Received: 29 November 2022 | Accepted: 25 January 2023
DOI: 10.1111/bjh.18685

ORIGINAL PAPER

BJHaem BRITISH JOURNAL OF HAEMATOLOGY

Characteristics and outcome of primary resistant disease in paediatric acute myeloid leukaemia

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Summary
A significant proportion of events in paediatric acute myeloid leukaemia (AML) are caused by resistant disease (RD). We investigated clinical and biological characteristics in 66 patients with RD from 1013 children with AML registered and treated according to the NOPHO-AML 93, NOPHO-AML 2004, DB AML-01 and NOPHO-DBH AML 2012 protocols. Risk factors for RD were age10 years or older and a white-blood-cell count (WBC) of 100×10^9/L or more at diagnosis. The five-year overall survival (OS) was 38% (95% confidence interval [CI]: 28%–52%). Of the 63 children that received salvage therapy with chemotherapy, 59% (N = 37) achieved complete remission (CR) with OS 57% (95% CI: 42%–75%) compared to 12% (95% CI: 4%–35%) for children that did not achieve CR. Giving more than two salvage chemotherapy courses did not increase CR rates. OS for all 43 patients receiving allogeneic

Abbreviations: AIEOP, The Associazione Italiana di Ematologia e Oncologia Pediatrica; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; BFM, Berlin-Frankfurt-Münster; CBF, core binding factor; CI, confidence interval; CNS, central nervous system; CR, complete remission; DB, Dutch-Belgian; DFS, disease free survival; EFS, event free survival; FAB, French-American-British classification; FLT3-ITD, fms-like tyrosine kinase receptor-3 internal tandem duplication; GvHD, graft versus host disease; HLA, human leukocyte antigen; HSCT, haemopoietic stem cell transplant; KMT2A-r, KMT2A rearrangement; MFC, multiparameter flow cytometry; MRC, The medical research council; MRD, minimal residual disease; MSD, matched sibling donor; NOPHO, Nordic Society for Pediatric Hematology and Oncology; NPM1, nucleophosmin gene; OS, overall survival; RD, resistant disease; TBI, total body irradiation; TRM, treatment-related mortality; WBC, white blood cells count.

haematopoietic stem cell transplantation (HSCT) was 49% (95% CI: 36%–66%). Those achieving CR and proceeding to HSCT had an OS of 56% (95% CI: 41%–77%, $N = 30$). This study showed that almost 40% of children with primary resistant AML can be cured with salvage therapy followed by HSCT. Children that did not achieve CR after two salvage courses with chemotherapy did not benefit from additional chemotherapy.

KEYWORDS
acute myeloid leukaemia, paediatric, resistant disease, survival

INTRODUCTION

During the last decades there has been a significant difference between event-free survival (EFS) and overall survival (OS) in both NOPHO (Nordic Society for Paediatric Haematology and Oncology) and other international paediatric protocols for acute myeloid leukaemia (AML).[1,2] To a large extent this reflects that of the approximately 40% of patients who relapse after primary therapy: Around 40% become long-term survivors following relapse therapy.[3–8] However, a significant proportion of events (5%–10%) are caused by resistant disease (RD)[9,10] and little is known about the clinical characteristics and factors associated with outcome in this patient group. Studies are scarce and the interpretation of data difficult due to small patient numbers, differences in definition of patient cohorts as well as in definition of RD.

The most effective treatment known today for children with RD is salvage therapy with chemotherapy followed by allogeneic haematopoietic stem cell transplantation (HSCT). In a cohort of 48 children, Quarello et al. reported a disease-free survival (DFS) of 31% for children that underwent HSCT versus 5% for children that did not receive HSCT.[11] O'Hare et al. reported an OS of 43% in 23 children with RD[12] and found a blast count of more than 30% in bone marrow before HSCT to be prognostically adverse, whereas acute graft-versus-host disease (GVHD) was favourable. Okamoto et al. could also show that the presence of more than 25% of blasts in bone marrow before transplantation was a poor prognostic factor, as was blasts in peripheral blood before HSCT.[13] However, it is still unclear how much salvage therapy should be given in the effort to achieve complete remission (CR) before HSCT.

In this study, we retrospectively investigated clinical and biological characteristics and treatment outcome in 66 children with AML and RD who were registered and treated according to the NOPHO-AML 93, NOPHO-AML 2004, DB AML-01 and NOPHO-DBH AML 2012 protocols (EudraCT 2012–002934-35). The aim of the study was to further clarify prognostic factors and important treatment elements for children with AML and RD.

METHODS

The study included children and adolescents aged 0–19 years in Sweden, Denmark, Norway, Finland, Iceland, Hong Kong, Latvia, Estonia, the Netherlands and Belgium with de novo AML and RD registered and treated according to the NOPHO-AML 93, NOPHO-AML 2004, DB AML-01 and the ongoing NOPHO-DBH AML 2012 protocol. Patients with Down syndrome, acute promyelocytic leukaemia (APL) and secondary AML were excluded. Between January 1993 and Jan 2018, 66 of 1013 (6.5%) children with de novo AML were identified with RD in the NOPHO-AML registry, which, at the time countries participated in the respective protocols, included all children with AML in the countries. Patients and/or guardians consented to the study, which was performed in accordance with the Declaration of Helsinki. The study was approved by the national ethics committees.

Clinical data on treatment of RD were collected through the AML registry and a questionnaire to the treating clinics. The questionnaire included more detailed information than the registry regarding chemotherapy and haematopoietic allogeneic stem cell transplant including human leukocyte antigen (HLA) typing and matching, stem cell source, conditioning regimen and occurrence and severity of acute and chronic GVHD.

Primary induction treatment

In the NOPHO-AML 93 protocol, induction therapy for *de novo* AML consisted of ATEDox (cytarabine, etoposide, thioguanine, doxorubicin). Patients with good response (<5% leukaemic cells on morphological examination of bone marrow) 2 weeks after the end of course one received a second course of ATEDox following haematological recovery, whereas patients with poor response (≥5% leukaemic cells on morphological examination) immediately received AM (cytarabine, mitoxantrone).[14]

In the NOPHO-AML 2004 protocol idarubicin was given instead of doxorubicin in the first induction course, AIET (cytarabine, etoposide, 6-thioguanine, idarubicin). If the response on day 15 after the first induction course was poor (≥5% leukaemic cells on morphological examination of bone marrow) the patients immediately received the second induction course, AM. If the response was good, they received AM after haematological recovery.[15]

The Dutch–Belgian protocol (DB AML-01 study) had identical induction therapy as NOPHO-AML 2004 but did not use HSCT in consolidation of any patients.[16]

In the ongoing NOPHO-DBH AML 2012 protocol, induction therapy was intensified and included randomized comparisons both in the first and second induction course.

The first randomization evaluated mitoxantrone versus liposomal daunorubicin, and MEC (mitoxantrone, etoposide, cytarabine) versus DxEC (liposomal daunorubicin, etoposide, cytarabine). The second randomization compared ADxE (cytarabine, liposomal daunorubicin, etoposide) versus FLADx (fludarabine, cytarabine, daunoxome). Patients with poor response on day 22 (≥5% leukaemic cells by minimal residual disease flow cytometry (MRD-flow) or, if MRD-flow was non-informative, bone marrow morphology) immediately received the second course. In case of good response after the first induction course the second course was given after haematological recovery.

In all protocols, patients with 5% or more leukaemic cells after the second induction course, as measured with flow-MRD if performed or else with bone marrow morphology, were classified as having RD.

Treatment for resistant disease

In the NOPHO-AML 93 protocol, children with RD were recommended a course of HA_2E (high-dose cytarabine and etoposide). If remission was achieved, HSCT was only recommended if a HLA-identical sibling donor was available.

In the NOPHO-AML 2004 protocol, children with RD were recommended FLAG (fludarabine, cytarabine, granulocyte colony-stimulating factor (G-CSF)). New recommendations, based on the results of the AML 2001/01 trial, were introduced in November 2009, recommending FLAG and liposomal daunorubicin (FLADx) as the first course and FLAG as the second course.[4] G-CSF was over time gradually omitted from the courses. The aim was to proceed to HSCT with any available donor after one or two courses after achieving CR.[15]

In the NOPHO-DBH AML 2012 protocol for children not randomized to FLADx as second primary induction course, guidelines for RD were identical to those in NOPHO-AML 2004. Those patients that received FLADx as a second course, received more diverse salvage therapy. The guidelines recommended individualized therapy with some main alternatives: MACE (amsacrine, cytarabine, etoposide with or without gemtuzumab ozogamacin) or CloEC (clofarabine, etoposide, cyclophosphamide) or CLARA-X (clofarabine, cytarabine, liposomal daunorubicin).[17–19] The aim was to proceed to HSCT with any available donor after one or two courses after achieving CR.

Definitions

The definition of RD was no CR after two induction courses. CR was defined as less than 5% leukaemic cells on morphological examination of a non-hypoplastic bone marrow, no leukaemic cells in peripheral blood and no evidence of extramedullary disease in patients treated according to NOPHO-AML 2004, NOPHO-AML 93 studies and the DB AML-01 study. In patients treated on the NOPHO-DBH AML 2012 protocol, CR was defined as less than 5% leukaemic cells, assessed with multiparameter flow cytometry (MFC) if an informative leukaemia associated immunophenotype was available or otherwise by morphology in the bone marrow, in addition to the other requirements.

Statistical methods

Analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 27.0.1.0 except for calculation of confidence intervals for survival data which was performed with R, version 4.2.0.

Differences in proportions between children with or without RD were assessed with Fisher's exact test and Pearson's chi-squared test. Median values were compared using the Mann–Whitney *U* test. Probabilities of OS and DFS were estimated according to the Kaplan–Meier method and differences between factors tested with the log-rank test. The date of RD was set to the date of diagnosis of AML. Hence, survival was calculated from the date of diagnosis to death of any cause. All living patients were censored at time of last follow-up but not later than 30 September 2021. For DFS analyses, relapse, second malignancy, refractory RD (i.e., failing to achieve CR) or death of any cause were considered as events. Those who did not enter CR were assigned event at day 0.

All *p* values are two-sided and considered statistically significant when smaller than 0.05. Estimates of survival are given as percentage probability of five-year survival with 95% CI. Cox regression with remission status as time-dependent covariate was used to assess the effect of achieving remission on survival. For calculating risk factors for RD, binary logistic regression analysis was used.

RESULTS

Patient characteristics

During the study period, a total of 1013 children with de novo AML were treated according to the NOPHO-AML 93, NOPHO-AML 2004, DB AML-01 and NOPHO-DBH AML 2012 protocols. Twenty-seven children died before evaluation for RD. Sixty-six children experienced RD, with a median age at diagnosis of 11 years (range 0–17 years). The clinical and biological characteristics of the patients are summarized in Table 1 and compared with children without RD. There were no differences in central nervous system (CNS) disease at diagnosis among children with RD and children without RD. RD was more common in older patients (children ≥10 years, $p < 0.001$), and they had a significantly higher white-blood-cell count at diagnosis ($p < 0.001$). RD was less common in children with favourable cytogenetic aberrations, *RUNX1*::*RUNX1T*, ($p = 0.017$) and *CBFB*::*MYH11* ($p = 0.037$) as well as among patients with *KMT2A* rearrangements (*KMT2A*-r) where only one patient with RD had a *KMT2A*::*MLLT3* fusion ($p = 0.010$) and one a *KMT2A* rearrangement with unknown fusion

TABLE 1 Presenting clinical and biological characteristics in patients with acute myeloid leukaemia (AML) with ($N=66$) or without ($N=947$) RD registered and treated according to the NOPHO-AML 93, NOPHO-AML 2004, DB AML-01 and NOPHO-DBH AML 2012 protocols.

	RD	No RD	*p* value
Patients, *N*	66	947	
Gender			
Male	37 (56%)	488 (52%)	0.476
Female	29 (44%)	459 (48%)	
Protocol			
NOPHO-AML 93	29 (44%)	253 (27%%)	0.009
NOPHO-AML 2004	21 (32%)	318 (34%)	
DB AML-01	7 (11%)	107 (11%)	
NOPHO-DBH AML 2012	9 (14%)	269 (28%)	
Age			
<2 years	7 (11%)	241 (25%)	<0.001
2–9 years	21 (32%)	367 (39%)	
≥10 years	38 (58%)	339(36%)	
WBC at diagnosis			
$<100\times10^9$/L	45 (68%)	806 (85%)	<0.001
$\geq100\times10^9$/L	20 (30%)	140 (15%)	
No data	1 (2%)	1 (0.1%)	
FAB type			
M0	6 (9%)	46 (5%)	0.01
M1	17 (26%)	111 (12%)	
M2	15 (23%)	204 (22%)	
M4	11 (17%)	187 (20%)	
M5	8 (12%)	219 (23%)	
M6	0	16 (2%)	
M7	1 (2%)	72 (8%)	
No data	8 (12%)	92 (10%)	
Genetic subgroups			
RUNX1::*RUNX1T1*	2/65 (3%)	121/944 (13%)	0.020
CBFB::*MYH11*	1/65 (2%)	87/944 (9%)	0.037
KMT2A::*MLLT3*	1/65 (2%)	102/944(11%)	0.010
Other *KMT2A* rearrangement	1/65 (2%)	139/944 (15%)	0.001
FLT3-ITD	11/45 (24%)	84/793 (11%)	0.004
Without *NPM1* mutation	10/45 (22%)	66/793 (8%)	0.002
With *NPM1* mutation[b]	0/45	18/793 (2%)	0.618
NPM1	0/44	41/794 (5%)	0.161
Without *FLT3* ITD	0	23/794 (3%)	0.627
With *FLT3* ITD[a]	0	18/794 (2%)	0.618
Other	31/47 (66%)	317/850 (37%)	<0.001
CNS at diagnose			
Yes	3 (5%)	89 (9%)	
No	62 (94%)	838 (89%)	0.384
No data	1 (2%)	20 (2%)	

Abbreviations: AML, acute myeloid leukaemia; CNS, central nervous system; FAB, French–American–British classification; ITD, internal tandem duplication; OS, overall survival; RD, resistant disease; WBC, white-blood-cell count.

[a]One patient with *NPM1* had no data on *FLT3*-ITD status.

[b]One patient with *FLT3*-ITD had no data on *NPM1* status.

partner ($p=0.001$). AML harbouring *FLT3*-internal tandem duplications (ITD) were more common in children with RD ($p=0.004$). Of the 11 patients with *FLT3*-ITD, 10 were tested for associated *NPM1* mutation and all had *NPM1* wild type. The French–American–British (FAB) subgroup M1 was the only subgroup in which the frequency of RD was increased.

Response to salvage treatment

Sixty-three of 66 patients were treated with chemotherapy, two went directly to HSCT and one died before treatment (Figure 1). Thirty-seven of 66 children (56%) achieved CR while 28 (42%), including the two who went directly to HSCT, never reached CR. There were missing data on CR in one patient. Of the children receiving chemotherapy, 28/63 (44%) achieved CR after the first course of chemotherapy (four patients missing data after the first course and one missing data on CR overall). Table 2 shows the frequency and remission rate for the different salvage therapy regimes given as course 1. Of the 34/62 patients (excluding the one with no data on CR) that did not achieve CR after the first course, 25 patients received a second salvage course and an additional nine patients achieved CR. No child obtained CR after three or more courses. Of the four children with missing data on CR after the first chemotherapy course, two achieved CR and two did not.

Among the two patients that went directly to HSCT, one patient died and one survived.

There were no differences in sex, age group (<2, 2–9 and ≥10 years), FAB-type, WBC ($WBC<100\times10^9$/L and $\geq100\times10^9$/L) and genetic subgroups among children ultimately obtaining CR or not responding.

Haematopoietic stem cell transplant

A total of 43 (65%) patients were treated with HSCT. Two received HSCT without prior chemotherapy of whom one survived.

The median time from diagnosis to HSCT was four months (range 2–8 months). A median of two salvage courses (range 0–4) was given before HSCT.

Six patients (14%) received total body irradiation (TBI)-based conditioning regimes and 36 patients (84%) chemotherapy alone (one patient missing data). Fourteen patients had a matched sibling donor (MSD) (33%), 24 a matched unrelated donor (MUD) (56%), two patients a haploidentical donor (5%) and three patients other donors (7%). The stem cell source was bone marrow in 25 patients (58%), peripheral blood in 11 (26%) and cord blood in four patients (9%) (three patients missing data). Bone marrow morphology within 3 weeks before HSCT was assessed and evaluable in 31 of 43 patients (72%). Twenty-one (68%) patients were in CR, five patients had 5%–25% (16%) blasts and four patients (13%) more than 25% blasts (one non-evaluable). MRD by flow cytometry was assessed and evaluable in only 13 of 43 patients (30%). Two patients had MRD less than 0.1%. Of the 11

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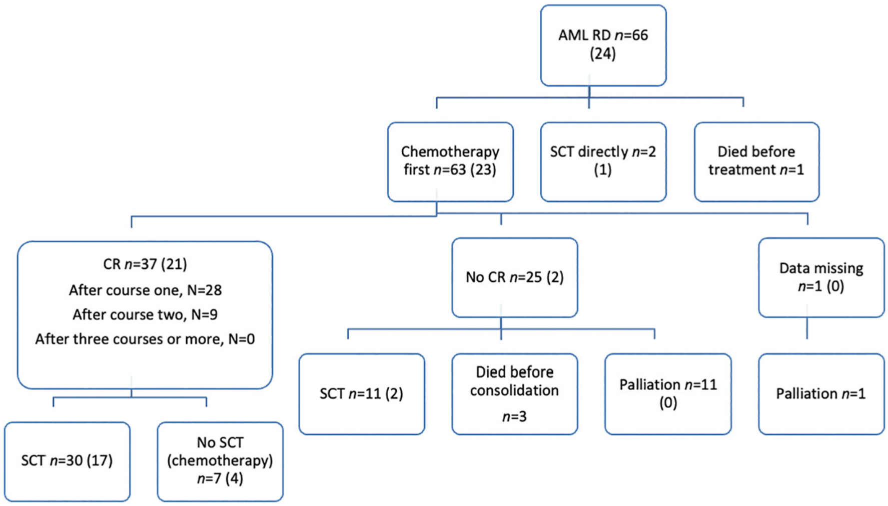

FIGURE 1 Flow diagram detailing the data on salvage therapy, remission status and consolidation therapy in 66 children with AML and RD. The number in parenthesis shows the number of children that survived in each group. AML, acute myeloid leukaemia; CR, complete remission; (H) SCT, (haematopoietic) stem cell transplantation; RD, resistant disease.

TABLE 2 Response rate in children with resistant AML after the first salvage course according to various regimens.

Remission achieved	FLAG/FLAG+ FLA/FLA+	CloEC	MACE	HA_2E	HA_1M	CLARA-DNX	Other	Total
Yes	18 (62%)	0	1 (33%)	6 (35%)	2 (33%)	1 (50%)	0	28 (44%)
No	9 (31%)	2 (100%)	1 (33%)	11 (65%)	3 (50%)	1 (50%)	3 (75%)	30 (48%)
Data missing	2 (7%)	0	1 (33%)	0	1 (17%)	0	1 (25%)	5 (8%)
Total	29	2	3	17	6	2	4	63

Note: The numerical subscript in courses denotes the dose in grams of each of six doses of cytarabine.
Abbreviations: AML, acute myeloid leukaemia; CLARA-DNX; clofarabine, cytarabine, liposomal daunorubicin; CloEC, clofarabine, etoposide, cyclophosphamide; FLA, fludarabine, cytarabine; FLAG, FLA with granulocyte colony-stimulating factor; FLA+, FLAG+, FLA and FLAG respectively with addition of idarubicin or liposomal daunorubicin; HA_2E, cytarabine, etoposide; HA_1M, cytarabine, mitoxantrone; MACE, amsacrine, cytarabine.

patients that had MRD 0.1% or more, six patients had MRD less than 5% and five patients had MRD 5% or more. Two of the five patients with MRD 5% or more survived. Four of the six patients with MRD 0.1%–4.9% survived.

Acute GVHD developed in 21 of 43 patients, (one patient missing data). Eleven patients had grade I, five grade II, three grade III and two grade IV acute GVHD. Chronic GVHD occurred in 10 of 43 (23%) patients, limited in eight patients and extensive in two (three patients missing data). Univariate analysis showed no significant difference in survival between children with acute GVHD and children without, OS 62% (95% CI: 44%–87%) versus 38% (95% CI: 22%–66%, $p = 0.272$).

When comparing donors, there was no significant difference in OS between MUD and MSD with OS 46% (95% CI: 29%–71%) and 43% (95% CI: 23%–79%) respectively ($p = 0.978$).

Overall outcome and survival

The estimated probability for overall five-year survival was 38% (95% CI; 28%–52%), (Figure 2). and the estimated probability for five-year DFS was 35% (95% CI: 25%–48%).

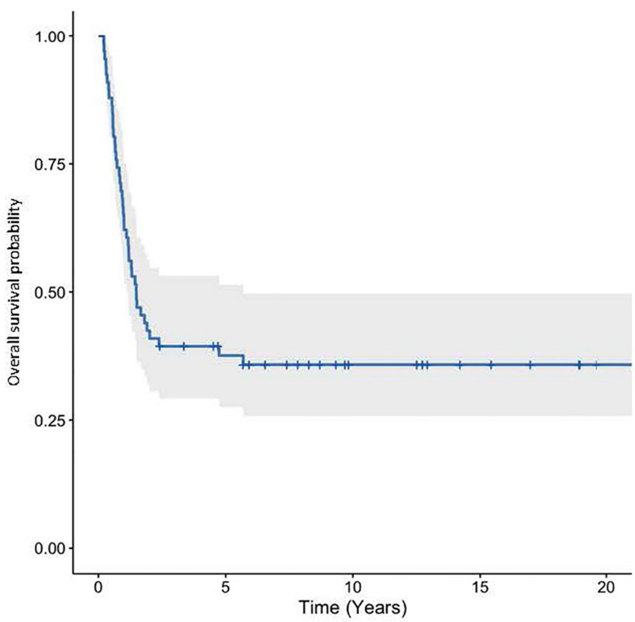

FIGURE 2 Probability of overall survival (OS) in all 66 patients with resistant acute myeloid leukaemia. OS at five years was 38% [95% confidence interval (CI): 28%–52%] and at 10 years 36% (95% CI: 26%–50%).

For the 24 patients that survived, the median follow-up time from diagnosis was 11.5 years (range 2–24 years). The median time to death for deceased patients was 12 months (range 2.5–68 months). Twenty-four of the 42 (57%) patients that did not survive died within a year and 38/42 (90%) within 2 years. The four patients dying after 2 years all experienced relapse.

Consolidation therapy and survival

The OSfor patients that reached CR was 57% (95% CI: 42%–75%, $N = 37$). In contrast, the children that did not reach CR had an OS of 14% (95% CI: 6%–35%, $N = 28$; Figure 3). Cox regression with remission status as time-dependent covariate showed that obtaining CR significantly reduced hazard rate for death (HR, 0.25; 95% CI: 0.13–0.48).

Of the 37 patients with CR, 30 patients proceeded to HSCT, with an OS of 56% (95% CI: 41%–77%). Thirteen died, of whom 10 experienced relapse and three patients died from treatment-related mortality (TRM).

Seven of the patients that entered CR received chemotherapy only as consolidation therapy. Six of these patients were treated according to the NOPHO-AML 93 protocol and one according to the NOPHO-AML 2004 protocol. In the NOPHO-AML 93 protocol only the patients in CR with an HLA-identical sibling were recommended HSCT. Of the patients treated with chemotherapy only, four survived, and two of these had core-binding factor (CBF) AML. The median follow-up time for these four patients was 13.5 years (range 7–19 years).

Of the 25 patients that did not achieve CR, 11 had HSCT of whom two survived with a follow-up exceeding 10 years. These survivors had 6% and 14% blasts respectively on bone marrow morphology before HSCT. Of the nine children with HSCT that died, eight experienced relapse and one died of TRM. None of the 14 non-CR patients without HSCT survived.

For the 43 patients that received HSCT, regardless of CR, OS was 49% (95% CI: 36%–66%) versus 22% (95% CI: 9%–53%; $p = 0.004$) for the 18 patients that did not receive HSCT.

Factors associated with risk for resistant disease

In univariate analyses age 10 years or older, WBC 100×10^9/L or higher at diagnosis, FAB-type M1 and the molecular aberration *FLT3*-ITD were significantly more common in children with RD (Table 1) whereas *RUNX1*::*RUNX1T*, *CBFB*::*MYH11*, *KMT2A*::*MLLT3* and other *KMT2A*-r were significantly less common. Since a significant number of cases had missing data on *FLT3*-ITD, we performed binary logistic regression on the 1007 patients with covariates age (over or under 10 years), WBC (more or less than 100×10^9/L), and presence of *RUNX1*::*RUNX1T*, *CBFB*::*MYH11* or *KMT2A*-r. Table 3 demonstrates that AML with CBF and *KMT2A*-r has a significantly lower risk for RD (odds ratio 0.10, 95% CI: 0.04–0.25) whereas age 10 years or older and WBC 100 or higher have higher risk with odds ratios of 2.0 (95% CI: 1.2–3.4) and 2.9 (95% CI: 1.6–5.1), respectively. We also analysed the 834 patients (43 with RD) with data also on *FLT3*-ITD included in the regression who failed to show

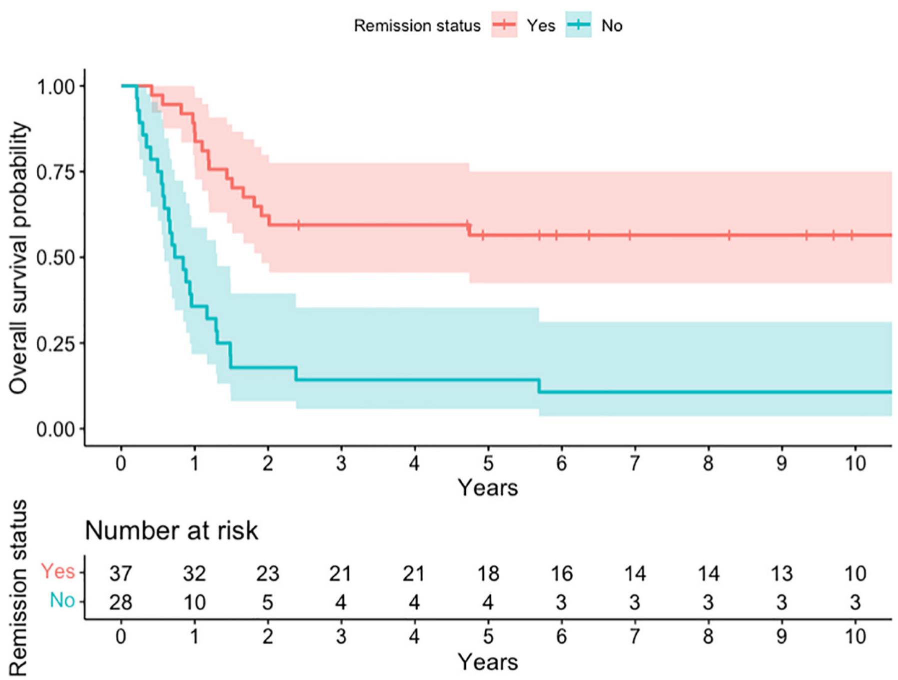

FIGURE 3 Probability of survival in patients who achieved complete remission (CR; $N = 37$) and those who did not achieve complete remission ($N = 28$) in children with resistant acute myeloid leukaemia. Patient with CR had an overall survival (OS) at five years of 57% [95% confidence interval (CI): 42%–75%] versus 14% (CI: 6%–35%).

TABLE 3 Binary logistic regression analyses of factors associated with resistant AML in children.

Variable	Hazard ratio 95% CI	*p* value
CBF or KMT2A	0.1 (0–0.3)	<0.001
WBC $\geq 100 \times 10^9$/L	2.7 (1.5–4.9)	0.001
Age $\geq$ 10 years	2.0 (1.2–3.3)	0.012
FAB M1	1.6 (0.9–3.1)	0.121

Abbreviations: 95% CI, 95% confidence interval; AML, acute myeloid leukaemia; CBF, core-binding factor; FAB, French–American–British classification; WBC, white-blood-cell count at diagnosis.

an independent effect of *FLT3*-ITD whereas the other factors retained their significance (data not shown).

DISCUSSION

We investigated a population-based cohort of 66 children with AML and primary RD treated according to the NOPHO-AML 93, NOPHO-AML 2004, DB AML-01 and NOPHO-DBH AML 2012 protocols.

In this population-based cohort 6.5% (66/1013) of the children had resistant AML. This is slightly lower compared to other studies and can at least partly be explained by differences in the definition of RD. Thus, the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP) AML 2002/01 study, defined RD as either more than 25% blasts at the end of the first induction course or more than 5% blasts at the end of the second induction course, and found a frequency of 10%.[11] The AML-Berlin–Frankfurt–Münster (BFM)-98 study and the AML-97 study from St Jude both employed the term non-responders without further specification, and reported rates of 8.5% and 7.5% respectively.[20,21] The Japanese AML-05 study demonstrated an RD frequency of 9.5% and is perhaps most comparable with our study since it used similar induction and had the same definition of RD.[10] The Medical Research Council (MRC) reported frequencies of 3% and 4% in the AML-10 and AML-12 studies but defined RD as more than 15% blasts in the bone marrow without further explanation, thus making comparisons with our study difficult.[22] Another factor that could potentially reduce the frequency of RD is that all protocols in our study used intensive timing of the second induction course in patients with a poor response to the first course.[14,15]

We found an OSof 38% which is higher than the AML-05 study and the AIEOP AML 2002/01 trial that reported three-year OS of 19% and 22% respectively.[10,11] In the MRC-AML-10 trial the five-year OS for children with RD from the start of course 2 was 23%, albeit using a different definition of RD.[22]

The Japanese Paediatric Leukaemia/Lymphoma Study Group found, in the AML-05 study, FAB M7 and *FLT3*-ITD to be more common in AML with RD (N = 43). In contrast, we found only one patient of 73 with FAB M7 and RD whereas 14% (17/124) with FAB M1 had RD. Very few of our patients with RD had CBF-AML or *KMT2A*-rearranged AML. Instead, the majority of cases had either *FLT3*-ITD of whom 11/45 patients had RD or were classified as having other genetic aberrations (31/47).

In univariate analyses, also age 10 years or above and WBC 100×10^9/L or higher were more common in children with resistant AML. To evaluate the independent effects of factors associated with RD, we performed a binary logistic regression. This confirmed that WBC 100×10^9/L or higher at diagnosis and age 10 years or above increased the risk of RD and even more pronounced that CBF-AML or *KMT2A*-rearranged AML was associated with very low risk of RD. Including the presence of *FLT3*-ITD in the regression analysis failed to show an independent effect of this aberration, while the other factors retained their significance. However, although there was a relation between *FLT3*-ITD and both age and WBC count, the power in this regression was low since only 838 patients (43 with RD) had data on *FLT3*-ITD. Furthermore, the missing data for *FLT3*-ITD were time-dependent in that almost all patients from 2007 onwards had data.

FLT3-ITD occurs in approximately 11% of children with *de novo* AML.[23,24] It is well known that children with *de novo* AML and *FLT3*-ITD have an inferior outcome.[23–25] Nonetheless, when comparing children with RD with and without *FLT3*-ITD, of whom none tested (10/11) had *NPM1* mutation, we found no difference in outcome. This was also observed by Quarello et al. in a study of 45 children with RD of whom 11 had *FLT3*-ITD.[11]

As of today, little is known about which salvage therapy is optimal for children with RD to achieve CR. The most common salvage therapy in this study was FLA-based courses with or without anthracyclines (FLA^+) This is consistent with the recommendation in the NOPHO-AML 2004 study to use FLA-based therapy and with the current NOPHO-DBH AML 2012 study that recommends FLA with anthracycline for all children that have not received this treatment already in the up-front second induction course. The second most common salvage therapy was HA_2E which was recommended in the NOPHO-AML 1993 protocol. There was no clear difference in CR rate in children that received any of these most common treatment regimens, but numbers are low.

The observed CR rate in our study of 59% is similar to those seen in the AIEOP AML 2002/01 and the AML-05 studies that reported a CR rate of 42% and 37% respectively.[10,11] Both in high-risk *de novo* AML and relapsed AML, CR before HSCT is one of the strongest prognostic factors for survival.[22,26,27] This is also true for children with resistant AML. In the present study, the five-year OS for children achieving CR was 56% compared with 14% in children who failed to reach CR. Cox regression with CR status as time-dependent covariate verified that achieving remission was strongly associated with increased survival. Historically, therapeutic options for patients with RD not responding to salvage therapy have been very limited. Therefore, many clinicians, in this clinical setting, have proceeded to HSCT despite a high disease burden.

Thirteen of our patients received HSCT even though they were not in remission. Three of these survived, all lacking any remarkable clinical features and with a follow-up exceeding 10 years. Similarly, in the Japanese AML-05 study, 22 patients went to HSCT without achieving CR of whom four survived.[10] In contrast, the AIEOP AML 2002/01 study had no survivors of the nine children that went to HSCT without remission.[11] Several studies in relapsed and refractory AML, not surprisingly, show that outcome after HSCT correlates with disease burden prior to transplant. Nonetheless, there is still no consensus of a defined cut-off level above which one should abstain from HSCT. Furthermore, since studies in general also show that some patients with high disease burden are cured and options to reduce disease burden in AML responding poorly to induction or reinduction therapy are few, clinicians often use HSCT as a last chance to cure the patient. This is perhaps even more pronounced in resistant AML not responding to salvage therapy. Importantly, we found that it appeared meaningless to give more than two chemotherapy courses when attempting to achieve CR. None of the children in our study reached CR only after three or more courses of chemotherapy ($N = 8$). Therefore, these patients, if they can tolerate further therapy, should be strongly considered for experimental studies or proceed to HSCT without being in CR. Today, many modern protocols for paediatric AML recommend a comprehensive geno-and phenotypic characterization already at diagnosis but if not performed earlier we strongly recommend such investigations in all patients with RD in search of targets for innovative treatment. Examples of current possible treatments are different small-molecule inhibitors of *FLT3*-ITD, menin or bcl-2, and immunotherapies as bridge therapy to HSCT [chimaeric antigen receptor (CAR) T cells and NK-CAR cells].[28]

Available data show that children with refractory AML, as well as children with relapsed AML, have little chance of cure without HSCT.[29] In our study, four of seven children who were treated with chemotherapy alone after obtaining CR survived. Six of these were treated in the AML-93 trial which only recommended HSCT with a MSD in this setting. All four children that survived had WBC less than 100×10^9 and no CNS disease. Survival in resistant AML with chemotherapy only is described in a few other studies. For example, in the AIEOP AML 2002/01 trial, one of 20 patients became a long-time survivor.[11] Probably, as in our study for the AML-93 patients, the presence of these patients in study cohorts more reflects that donor selection was more restricted in the past and that the diagnosis of RD was less certain since it relied only on bone marrow morphology in which immature cells may be interpreted as leukaemic cells. Therefore, no obvious criteria exist to select patients in whom HSCT might not be needed in RD.

In conclusion, 38% of children with RD can be cured with intensive reinduction therapy followed by HSCT. Only in rare instances can cure be achieved without HSCT and there is no established selection algorithm. Children that do not reach CR after two salvage courses with chemotherapy do not benefit from additional, conventional chemotherapy.

Although a significant proportion of children with RD responds to conventional therapy and can be cured, the overall poor outcome warrants that novel targeted or immune-directed therapies should be pursued in this patient group. Due to the small numbers, large intergroup studies with homogeneous definition of resistant AML and well-defined patient cohorts are necessary to evaluate novel treatment strategies and further define prognostic factors.

AUTHOR CONTRIBUTIONS

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ACKNOWLEDGEMENTS

The study was financed by grants from the Swedish Childrens´ Cancer foundation and the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALFGBG-966256).

FUNDING INFORMATION

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CONFLICT OF INTEREST STATEMENT

The authors report no potential conflict of interest.

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REFERENCES

1. Zwaan CM, Kolb EA, Reinhardt D, Abrahamsson J, Adachi S, Aplenc R, et al. Collaborative efforts driving progress in pediatric acute myeloid leukemia. J Clin Oncol. 2015;33(27):2949–62.

2. Rubnitz JE, Inaba H, Dahl G, Ribeiro RC, Bowman WP, Taub J, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. Lancet Oncol. 2010;11(6):543–52.
3. Abrahamsson J, Clausen N, Gustafsson G, Hovi L, Jonmundsson G, Zeller B, et al. Improved outcome after relapse in children with acute myeloid leukaemia. Br J Haematol. 2007;136(2):229–36.
4. Kaspers GJ, Zimmermann M, Reinhardt D, Gibson BE, Tamminga RY, Aleinikova O, et al. Improved outcome in pediatric relapsed acute myeloid leukemia: results of a randomized trial on liposomal daunorubicin by the international BFM study group. J Clin Oncol. 2013;31(5):599–607.
5. Nakayama H, Tabuchi K, Tawa A, Tsukimoto I, Tsuchida M, Morimoto A, et al. Outcome of children with relapsed acute myeloid leukemia following initial therapy under the AML99 protocol. Int J Hematol. 2014;100(2):171–9.
6. Rubnitz JE, Razzouk BI, Lensing S, Pounds S, Pui CH, Ribeiro RC. Prognostic factors and outcome of recurrence in childhood acute myeloid leukemia. Cancer. 2007;109(1):157–63.
7. Karlsson L, Forestier E, Hasle H, Jahnukainen K, Jonsson OG, Lausen B, et al. Outcome after intensive reinduction therapy and allogeneic stem cell transplant in paediatric relapsed acute myeloid leukaemia. Br J Haematol. 2017;178(4):592–602.
8. Hoffman AE, Schoonmade LJ, Kaspers GJ. Pediatric relapsed acute myeloid leukemia: a systematic review. Expert Rev Anticancer Ther. 2021;21(1):45–52.
9. Pession A, Masetti R, Rizzari C, Putti MC, Casale F, Fagioli F, et al. Results of the AIEOP AML 2002/01 multicenter prospective trial for the treatment of children with acute myeloid leukemia. Blood. 2013;122(2):170–8.
10. Miyamura T, Moritake H, Nakayama H, Tanaka S, Tomizawa D, Shiba N, et al. Clinical and biological features of paediatric acute myeloid leukaemia (AML) with primary induction failure in the Japanese Paediatric Leukaemia/Lymphoma Study Group AML-05 study. Br J Haematol. 2019;185(2):284–8.
11. Quarello P, Fagioli F, Basso G, Putti MC, Berger M, Luciani M, et al. Outcome of children with acute myeloid leukaemia (AML) experiencing primary induction failure in the AIEOP AML 2002/01 clinical trial. Br J Haematol. 2015;171(4):566–73.
12. O'Hare P, Lucchini G, Cummins M, Veys P, Potter M, Lawson S, et al. Allogeneic stem cell transplantation for refractory acute myeloid leukemia in pediatric patients: the UK experience. Bone Marrow Transplant. 2017;52(6):825–31.
13. Okamoto Y, Kudo K, Tabuchi K, Tomizawa D, Taga T, Goto H, et al. Hematopoietic stem-cell transplantation in children with refractory acute myeloid leukemia. Bone Marrow Transplant. 2019;54:1489–98.
14. Abrahamsson J, Forestier E, Heldrup J, Jahnukainen K, Jonsson OG, Lausen B, et al. Response-guided induction therapy in pediatric acute myeloid leukemia with excellent remission rate. J Clin Oncol. 2011;29(3):310–5.
15. Wareham NE, Heilmann C, Abrahamsson J, Forestier E, Gustafsson B, Ha SY, et al. Outcome of poor response paediatric AML using early SCT. Eur J Haematol. 2013;90(3):187–94.
16. Moerloose B, Reedijk A, Bock GH, Lammens T, Haas V, Denys B, et al. Response-guided chemotherapy for pediatric acute myeloid leukemia without hematopoietic stem cell transplantation in first complete remission: results from protocol DB AML-01. Pediatr Blood Cancer. 2019;66(5):e27605.
17. van Eijkelenburg NKA, Rasche M, Ghazaly E, Dworzak MN, Klingebiel T, Rossig C, et al. Clofarabine, high-dose cytarabine and liposomal daunorubicin in pediatric relapsed/refractory acute myeloid leukemia: a phase IB study. Haematologica. 2018;103(9):1484–92.
18. Messinger Y, Boklan J, Goldberg J, DuBois SG, Oesterheld J, Abla O, et al. Combination of clofarabine, cyclophosphamide, and etoposide for relapsed or refractory childhood and adolescent acute myeloid leukemia. Pediatr Hematol Oncol. 2017;34(4):187–98.
19. Burnett AK, Russell NH, Hills RK, Knapper S, Freeman S, Huntly B, et al. Defining the optimal Total number of chemotherapy courses in younger patients with acute myeloid leukemia: a comparison of three versus four courses. J Clin Oncol. 2021;39(8):890–901.
20. Creutzig U, Zimmermann M, Lehrnbecher T, Graf N, Hermann J, Niemeyer CM, et al. Less toxicity by optimizing chemotherapy, but not by addition of granulocyte Colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. J Clin Oncol. 2006;24(27):4499–506.
21. Ribeiro RC, Razzouk BI, Pounds S, Hijiya N, Pui CH, Rubnitz JE. Successive clinical trials for childhood acute myeloid leukemia at St Jude Children's research hospital, from 1980 to 2000. Leukemia. 2005;19(12):2125–9.
22. Gibson BE, Wheatley K, Hann IM, Stevens RF, Webb D, Hills RK, et al. Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. Leukemia. 2005;19(12):2130–8.
23. Meshinchi S, Stirewalt DL, Alonzo TA, Boggon TJ, Gerbing RB, Rocnik JL, et al. Structural and numerical variation of FLT3/ITD in pediatric AML. Blood. 2008;111(10):4930–3.
24. Zwaan CM, Meshinchi S, Radich JP, Veerman AJ, Huismans DR, Munske L, et al. FLT3 internal tandem duplication in 234 children with acute myeloid leukemia: prognostic significance and relation to cellular drug resistance. Blood. 2003;102(7):2387–94.
25. Brown P, Meshinchi S, Levis M, Alonzo TA, Gerbing R, Lange B, et al. Pediatric AML primary samples with FLT3/ITD mutations are preferentially killed by FLT3 inhibition. Blood. 2004;104(6):1841–9.
26. Tierens A, Bjorklund E, Siitonen S, Marquart HV, Wulff-Juergensen G, Pelliniemi TT, et al. Residual disease detected by flow cytometry is an independent predictor of survival in childhood acute myeloid leukaemia; results of the NOPHO-AML 2004 study. Br J Haematol. 2016;174(4):600–9.
27. Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, et al. Treatment stratification based on initial in vivo response in acute myeloid leukaemia in children without Down's syndrome: results of NOPHO-AML trials. Br J Haematol. 2003;122(2):217–25.
28. Zarnegar-Lumley S, Caldwell KJ, Rubnitz JE. Relapsed acute myeloid leukemia in children and adolescents: current treatment options and future strategies. Leukemia. 2022;36(8):1951–60.
29. Davila J, Slotkin E, Renaud T. Relapsed and refractory pediatric acute myeloid leukemia: current and emerging treatments. Paediatr Drugs. 2014;16(2):151–68.

How to cite this article: Karlsson L, Cheuk D, De Moerloose B, Hasle H, Jahnukainen K, Juul-Dam KL, et al. Characteristics and outcome of primary resistant disease in paediatric acute myeloid leukaemia. Br J Haematol. 2023;201(4):757–765. https://doi.org/10.1111/bjh.18685