Etoposide Combined with FLAG Salvage Therapy Is Effective in Multiple Relapsed/Refractory Acute Myeloid leukemia

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Keywords

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Abstract

Managing acute myeloid leukemia (AML) is often hampered by repeated failure to achieve complete remission as well as recurrent relapse that causes an emergent need for alternative salvage therapies. The efficacy of most salvage therapies is based on anthracycline combinations. In highly pretreated patients who are not eligible for anthracycline-based protocols therapeutic alternatives are limited. For this particular group we evaluated the efficacy and safety of fludarabine, cytarabine, granulocyte colony-stimulating factor (FLAG) in combination with etoposide (FLAG-Eto) in 36 patients. The complete remission rate (CR) was 25.7% with a median overall survival of 6 months (95% CI 4.5–7.7). The median disease-free survival for CR/CRi/MLFS (CR/CR with incomplete hematological recovery/morphologic leukemia-free state) patients was 8 months (95% CI 0.6–15.5). The mortality rate on day 30 was 8% and increased on day 60 to 17%. Our results show meaningful anti-leukemic activity of the FLAG-Eto reg-

imen with a moderate toxicity profile in heavily pretreated relapsed/refractory AML patients enabling consolidating allogeneic stem cell transplantation.

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Introduction

Over decades no significant increase in the therapeutic response in acute myeloid leukemia (AML) could be documented [1], while improvement in outcome, in particular for younger patients, is more likely due to improved supportive care. Only recently, a number of targeted compounds have shown significant improvement in response rates with meaningful, but not substantial improvement in long-term outcome in AML [2]. Relapse or refractory disease is still a frequently occurring issue in AML. For relapsed/refractory AML patients the current curative treatment approach usually includes anthracycline-based re-induction chemotherapy to achieve deep remission followed by consolidating allogeneic stem cell transplantation. However, even with intensive salvage treatment protocols only half of the patients achieve re-

mission with a median disease-free survival of only 7–17 months [3–5]. Therefore, salvage therapies are required in managing AML patients. Multiple relapsed/refractory AML patients, however, frequently have reached the maximum cumulative anthracycline doses and often show a compromised performance status which again hampers following consolidation therapies. Hence, well-tolerable salvage therapies considering the requirements of heavily pretreated AML patients are urgently needed. Many salvage treatment protocols are based on the combination of fludarabine, cytarabine, and granulocyte stimulating factors, abbreviated to FLAG [6–8]. Recently, we have demonstrated the efficacy and safety of the FLAG protocol combined with idarubicin in a large patient cohort of primary refractory and first-time relapsed AML patients reaching overall complete remission rates (CR + CR with incomplete hematological recovery [CRi]) of 56% with a median overall survival of 15 months (95% CI 5.7–25.1) [9]. For heavily pretreated patients who are not eligible for further anthracycline administration we now introduce another FLAG-based salvage therapy protocol combined with the topoisomerase II-inhibitor etoposide (FLAG-Eto). In this study, we analyzed the efficacy and toxicity profile of the FLAG-Eto regimen in this special patient population. This study finally paves the way for chemotherapy-based salvage treatment protocols tailored to the needs of different subsets of AML patients.

Patients and Methods

Patients

Thirty-six patients with relapsed/refractory AML had been treated according to the FLAG-Eto protocol between 1996 and 2016 and were retrospectively analyzed. Patients with AML M3 were not included. Cytogenetic risk groups were defined according to the Medical Research Council (MRC) criteria of Grimwade et al. [10], while complex aberrant karyotypes were defined as ≥ 3 aberrations [11–14]. Preceding hematological disorders diagnosed at least 6 months before the diagnosis of AML were marked as secondary AML.

Treatment Protocols

The FLAG-Eto protocol consisted of fludarabine (30 mg/m²/day) and cytarabine (2 g/m²/day) for 5 days, during which etoposide (150 mg/m²/day) was administered on 3 days; granulocyte colony-stimulating factor (G-CSF) was given one day prior to chemotherapy for 6 days in cases where leukocytes were below 20/nL.

Safety and Response Evaluation

Safety was retrospectively evaluated according to the established Common Terminology Criteria for Adverse Events (CTCAE, version 4.0) [15]. Values of creatinine and liver enzymes were considered until day 28 after the last chemotherapy application.

For patients with elevated liver enzymes before starting therapy, only further elevations up to the next CTCAE grade were taken into account. Analysis of hematological recovery was only performed in patients achieving partial remission or better. Duration of neutropenia was defined as the latency to neutrophil recovery ≥ 0.5 /nL. The duration of hospitalization was calculated from the first day of therapy until discharge or in cases of continuous hospitalization until initiation of a new treatment, e.g., allogeneic transplantation. Response to treatment was assessed according to established remission criteria [12, 16]. Two patients fulfilling all CR criteria but whose hematopoietic regeneration could not be retrospectively evaluated due to unavailability of related peripheral blood counts were therefore declared as CRi/MLFS (morphologic leukemia-free state). Of note, these 2 patients showed long-term survival over 200 months. Blast reduction was defined as at least 50% reduction of leukemic burden with maximum 5–25% residual bone marrow blasts without regeneration of neutrophils (<1 /nL) and/or platelets (<100 /nL).

Statistical Analyses

Statistical analyses were performed by IBM SPSS Statistics Software version 22.0 (SPSS, Chicago, IL, USA). Overall survival was defined as duration from the first day of salvage therapy until death or alive at last follow-up (censored). Disease-free survival was only measured for CR/CRi/MLFS patients and was defined as duration from first day of documented remission after re-induction therapy until relapse, death (censored), or alive without relapse at last follow-up (censored). Overall survival and disease-free survival were estimated by Kaplan-Meier method. The log-rank test or log-rank test for trend was used to compare survival distributions in subgroups. Distributions of patient characteristics and response rates in subgroups were compared in the case of categorical variables by χ^2 test, or if indicated by Fisher's exact test or linear-by-linear association. In the case of continuous variables and medians of ordinal variables the *t* test or Mann-Whitney U test were used. All *p* values were 2-sided and were considered significant at a level <0.05 .

Results

Patient Characteristics

In total, 36 patients received the FLAG-Eto protocol, of whom at least 91.7% completed the treatment. Dose modifications of at least 20% of one substance occurred in 25% of FLAG-Eto-treated patients. Table 1 presents patients' demographic and baseline characteristics. The median age was 57 years (range 28–71) with approximately 30% of patients over 60 years of age. All patients received intensive induction chemotherapy at initial diagnosis of AML. Median duration of last remission was 10.7 months (range 1.5–125.9 months). Early relapse within the first 12 months occurred in 64% ($n = 14/22$) of patients; 16.7% of treated AML patients were primary refractory while almost all showed minor or no response to the first or second induction therapy. Strikingly, 36%

Table 1. Patient characteristics ($n = 36$)

	FLAG-Eto		FLAG-Eto
Age at relapse, years		Duration of last CR/CRI	
Mean	53±11	≤12 months	14/22 (63.6)
Median	57 (28–71)	>12 months	8/22 (36.4)
Age at relapse		Median prior relapse therapies ^a , <i>n</i>	1 (0–2)
≤60 years	25 (69.4)	Prior relapse therapies ^a	
>60 years	11 (30.6)	0	13 (36.1)
Gender		1	16 (44.4)
Male	18 (50)	2	7 (19.4)
Female	18 (50)	Previous allo-HSCT	
Subtype		Yes	16 (44.4)
De novo	29 (80.6)	No	20 (55.6)
Secondary	7 (19.4)	FLAG-Ida prior to FLAG-Eto	
Previous BMD	1 (2.8)	Yes	11 (30.6)
Therapy-related	6 (16.7)	No	25 (69.4)
Cytogenetic risk group at diagnosis			
Favorable	4 (11.1)	Data are presented as <i>n</i> (%), mean ± SD, or median (range), as appropriate. allo-HSCT, allogeneic hematopoietic stem cell transplantation; BM, bone marrow; BMD, bone marrow disease; CCI, Charlson Comorbidity Index; CR, complete remission; CRI, CR with incomplete hematologic recovery; ECOG, Eastern Cooperative Oncology Group; Hb, hemoglobin; LDH, lactate dehydrogenase; PIF, primary induction failure; Plt, platelets; PS, performance status; SD, standard deviation; WBC, white blood cell count. ^a prior to 1st cycle of FLAG-Eto. ^b duration of last CR/CRI prior to FLAG-Eto, only measured in patients who achieved a CR/CRI prior to FLAG-Eto.	
Intermediate	23 (63.9)		
Unfavorable	7 (19.4)		
Not available	2 (5.6)		
NPM1 mutation	9 (25.0)		
Missing	18 (50.0)		
FLT3-ITD mutation	5 (13.9)		
Missing	18 (50.0)		
CEBPA mutation	1 (2.8)		
Missing	31 (86.1)		
Blood counts at relapse			
WBC, 1/nL	4.30 (0.38–94.13)		
Missing	5 (13.9)		
Plt, 1/nL	39.0 (1.0–427.0)		
Missing	5 (13.9)		
Hb, g/dL	10.10 (7.40–15.80)		
Missing	5 (13.9)		
LDH, U/L	371 (92–4,562)		
Missing	5 (13.9)		
Blasts in BM at relapse, %	45 (10–91)		
Missing	15 (41.7)		
Median ECOG PS at relapse	1 (0–4)		
ECOG PS at relapse			
≤2	21 (58.3)		
>2	4 (11.1)		
Not available	11 (30.6)		
Median CCI at relapse	0 (0–5)		
CCI at relapse			
0	27 (75.0)		
1	3 (8.3)		
2	4 (11.1)		
3	0 (0.0)		
4	1 (2.8)		
5	1 (2.8)		
Disease status ^a			
PIF	6 (16.7)		
1st relapse	17 (47.2)		
≥2nd relapse	13 (36.1)		
Median duration of last CR/CRI ^b , months	10.74 (1.48–125.93)		

(*n* = 13) of patients treated according to the FLAG-Eto protocol experienced at least one prior relapse and correspondingly the median number of prior salvage therapies was 1 (range 0–2). These salvage therapies comprised intensive chemotherapy protocols; 4 patients received hypomethylating agents. Further, 44% (*n* = 16) of FLAG-Eto-treated patients previously underwent allogeneic stem cell transplantation. In line with the number of preceding relapses and intensive treatments, patients receiving FLAG-Eto showed an impaired performance status with 11.1% (*n* = 4) of patients having an ECOG status of ≥3.

Flag-Eto Shows Anti-Leukemic Activity in Heavily Pretreated Relapsed/Refractory AML Patients

Nine patients (25.7%) receiving the FLAG-Eto protocol achieved CR, 5.7% CRI (*n* = 2), 2.9% (*n* = 1) MLFS, 5.7% (*n* = 2) CRI/MLFS, and 5.7% (*n* = 2) partial remission (Fig. 1a). This resulted in a 1-year disease-free survival of 40% (95% CI 9.6–70.3) with a median disease-free survival of 8 months (95% CI 0.6–15.5) for CR/CRI/MLFS.

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Fig. 1. Response rates (a), and Kaplan-Meier curves for disease-free survival (b) and overall survival (c) for FLAG-Eto-treated patients. OS, overall survival; DFS, disease-free survival; CR, complete remission; CRi, CR with incomplete hematological recovery; MLFS, morphologic leukemia-free state; PR, partial remission; RD, refractory disease.

Fig. 2. Kaplan-Meier curves for overall survival subdivided by remission state. CR, complete remission; CRi, CR with incomplete hematological recovery; MLFS, morphologic leukemia-free state; PR, partial remission; BLR, blast reduction; TF, treatment failure.

patients (Fig. 1b). The median overall survival for all treated patients was 6 months (95% CI 4.5–7.7) with an overall survival rate at 1 year of 33% (95% CI 17.6–49.4) considering a median follow-up time of 38 months (range

4–246; Fig. 1c). As expected, overall survival depended on remission status with a median of 17.2 months (95% CI 3–25.5) for patients achieving CR (Fig. 2; online suppl. Tables S1–3; for all online suppl. material, see www.karger.com/doi/10.1159/000503056).

The response to FLAG-Eto treatment did not depend on age; patients over 60 years of age showed CR/CRi/MLFS rates of 36% ($n = 4/11$) compared to 42% ($n = 10/24$) in patients ≤ 60 years with a comparable median overall survival for both groups (6.2 vs. 6.1 months for patients >60 vs. ≤ 60 years; online suppl. Tables S1, S2). It remains elusive to what extent the number of prior salvage therapies influenced the response to treatment. Of note, 44.4% of FLAG-Eto-treated patients received 1 prior salvage treatment and 19.4% received 2. While 61.5% ($n = 8/13$) of patients who received FLAG-Eto as a first salvage treatment achieved CR/CRi/MLFS, this rate decreased to 40% ($n = 6/15$) among patients who received FLAG-Eto as a second salvage regimen, and finally none of the 7 patients with 2 prior salvage therapies achieved CR/CRi/MLFS ($p = 0.01$; online suppl. Table S1). However, in this limited cohort size disease-free and overall survival did not differ significantly in dependence of the number of prior salvage therapies (online suppl. Fig. S1). Focusing on patients who relapsed after preceding allogeneic stem cell transplantation, 25% ($n = 4/16$) of patients treated with the FLAG-Eto protocol still achieved CR/CRi/MLFS (online suppl. Table S1) with a median overall survival for CR/CRi/MLFS patients of 5 months (95% CI 0–46.8) compared to 14 months (95% CI 5.1–23.4) in not previously transplanted patients. In conclusion, FLAG-

Table 2. Documented infections in FLAG-Eto-treated patients ($n = 36$)

	n (%)	n/a
Infection	34 (94.4)	0
FUO ^a	2 (6.1)	3
Diarrhea ^a	4 (11.1)	0
Mucositis ^a	6 (16.7)	0
Pneumonia		
Grade 4	2 (5.6)	0
Grade 5	2 (5.6)	
Sepsis		
Grade 4	2 (5.6)	0
Grade 5	3 (8.3)	

CTC, common toxicity criteria; FUO, fever of unknown origin; n/a, not available. ^a Any CTC grade.

Eto showed significant anti-leukemic activity which did not depend on age but correlated with the number of preceding salvage therapies.

Toxicity Profile of FLAG-Eto Salvage Therapy

Nearly all patients experienced grade 3–4 myelosuppression with a median duration until neutrophil recovery (≥ 0.5 nL) of 24.5 days (range 19–30). Patients treated with FLAG-Eto received a median of 8 (range 0–20) red blood cell units and 9 (range 3–33) platelet units; 5.7% ($n = 2$) suffered from grade 3 bleeding complications and almost all patients experienced infections of any severity according to CTCAE (Table 2). Four FLAG-Eto-treated patients (11.1%) got grade 4/5 pneumonia and 13.8% ($n = 5$) had sepsis, of whom 8.3% ($n = 3$) died (Table 2). Considering other organ functions, only few cases with higher-grade toxicities were observed (Table 3). The median duration of hospitalization reached 38.5 days (range 27–90). The 30-day mortality was 8.3% ($n = 3$) and increased to 16.7% ($n = 6$) at day 60. Cause of death during the first 30 days after treatment initiation was sepsis (66%, $n = 2$) and resistant disease with meningeal infiltration (33%, $n = 1$). Within the following 30 days, 2 further patients succumbed to infectious complications and 1 patient had relapse. Importantly, there were no significant differences in higher-grade toxicity in dependence of age or number of prior salvage therapies.

Post-Remission Treatment

We have recently confirmed allogeneic stem cell transplantation as the consolidation treatment of choice for first-time relapsed/refractory AML patients to achieve

Table 3. Nonhematological grades 3 and 4 toxicities according to the common toxicity criteria

	FLAG-Eto ($n = 36$)		
	grade 3	grade 4	assessed
Nephro- and hepatotoxicity			
Creatinine	2 (6.5)	0 (0)	31
Bilirubin	2 (6.5)	0 (0)	31
AP	0 (0)	0 (0)	21
GGT	7 (23.3)	0 (0)	30
AST	6 (19.4)	0 (0)	31
ALT	4 (12.9)	0 (0)	31
Cardiotoxicity			
Arrhythmia	0 (0)	1 (2.8)	35
Myocardial infarction	0 (0)	0 (0)	35
EF reduction abs. ^{a, c}	1 (8.3)	0 (0)	12
EF reduction diff. ^{b, c}	0 (0)	0 (0)	5

Data are presented as n (%). AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EF, ejection fraction; GGT, gamma-glutamyltransferase; n/a, not available. ^a EF reduction absolute. ^b EF reduction in difference (in cases where EF before Flag-Eto was available). ^c EF reduction in sepsis not included.

long-lasting relapse-free survival [9]. We next evaluated post-remission treatment among those patients who achieved at least partial remission after FLAG-Eto treatment. In fact, the majority of patients could continue with consolidation therapy: 33% (5/15) of the evaluable patients underwent allogeneic stem cell transplantation, 27% ($n = 4$) received donor lymphocytes, and 13% ($n = 2$) were consolidated with chemotherapy. The low number of patients in each consolidation treatment subgroup did not allow firm conclusions on the prognostic value of each therapy modality in multiple relapsed/refractory patients. However, it appears that any consolidation therapy positively affected overall survival (online suppl. Fig. S2, Tables S2–3).

Discussion

Given the high rate of multiple occurring relapse and refractoriness to chemotherapy in AML, different salvage protocols are urgently needed. The requirements for such therapies, apart from high efficacy, are good tolerability with maintenance of good performance status to enable any consolidating intensive treatment. Frequently, cumulative anthracycline dosages have been reached in multiple relapsed/refractory AML patients which built

Table 4. Overview of studies on etoposide-containing treatment protocols for relapsed/refractory AML patients

Regimen	PRT, yes/ no	n	Age (median, range), years	Secondary AML, %	Adverse genetics, %	Patients with 2nd or more relapse, %	Number of previous relapse therapies, %	Previous SCT, %	Response rates, %	DFS/RFS ^a (median, range), months	OS ^a (median, range), months	Length of neutropenia to neutrophil recovery ^a (median, range), days	30- or 60-day mortality ^g , %	Ref.
Anthracycline-free protocols														
HIDAC + Eto + Cisplatin (HAEP)	No	49	42 (18–71)	18	25	27	1:88 ≥2:6	n/a	16 (CR) 15 (CRp)	n/a	2.9	In patients with recovered cell count: 32 (19–99) ^{b,c}	30: 33	Lee et al. [18], 2014
Eto + CTX	No	34	48 (25–70)	n/a	56	n/a	0:35 1:38 2:21 3:6	15	32 (CR)	5 (median duration of CR)	n/a	n/a	n/a	Trifilio et al. [19], 2013
CTX + Eto + Carbo- platin + Ara-C (CECA)	No	25	54 (24–79)	n/a	52	n/a	≥1:36	n/a	12 (CR)	n/a	n/a	n/a	n/a	Komblau et al. [21], 1998
Flu + Ara-C + Eto (FCE)	No	20	46 (26–61)	n/a	60	15	1:15	5	70 (CR) 5 (CRi)	n/a	n/a	24 ^{c,d}	30: 0 60: 5	Aldoss et al. [17], 2014
Anthracycline-containing protocols														
Lenalidomide plus MTZ + Eto + Ara-C	No	35	61	n/a	51	0	n/a	22	34 (CR + CRi)	6.4 (<1–32) for CR patients	11.5	For CR patients: 29 (11–81) ^d (time to recovery from treatment initiation)	30: 6 60: 13	DeAngelo et al. [22], 2018
MTZ + Eto + Ara-C following epigenetic priming with decitabine	No	52	55 (19–72)	17.3	40.4	n/a	n/a	25	21.2 (CR) 13.5 (CRp) 1.9 (CRi) 13.5 (MLS)	5	5	For CR-patients after receipt decitabine/MEC at/above rec- ommended phase 2 dose: 29 (27–66) ^{b,c}	28: 17.3 60: 21.2	Halpern et al. [23], 2017
MTZ + Eto (ME)	No	91	56.8 (21.9–75.8) ^e	8.8	20	0	n/a	n/a	25 (CR + CRi)	12.7 (for CR patients)	7.4	35 (21–63) ^{b,f}	30: 4 60: 13	Im et al. [24], 2016
MTZ + Eto + IDAC (MEC) (A) vs. MTZ + Eto (ME) (B)	No	65 A: 29 B: 36	A: 54 (21–77) B: 58 (24–79)	n/a	A: 39 B: 32	n/a	≥1 A: 41 B: 42	A: 56 B: 21	A: 59 (CR) B: 34 (CR)	n/a	n/a	n/a	n/a	Trifilio et al. [25], 2012
IDAC + Ida + Eto	No	19	28 (15–61)	n/a	n/a	n/a	n/a	n/a	Threshold neu- trophils for CR: >1.5/nL 37 (CR)	6.7 (2.5–61.4+) (median duration of CR for CR patients)	4.6 (0.4–62.6+)	In CR patients: 28 (25–59) ^{c,d}	n/a	Bahng et al. [26], 2001
MTZ + Eto + Ara-C (EMA) + GM-CSF (A) vs. EMA (B)	Yes	192 A: 95 B: 97	A: 47 (17–65) B: 46 (16–65)	A: 13.7 B: 16.5	A: 30.5 B: 22.7	n/a	n/a	Auto: A: 6.3 B: 6.2	A: 65 (CR) B: 59 (CR)	A: 8.2 (251 days) B: 7.9 (240 days)	A: 10 (303 days) B: 8.3 (254 days)	Neutrophil recovery from ini- tiation of chemotherapy A: 38 (7–82) ^d B: 37 (29–66) ^d	n/a	Thomas et al. [27], 1999

Ara-C, cytarabine; CTX, cyclophosphamide; Eto, etoposide; Flu, fludarabine; HIDAC, high-dose cytarabine; AML, acute myeloid leukemia; CR, complete remission; CRp, complete remission with incomplete platelet recovery; DFS, disease-free survival; n/a, not available; OS, overall survival; PRT, prospective randomized trial; RFS, relapse-free survival; SCT, stem cell transplantation. ^a Range given only if available in original article. ^b Threshold 1/nL. ^c Median time to recovery of neutrophils (no definition given). ^d Threshold 0.5/nL. ^e Median age at relapse (in original article also "at diagnosis" given). ^f Median time to recovery of neutrophils (defined as: from completion of treatment to neutrophils > threshold). ^g 30-day, 4-week, and 1-month mortality as well as 60-day, 8-week, and 2-month mortality were equalized and all listed as 30-day or 60-day mortality rate.

the rationale to introduce the here presented FLAG-Eto protocol for patients not eligible for further anthracycline treatment. In this heavily pretreated patient cohort 25.7% achieved CR and 14.3% CRi/MLFS with a 1-year disease free survival of 40%. Noteworthy, long-term outcome for patients who achieved remission under FLAG-Eto was inferior compared to long-term survival data of our recently published cohort of FLAG-Ida-treated mainly first-time relapsed/primary refractory AML patients [9]. These obvious differences in the quality of remission are most likely not due to the chemotherapeutic regimen itself but rather rely on the number of prior relapses and the biology of disease. This is supported by the here presented decreasing response rates along with the increasing number of preceding salvage therapies. Despite the impaired performance status among FLAG-Eto-treated patients at baseline we did not observe a significant increase in the frequency of grade 3/4 toxicities compared to the toxicity profile of our previously published FLAG-Ida regime [9].

To our knowledge, there is only one published study on the efficacy of the FLAG-Eto protocol in 20 patients, of whom only 15% received one prior salvage therapy and 5% previously underwent allogeneic stem cell transplantation [17]. In this patient cohort higher anti-leukemic activity with CR/CRi rates of 75% and a 1-year overall survival of 71% has been achieved with a more beneficial toxicity profile. These results raise the question of the role of the FLAG-Eto protocol for up-front salvage treatment. Focusing on studies which applied salvage therapies in multiple relapsed/refractory AML patients, CR or CR/CRp (CR with incomplete platelet counts) was achieved in 31–36% of patients with a median overall survival of about 3 months (Table 4) [18–20], which suggests that

remission rates achieved with the FLAG-Eto regimen range above average. However, randomized trials are required to define optimal chemotherapeutic combinations for different subsets of relapsed/refractory AML.

In conclusion, FLAG-Eto for multiple relapsed/refractory AML patients is an effective salvage regimen, also eligible for selected elderly patients. The impaired performance status and higher comorbidity index of heavily pretreated patients at baseline were not worsened through FLAG-Eto. This regimen still enabled implementation of further consolidation therapies such as allogeneic stem cell transplantation.

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Statement of Ethics

The study was approved by the relevant Ethics Committee and followed guidelines set out in the Declaration of Helsinki.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

J.W., R.N., C.S., M.F., U.D., and M.H. collected and analyzed the data. J.W. and M.H. wrote the manuscript.

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