001$  (relapse within 12 months of initial diagnosis for patients with a refractory first relapse and relapse within 12 months of diagnosis of first relapse for patients with a second relapse). (B) Overall survival of patients with FLT3-ITD mutations. Patients were grouped into those with FLT3-ITD alone or in combination with a WT1 mutation; patients with no mutation, an ALM mutation in FLT3 and those with a FLT3-ITD mutation and NPM1); and patients for which information on FLT3 status was unavailable.  $p = 0.023$ . (C) Overall survival of patients grouped based on the type of third-line treatment they received. For the purpose of analysis treatments were grouped into the categories: palliative, chemotherapy alone, chemotherapy in combination with SCT, immunotherapy/DLI and patients for which no treatment information was available.  $p < 0.001$ . ALM, activation loop mutation; Chemo, chemotherapy; ITD, internal tandem duplication; NPM1, nucleophosmin 1; SCT, stem cell transplantation; WT1, Wilm's tumour gene [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

first relapse<sup>5,7,8,20</sup> and what was recently identified by Rasche *et al.*<sup>11</sup> for second relapse, time to relapse and third-line treatment with chemotherapy and SCT were the most important predictors of favourable prognosis in our patient cohort. The latter finding must be interpreted with caution due to the bias

inherent in comparing patients who did and did not receive SCT. Namely, in order to be eligible for SCT these patients would have to be in good clinical condition and, in most cases, have a good early response to re-induction treatment and remain in remission until SCT. Unfortunately, as we did

**TABLE 5** Multivariable Cox regression analysis of factors influencing survival in patients with a refractory first relapse ( $n = 98$ )

	Hazard ratio (95% CI)	<i>p</i> -value
SCT in CR1		
Yes	1	0.63
No	1.23 (0.53, 2.89)	
Protocol		
NOPHO-AML 2012	1	0.19
NOPHO-AML 2004	1.76 (0.96, 3.22)	
DB-AML 01	1.53 (0.58, 4.00)	
Early <i>versus</i> late relapse		
Early (<1 year)	1	<0.001
Late (>1 year)	0.36 (0.20, 0.65)	
Third-line treatment		
Palliative	1	<0.001
Chemotherapy	0.57 (0.23, 1.41)	
Chemotherapy + SCT	0.10 (0.04, 0.30)	
Immunotherapy/DLI	0.89 (0.17, 4.68)	
Unknown	0.40 (0.16, 1.01)	

A hazard ratio of 1 indicates that that particular factor was the reference category. Abbreviations: AML, acute myeloid leukaemia; CI, confidence interval; CR1, first complete remission; SCT, stem cell transplantation.

**TABLE 6** Multivariable Cox regression analysis of factors influencing survival in patients with a second relapse ( $n = 59$ )

	Hazard ratio (95% CI)	<i>p</i> -value
SCT in CR1/CR2		
Never	1	0.64
CR1	2.01 (0.52, 7.78)	
Following first relapse	0.98 (0.29, 3.26)	
CR1 & following first relapse	0.88 (0.19, 4.20)	
Protocol		
NOPHO-AML 2012	1	0.63
NOPHO-AML 2004	1.12 (0.34, 3.74)	
DB-AML 01	1.73 (0.42, 7.04)	
Early <i>versus</i> late relapse		
Early (<1 year)	1	<0.001
Late (>1 year)	0.24 (0.11, 0.53)	
Third-line treatment		
Palliative	1	0.01
Chemotherapy	0.32 (0.12, 0.88)	
Chemotherapy + SCT	0.20 (0.06, 0.63)	
Immunotherapy/DLI	0.18 (0.06, 0.57)	
Unknown	0.72 (0.29, 1.81)	

A hazard ratio of 1 indicates that that particular factor was the reference category. Abbreviations: AML, acute myeloid leukaemia; CI, confidence interval; CR1, first complete remission; SCT, stem cell transplantation.

not have information on the SCT date in most patients, we could not correct for the immortal time bias in our analyses. In contrast to what is seen for a first relapse, SCT prior to

relapse did not appear to have a statistically significant effect on survival in our patient cohort. Given that SCT in CR1 is an important predictor of a poor prognosis following first relapse,<sup>5,8,20</sup> it is possible that the lack of observed effect was a result of the limited number of patients ( $n = 17$ ) in the cohort who received SCT in CR1. *FLT3* mutational status was also found to be a predictor of prognosis. As both *FLT3-ALM* and *FLT3-ITD* in combination with an *NPM1* mutation have been shown to have a similar prognosis to patients without a mutation in *FLT3*,<sup>17,21,22</sup> these three groups were grouped together for analysis. These patients had a superior prognosis compared to patients with a *FLT3-ITD* mutation either alone or in combination with a mutation in *WT1*. This supports what is seen at initial diagnosis<sup>17,21-23</sup> and after first relapse.<sup>10,23,24</sup> Finally, patients treated originally according to one of the two older protocols—NOPHO-AML 2004 or DB-AML 01—had inferior survival outcomes following a refractory first relapse or second relapse in comparison to patients originally treated according to NOPHO-AML 2012. We repeated this analysis, using date of diagnosis (before or after 2012) rather than protocol, with comparable results (Table S4). This suggests that improvements in supportive care, the availability of better salvage treatments and SCT regimens in recent years, rather than the original treatment protocols themselves, resulted in the superior survival.

In summary, the present analysis shows that five-year pOS following third-line treatment for a refractory first relapse or second relapse is currently approximately  $14 \pm 3\%$ . In selected patients who were transplanted following salvage therapy, 5-year pOS was 40%. This baseline pOS can be used for evaluating the effectiveness of emerging therapies for paediatric AML. Patient and disease characteristics associated with improved survival in this cohort included: original treatment according to NOPHO-AML 2012, favourable *FLT3* mutational status, late relapse (>12 months) and curative treatment following relapse. This information can be used to stratify patients to the best possible treatment strategy considering patient and disease characteristics of the individual patient. More research is needed to examine the observed effects in larger cohorts of patients for whom information on early treatment response and achievement of CR is available.

## ACKNOWLEDGEMENTS

The authors would like to thank the patients and their families for agreeing to the use of their data and making this research possible.

## CONFLICT OF INTEREST


The authors declare no competing financial interests.

## AUTHOR CONTRIBUTIONS

BG and GK designed the study and helped write the manuscript. TW wrote the manuscript. BG, GK, JA, NAC, JF, SH, HH, BM, MZ enrolled patients, collected patient data and helped write the manuscript. TW and DC conducted statistical analyses. All authors provided final approval of the manuscript.

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## SUPPORTING INFORMATION

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**How to cite this article:** White T, Kaspers G, Abrahamsson J, Arad-Cohen N, Cianci D, Fernandez J, Ha S-Y, et al. (2022). Clinical outcomes of second relapsed and refractory first relapsed paediatric AML: A retrospective study within the NOPHO-DB SHIP consortium. *Br J Haematol*. 2022;197:755–765. doi:[10.1111/bjh.18039](https://doi.org/10.1111/bjh.18039)