Leukemia Research 85 (2019) 106192

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Low-dose melphalan in elderly patients with relapsed or refractory acute myeloid leukemia: A well-tolerated and effective treatment after hypomethylating-agent failure

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ARTICLE INFO

Keywords:
Relapsed or refractory acute myeloid leukemia
Melphalan
Complex karyotype
Palliative treatment
Elderly patients
Hypomethylating agents

ABSTRACT

Relapsed or refractory (R/R) disease remains challenging in acute myeloid leukemia (AML), especially in elderly patients not considered eligible for intensive treatment options. We retrospectively evaluated the safety and efficacy of low-dose melphalan (LD-Mel) in a multicenter analysis in patients over 65 years with R/R AML, who previously had received ≥1 non-curative treatment line. The study included 31 patients (median age 77 years) with 1–4 previous treatment lines. Three patients (9.7%) achieved a complete remission. Two patients (6.5%) achieved a partial remission, nine patients (29.0%) had disease stabilization with reduction of peripheral or bone marrow blast burden, resulting in an overall response rate of 16.1% and 45.2% achieved clinical benefit. Responders showed a significantly longer median overall survival than non-responders (16.3 vs. 2.3 months, $p < 0.001$). Multivariate analysis identified complex karyotype as the only risk factor associated with inferior survival ($p < 0.001$), whereas prior treatment with hypomethylating agents (HMAs) in 25 of 31 patients was associated with superior OS, regardless of prior response to HMAs ($p = 0.03$). LD-Mel was well tolerated, with mild myelosuppressive side effects. Conclusively, LD-Mel is an effective treatment option in elderly patients with R/R AML, particularly after HMA therapy and in the absence of a complex karyotype.

1. Introduction

Acute myeloid leukemia (AML) is a malignant hematopoietic stem cell disorder that arises from clonal expansion of myeloid precursors in the bone marrow [1]. More than half of the newly diagnosed patients with AML are > 65 years old [2] and increasing age negatively impacts survival rates [2–4] due to comorbidities and decreased tolerance of intensive treatment regimens [5,6]. Although on average 50–60% of all patients beyond 60 years achieve complete remission (CR) after intensive induction therapy, this only translates into a 2 years survival of 15–20% [7], thus leaving a high proportion of patients with relapsed and primarily refractory (R/R) disease.

Management of R/R AML in elderly patients is challenging and outcomes are generally poor, with a median survival of less than 1 year [3,8]. Despite intense efforts in developing new anti-leukemic drugs, palliative treatment options are still restricted to a few approved drugs and clinical trial participation is a highly recommended option by guideline. While novel therapies targeting specific acquired mutations or surface antigens are under development, cytidine analogs like low-dose cytarabine (LDAC) or hypomethylating agents (HMA) continue to be the major treatment modalities for elderly R/R AML patients.

Melphalan, an alkylating agent developed in the mid-1950s, was first documented to be clinically active at low doses in patients with

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https://doi.org/10.1016/j.leukres.2019.106192
Received 28 March 2019; Received in revised form 14 June 2019; Accepted 14 July 2019
Available online 10 August 2019

high-risk myelodysplastic syndrome (MDS) or AML in 1996 [9]. Until today, published data regarding the efficacy of low-dose (LD) melphalan (Mel) in high-risk MDS or AML is limited to small cohort studies or case reports from a total of 78 patients [10–14] with a reported overall response rate of approximately 45% [9,15,16].

However, LD-Mel was used as a front-line palliative treatment in almost all published cases (n = 76) and only 2 patients received LDAC or HMA prior to LD-Mel therapy. The efficacy of LD-Mel as a second-line treatment option or beyond in relapsed or refractory (R/R) AML is not known.

Due to the promising antileukemic efficacy and the favorable toxicity profile of this widely available therapy, we reasoned that LD-Mel might be an attractive treatment option in elderly patients with R/R AML. We therefore conducted a registry-based multicenter retrospective analysis to identify elderly patients (defined as > 65 years) with R/R AML who were treated with LD-Mel in second line or beyond.

2. Patients, materials and methods

2.1. Patients and treatment

Patients were identified in a registry search based on data of the German Study Alliance Leukemia (SAL) network and the AML registry of the Div. Hematology, Oncology and Stem Cell Transplantation, University of Freiburg Medical Center, Freiburg, Germany. Eligibility criteria for this retrospective analysis were: patients aged > 65 years with de novo or secondary AML (except acute promyelocytic leukemia) who had failed to achieve disease remission after one or more previous therapy lines (refractory disease) or relapsed after previous CR. All patients had received at least one non-curative treatment line. All patients were considered ineligible for intensive treatment options including allogeneic stem cell transplantation at the time of LD-Mel initiation. Patients who had received high-dose melphalan (> 100 mg/m^2) following autologous stem cell transplantation (ASCT) for AML treatment as well as patients who had received melphalan as a front-line palliative treatment were excluded from this analysis.

Melphalan was administered orally at 2 mg per day in a continuous 28-day cycle until disease progression (in the absence of clinical benefit on the discretion of the treating physician), intolerable toxicity or death according to the protocol provided by Omoto et al [9]. Concomitant or intermittent treatment with other antineoplastic agents including hydroxyurea was not allowed. The use of growth factors and other supportive measures was at the discretion of the treating physician and was not recorded for this analysis.

Cytogenetic analysis and molecular data including mutations of *NPM1, FLT3, MLL* and *RUNX1* genes were collected where available and stratified according to the 2017 European LeukemiaNet (ELN) recommendations [17]. Approval from the responsible institutional review board was obtained before data collection (approval number: EK 98032010). All procedures performed were in accordance with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included into this study.

2.2. Efficacy assessment

Endpoints of this study were time on treatment (ToT), overall survival (OS), clinical benefit rate (CBR, for definition see below), overall response rate (ORR) and rate of complete (CR) and partial remission (PR). Time on treatment was defined as the time period from treatment initiation to the last documented dose application. Overall survival was calculated from the first day of LD-Mel treatment until death from any cause.

The CBR was defined as the proportion of patients not showing refractory disease to LD-Mel therapy. The ORR was defined as the proportion of patients with confirmed PR, complete remission with incomplete blood count recovery (CRi) and CR. Disease evaluation was based on the revised recommendations of the international working group for standardization of response criteria in acute myeloid leukemia [18]. In addition, patients with a bone marrow blast reduction not fulfilling PR criteria and patients with suspected response to LD-Mel in the absence of confirming bone marrow aspirates were defined as “stable disease” (SD).

2.3. Toxicity assessment

The electronic charts were carefully reviewed for LD-Mel related toxicities, focusing on documented infections and other potentially life-threatening events. All patients received regular clinical follow-up and laboratory workup to detect relevant toxicities on the discretion of the treating physicians.

2.4. Statistical analysis

Graph Pad Prism Version 6.01, SPSS Version 25.0 and R Version 3.4.2 were used for statistical analysis and reporting of the data collected for this study. The number of all included patients and recorded variables were reported descriptively. Survival analyses were performed using the Kaplan-Meier method for estimation of the percentage of surviving patients and the log rank test for comparing patient groups. Cox robust regression (marginal WLW model) (Venables & Ripley, 2011) was used for uni- and multivariate analyses. Age (dichotomized at the median), presence of a complex karyotype, prior HMA therapy, intensive pretreatment, and de novo vs secondary AML were used as independent linear predictors. Proportional hazards assumption and residuals were checked formally [19,20] and graphically. Schoenfeld residuals for all covariates were verified to be independent of time. A p-value below 0.05 was considered statistically significant.

3. Results

3.1. Patients

We identified 31 AML patients in the databases who met the inclusion criteria and were treated with LD-Mel between 2002 and 2018 at 4 tertiary treatment centers. Table 1 depicts the patient and disease characteristics. Median age at treatment initiation was 77 years, 61.3% of all treated patients were older than 75 years and 16.1% were older than 80 years, respectively. All but one patient had either ELN intermediate or adverse risk scores by genetics at the time of treatment initiation (n = 7 unknown). All patients were either pretreated with cytarabine or an HMA and approximately one-third of all patients were treated with both antineoplastic agents prior to LD-Mel. Of those patients who have ever received HMAs (n = 25; n = 17, decitabine; n = 8, azacytidine), only one patient discontinued due to unacceptable toxicity and 24 patients subsequently showed disease progression. Eighteen patients (58.1%) had participated in clinical trials before LD-Mel treatment. Approximately two-thirds of all patients had received ≥2 previous therapy lines. No patient had received, or was subsequently planned, for allogeneic stem cell transplantation. Eleven patients (35.5%) received treatment after melphalan failure. Median time from first diagnosis to LD-Mel initiation was 10.5 months (range, 0.3–73 months). A total of 108 treatment cycles were administered on 3027 treatment days, with a median of 1.6 treatment cycles (range, 0.4–14 cycles) per patient.

3.2. Toxicity of LD-Mel

Detailed review of patients´ charts revealed overall mild side effects, with mainly hematologic toxicities. LD-Mel therapy was paused either because of pancytopenia (5 patients, 16.1%), infection (3 patients, 10.0%), one patient each (3.2%) with infectious colitis, fungal pneumonia, and neutropenic fever. Treatment was resumed in 3 of these patients (1 each with neutropenic fever, infectious colitis, pancytopenia). The remaining patients died of the stated infection or showed subsequent progressive disease. One patient died during his 4th cycle of

Table 1
Summary of patient characteristics.

	n = 31 (100.0%)
Gender	
female	16 (51.6%)
male	15 (48.4%)
Age (years)	
median (range)	77 (66-87)
AML characteristics	
de novo	16 (51.6%)
secondary	15 (48.4%)
WBC x 10^9 cells/L (median, range)	1.9 (0.4-45.2)
bone marrow blast count (median, range) in %	35 (10-75)
Prior lines of therapies	
1	12 (38.7%)
2	14 (45.2%)
3	4 (12.9%)
4	1 (3.2%)
median	2
Prior intensive chemotherapy	
yes	9 (29.0%)
no	22 (71.0%)
Proportion of patients who were previously treated with	
HMA (no cytarabine)	14 (45.2%)
cytarabine (no HMA)	6 (19.4%)
HMA, cytarabine	11 (35.5.0%)
ELN risk stratification by genetics	
favorable	1 (3.2%)
intermediate	12 (38.7%)
adverse	11 (35.5%)
complex karyotype	8 (25.8%)
UKN	7 (22.6%)

AML, acute myeloid leukemia; HMA, hypomethylating agent; ELN, European LeukemiaNet; UKN, unknown.

LD-Mel treatment of indeterminate cause. There were no laboratory signs or symptoms of melphalan-related organ toxicities.

3.3. Efficacy of LD-Mel and survival analyses

Bone marrow aspirates to evaluate disease response were available in 15 out of 31 patients (48.4%) and 9 out of 14 patients (64.3%) with suspected disease response. Bone marrow biopsies were performed after a median of 49 days (range, 26–111 days) calculated from LD-Mel treatment initiation. The remaining 5 patients showed peripheral blast clearance upon LD-Mel treatment but lacked bone marrow evaluation to assess suspected response. Three out of 31 patients (9.7%) achieved a complete remission (CR/CRi), of which one had a full blood count recovery (CR), see Table 2. Two patients (6.5%) achieved a PR with at least 50% blast reduction in the bone marrow. Median time to treatment evaluation in patients with CR (CRi) or PR was 63 days (43–92 days). Among those patients who fell into the SD group (n = 9; 29%), 2 recovered with incomplete blood counts and remained transfusion-free for more than 10 months. Four additional patients showed improvement of hemoglobin levels of more than 1 g/dl and remained transfusion-free for at least 3 months, 2 of which fully recovered their absolute neutrophil count. The remaining 3 patients showed peripheral blast clearance without further evidence of hematologic improvement. Overall response rate was 16.1% and CBR was 45.2%.

Table 2
Best treatment response.

	31 (100%)
CR/CRi	3 (10.0%)
PR	2 (6.5%)
SD	9 (29.0%)
RD	17 (54.8%)

CR, complete remission; CRi, complete remission with incomplete blood count recovery; PR, partial remission; SD, stable disease; RD, resistant disease.

Median ToT of all study subjects was 1.5 months (95% CI, 0.4–2.6 months). Treatment responders (SD, PR, CRi, CR) had a longer time on treatment than non-responders (median ToT, 4.2 vs. 1.0 months; HR 0.27; 95% CI 0.05-0.28; $p < 0.001$). Median OS of all patients was 3.7 months (95% CI, 2.6–4.8 months) and 3.7 months (95% CI, 1.7–5.7 months) when censored at the time of starting the next treatment line, see Fig. 1A and B. Median OS of patients in CR, CRi and PR was significantly longer than in patients with disease stabilization only (median OS, 16.3 vs. 5.4 months; HR 0.38; 95% CI 0.11-0.89; $p = 0.04$), which in turn was significantly longer than in non-responders (median OS, 5.4 vs. 2.3 months; HR 0.34; 95% CI 0.12-0.57; $p = 0.002$), see Fig. 1C. Ten out of 31 patients (32.3%) survived 6 months and 7 patients survived 12 months (22.6%) after treatment initiation, respectively.

Twelve patients (38.7%) died within one month after termination of LD-Mel therapy, either because of disease progression (n = 6) or uncontrollable infection (n = 1, fungal pneumonia). For the remaining 5 patients, no definite cause of death could be identified. Median survival calculated from first diagnosis in all patients was 17.0 months (95% CI, 12.5–21.5 months).

All patients with complex karyotype showed refractory disease to LD-Mel, progressed rapidly and died after a median survival time of only 1.5 months (95% CI, 0.1–3.4 months), see Fig. 1D.

3.4. Long-term responders

Five patients (median age 84 years, range 75–87 years) remained on LD-Mel therapy for longer than 10 months (median ToT of 12.2 months; 95% CI, 9.6–14.7 months). Due to their advanced age, none of these patients was ever considered for intensive treatment. Their disease and patient characteristics are summarized in Table 4. Molecular genetics were wild-type for *NPM1* and *FLT3* in all 5 patients. All of these patients had been pretreated with HMAs (disease response to HMA therapy: 2 CR, 1 PR, 1 SD, 1 PD). One of them had also received prior cytarabine therapy (refractory disease documented after two cycles of LDAC). Complete remission was confirmed in two patients (1 CR, 1 CRi). One patient had a PR documented after one cycle of LD-Mel, she subsequently fully recovered with her peripheral blood counts for 10.7 months, but further bone marrow assessments to confirm suspected CR were not performed. Two patients were categorized SD in the absence of bone marrow aspirates, both of them recovered with incomplete blood counts. Fig. 2 illustrates the blood counts while on LD-Mel therapy for the 5 long-term responders.

Three of the five long-term responders received further treatment after LD-Mel: One patient achieved a second CR on LDAC therapy following LD-Mel failure after 11 months of treatment. Two patients were included in clinical trials and received investigational agents after 10.7 and 12.4 months on LD-Mel, respectively. Two patients (ToT, 13.5 and 12.2 months) received best supportive care and died shortly after LD-Mel was stopped.

3.5. Uni- and multivariate analyses for survival

Evaluated by uni- and multivariate analysis, presence of complex karyotype was the only significant and independent risk factor associated with inferior survival in our study cohort (HR, 7.13; 95% CI, 2.3–22.1; $p < 0.001$), see Table 3. There was also a strong trend for inferior survival in patients older than the population median (HR, 2.18; 95% CI, 1.0–5.0; $p = 0.06$). Pretreatment with HMA, on the contrary, was the only beneficial factor for superior survival in multivariate analysis (HR, 0.35; 95% CI, 0.1-0.9; $p = 0.03$, Table 3) and in univariate analysis when patients with complex karyotype were excluded (Fig. 1E, F). The beneficial effect of HMA pretreatment was independent of response to prior HMA therapy (survival of patients who responded to HMA therapy vs non-responder; HR, 1.3; 95% CI, 0.49–3.26; $p = 0.63$). Information on bone marrow cellularity was not

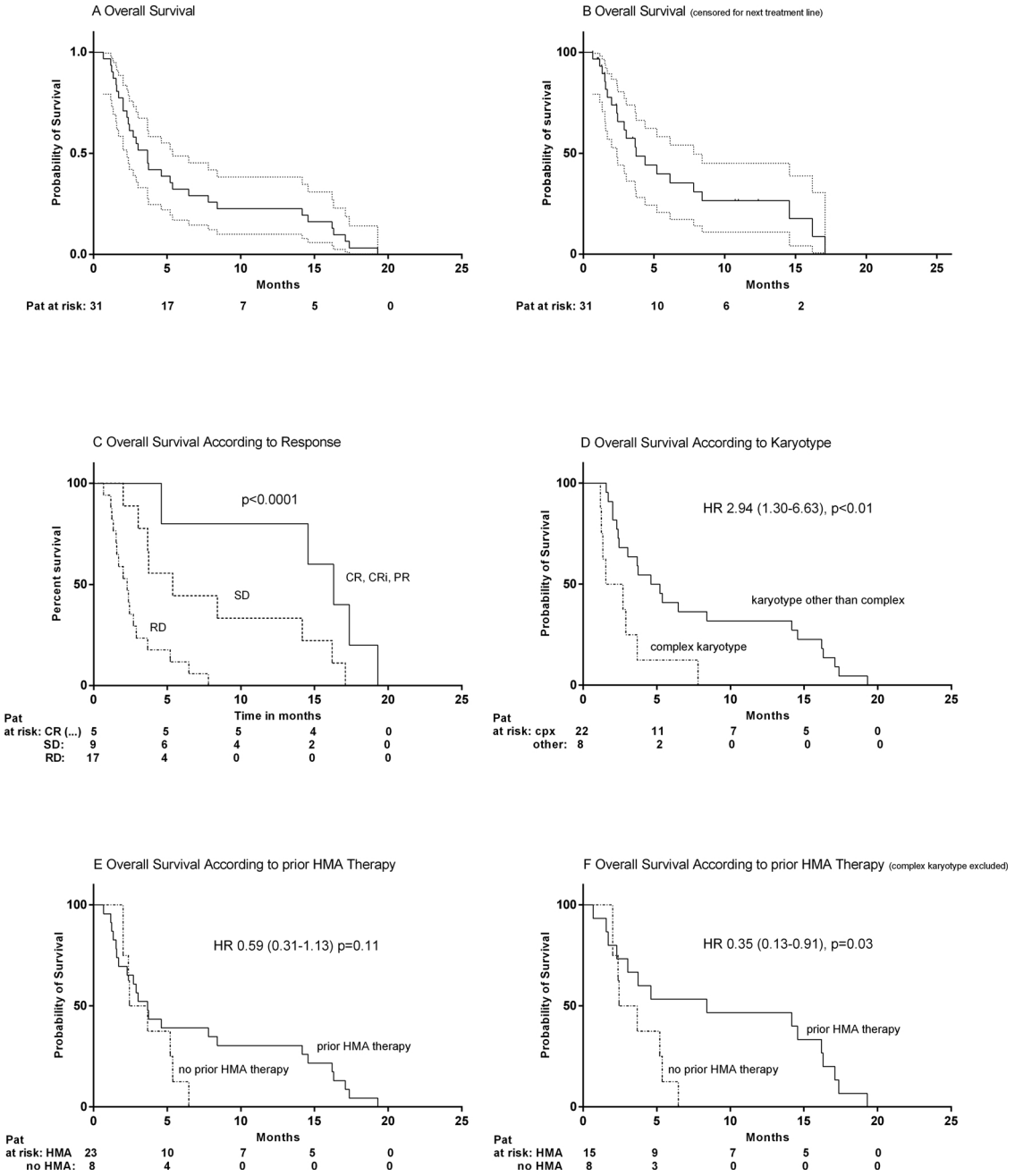

Fig. 1. Survival estimates; A: Overall Survival of all patients treated with low-dose melphalan, dotted pattern indicates 95% confidence interval; B Overall Survival According to Response to LD-Mel of all patients, defined as patients achieving CR, CRi, PR, (black line), SD (dotted pattern and non-responders (PD)(dotted/lined pattern); C Overall Survival According to Karyotype; D Overall Survival According to prior HMA Therapy in the whole study population; E Overall Survival According to prior HMA therapy (complex karyotype excluded).

regularly reported and could not be considered in the statistical analyses. There were no censored events in the ToT or survival analysis.

4. Discussion

Relapsed and refractory AML patients have a dismal prognosis, and effective and standardized treatment options are sparse in this difficult-to-treat situation, especially in elderly patients not considered eligible for intensive treatment options.

Here we report on the safety and efficacy of LD-Mel in 31 elderly patients with R/R AML who were identified in two German leukemia registries. The study population was of higher age, all but one patient were categorized as intermediate or adverse ELN risk. All patients had received cytarabine and/or HMA and 58% had received investigational agents prior to LD-Mel therapy, thus representing a heavily pretreated patient cohort with adverse prognostic factors [21].

In line with previous reportings [9,13,15], LD-Mel was very well tolerated in our study cohort. Potential side effects were mainly either hematotoxicity or subsequent complications related to myelosuppression, predominantly infections. In particular, we found no evidence for gastrointestinal, renal or hepatic toxicity related to LD-Mel, even in long-term responders who received high cumulative doses of melphalan. Although adverse events were only reported at the discretion of the treating physician and therefore might be underreported here, we have consistently experienced LD-Mel as a remarkably safe and easy-to-handle agent in frail patients, even when presenting with low performance status and persisting toxicities from previous antineoplastic therapies.

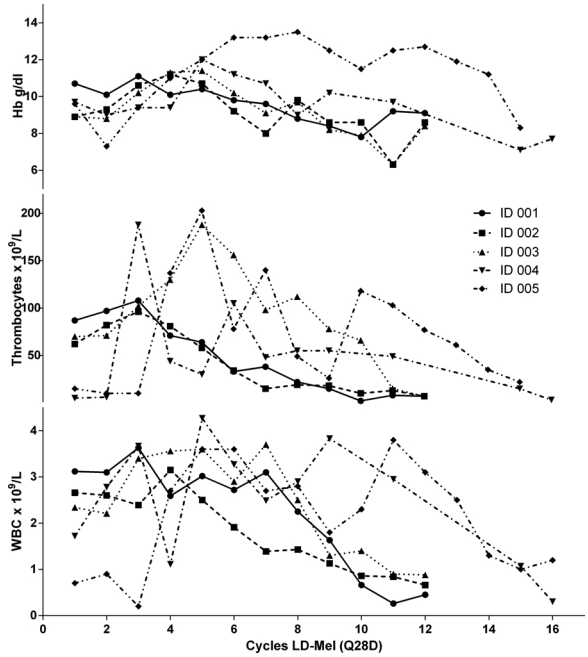

Fig. 2. Blood counts in long-term responders: hemoglobin (Hb), thrombocyte and white blood cell count (WBC) in five long-term responders treated with LD-Mel; Curves show blood counts from first medcation intake till last dose; ID, patients identification corresponding with Table 4.

LD-Mel demonstrated encouraging single-agent antileukemic efficacy with an ORR of 16.1% and a CBR of 45.2%. Data on response and survival outcomes in AML after HMA failure is sparse, especially in patients not eligible to intensive treatment options but overall subsequent therapy response seems to be limited to a few cases and survival is exceptionally poor [22,23].

Median overall ToT in responders was only 4.2 months, however, five long-term responders were on treatment for > 10 months. In the absence of sequential bone marrow assessments, three of these patients were categorized PR (n = 1) or SD (n = 2), thus the ORR in our study may be an underestimation. All long-term responders were categorized as intermediate ELN risk by genetics and had a low leukemic burden, which has been previously identified as a beneficial predictive marker in patients treated with LD-Mel [15]. Indeed, presence of complex karyotype (adverse ELN risk classification) was the only adverse prognostic risk factor for inferior survival following LD-Mel treatment in uni- and multivariate analyses. Our results further indicate that pre-treatment with HMA may be a new independent beneficial factor for superior survival. Importantly, this relationship could be determined regardless of previous response to HMA therapy, indicating that superior survival following LD-Mel therapy is not a result of a positive selection of treatment responders.

Although the specific mechanism of action of melphalan is still not fully elucidated, there is some evidence derived from in vitro myeloma cell studies that melphalan maintains anti-tumour activity dependent on wild-type *TP53,* and its loss-of-function confers inherited or acquired resistance [24,25]. In addition, a total of five patients with high-risk MDS or AML who were treated with LD-Mel showed acquired a 17p deletion on disease progression in two published series [10,26]. Unfortunately, *TP53* mutation status was not routinely assessed in our cohort, only one patient with complex karyotype harbored a 17p (and therefore *TP53*) deletion and did not respond to LD-Mel therapy.

Haferlach et al and others could demonstrate that there is a high incidence of *TP53* mutations (60–80%) in AML with complex-aberrant karyotype, whereas *TP53* mutations are only infrequently observed in all other cytogenetic subgroups [27–29]. In consequence, we speculate that non-response to LD-Mel in patients with complex karyotype may at least partially be attributed to *TP53* inactivation, and these patients might rather benefit from alternative treatment options, such as HMAs, that have been shown to induce favorable responses in AML with *TP53* mutation or unfavorable cytogenetics [30,31]. This is also in line with clonal evolution analyses that commonly show a (transient) clearance of *TP53* mutant AML subclones induced by HMA therapy [32]. Following this reasoning, superior survival seen in patients pretreated with HMAs might be explained by differences in subclonal disease eradication.

Additionally, the sequence of HMAs and other alkylating agents provides preclinical evidence for synergistic anti-tumor activity: It has been shown that HMAs upregulate CD40 and CD86, important co-stimulatory surface molecules for T-cell interaction and proinflammatory T-cell priming on dendritic cells [33]. The use of (low-dose) alkylating agents, in turn, has been associated with a selective expansion and activation of effector T cells, while immunosuppressive regulatory T and B cells are proportionally depleted [34], The combination of enhanced MHC class-II restricted neoantigen presentation induced by HMAs with a proinflammatory lymphocyte environment induced by alkylating agents may also contribute to enhance anti-leukemic activity. While the existence and biological mechanism of a synergistic sequential HMA/LD-Mel treatment approach suggested in this study is highly speculative, it provides some rationale for further mechanistic studies and clinical combination or sequence studies.

LD-Mel was administered *per os*, providing the convenience of an orally available medication, limiting in- and outpatient hospital contact and lacking the necessity of invasive subcutaneous or intravenous drug application. The dosing schedule, however, is quite atypical for cell-cycle non-specific agents, whose fraction of tumor-cell-kill usually increases linearly with the dose of the drug.

Of interest, pulsatile dosing schedules of melphalan are common in other hematologic or solid malignancies (e.g. [35–38]), but have not been applied to patients with AML in the non-curative setting. There are two clinical trials that evaluated high-dose melphalan (HD-Mel) following autologous stem cell transplantation for post-remission or salvage therapy in AML [39,40]. Notably, in a pilot study by Bug et al., 13 out of 14 patients (93%), including all patients with complex karyotype achieved a second CR

Table 3
Univariate and multivariate analysis of overall survival in AML patients (calculated from LD-Mel initiation).

		Univariate analysis			Multivariate analysis		
All patients (n = 31)	Parameter	HR	95% CI	p value	HR	95% CI	p value
	prior vs no prior therapy with HMA	0.59	0.31-1.13	0.11	0.35	0.13-0.90	0.03
	complex karyotype vs other	2.94	1.30-6.63	< 0.01	7.13	2.30-22.14	< 0.001
	age < 77 vs ≥ 77 years	1.46	0.72-2.98	0.30	2.18	0.96-4.98	0.06
	de novo vs secondary AML	1.62	0.79-3.31	0.19	2.18	0.92-5.17	0.08
	intensive vs palliative pretreatment	1.57	0.82-3.02	0.17	1.03	0.45-2.35	0.95
no complex karyotype	prior vs no prior therapy with HMA	0.36	0.15-0.87	0.02	0.35	0.13-0.91	0.03
	age < 77 vs ≥ 77 years	1.94	0.81-4.61	0.14	2.58	1.00-6.68	0.05
	de novo vs secondary AML	2.18	0.92-5.21	0.08	2.51	1.01-6.21	0.05
	intensive vs palliative pretreatment	2.11	0.94-4.73	0.07	0.77	0.34-1.76	0.54

AML, acute myeloid leukemia; LD-Mel, low-dose melphalan; HMA, hypomethylating agents; HR, hazard ratio; CI, confidence interval.

Table 4
Patient and disease characteristics of long-term responders.

Pat ID	Gender	age	type of AML	karyotype	ELN risk	bone marrow cellularity (blast count)	previous TL	previous BR to HMA	ToT [months]	cumulative LD-Mel dose [mg]	OS [months]	BR
001	f	77	secondary	46,XY [20]	INT	normocellular (37%)	DEC	CR	10.9	621	19.3	Cri (d43)
002	f	84	de novo	46,XY [20]	INT	normocellular (41%)	Cyt,DEC	PR	10.7	372	16.3	PR (d63)
003	f	87	secondary	45,X,-X [4]; 46,XX [16]	INT	normocellular (42%)	DEC	RD	12.4	622	17.4	CR (d63)
004	f	84	de novo	UKN	UKN	normocellular (50%)	DEC	SD	13.5	792	16.2	SD (d59)
005	m	75	secondary	46,XY [20]	UKN	hypocellular (29%)	DEC	CR	12.2	730	17.1	SD (d49)

Pat ID, patient identification number; f, female; m, male; AML, acute myeloid leukemia;TL, therapy line; BR, best response; DEC, decitabine; Cyt, cytarabine; CR, complete remission; CRi, complete remission with incomplete blood count recovery; PR; partial remission; PD, progressive disease; SD, stable disease; ToT, time on treatment; OS, overall survival calculated from start of LD-Mel; ELN, European LeukemiaNet; UKN, unknown; INT, intermediate; d, day of bone marrow evaluation;

with HD-Mel (200 mg/m^2) following autologous stem cell transplantation in R/R AML [40]. Therefore, a more dose-dense melphalan administration may conceivably overcome inherent resistance which was seen in our patients with complex karyotype AML. However, the role of inactivating *TP53* mutations remains unclear in this situation, and increased myelosuppressive and other side effects of a more intensified treatment schedule must carefully be considered.

In this regard, the novel aminopeptidase-potentiated alkylating agent melflufen is a promising candidate in the landscape of new anti-leukemic drug development [41,42]. Under the action of aminopeptidases which are commonly upregulated in myeloid blasts [43], melflufen is hydrolyzed, releasing its active form melphalan at high intracellular concentrations. Safety was successfully evaluated in a phase I/IIa clinical trial in patients with advanced solid malignancies [44] and a phase III trial in patients with R/R multiple myeloma started recruiting in June 2017 (NCT03151811, www.clinicaltrials.gov). Our data suggest that clinical trials evaluating melflufen in R/R AML are warranted.

We fully acknowledge the important limitations imposed on this preliminary pilot study by its retrospective design. Patient inclusion, initiation and continuation of treatment as well as documentation were at the discretion of the individual physicians. Selection and documentation biases place restrictions on the reliability of our results, and on their generalizability beyond the setting at which they were evaluated. Patient comorbidities and performance status were only irregularly assessed and could not be considered for analysis.

5. Conclusion

In conclusion, LD-Mel is a safe regime that induces responses in a considerable number of elderly patients with R/R AML. Although CR rates are sort of modest, the simplicity of administration and good tolerance in elderly patients is a clear advantage. Oral melphalan is widely available and inexpensive, thus broadening the landscape of agents available outside of clinical trials in this difficult-to-treat advanced stage of AML. Patients with intermediate ELN classification and previous HMA therapy might benefit the most from LD-Mel therapy, whereas patients with complex karyotype (presumably also with *TP53* inactivation) probably show intrinsic drug resistance.

On the basis of our experiences, we recommend considering LD-Mel in elderly patients with R/R AML ineligible for intensive treatment or clinical trials, who have either progressed on approved therapeutic options or for whom no other standard of care is applicable. Our findings need confirmation in prospective clinical trials.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

We would like to express our gratitude to all the participants of the SAL registry network, as well to the members of the DKTK consortium, who supported and contributed to this manuscript. We thank Prof. Eva. Hartmann from the Institute of Biostatistics, Goethe University Hospital Frankfurt, for her support at various stages of this research.

References

[1] E.A. McCulloch, Stem cells in normal and leukemic hemopoiesis (Henry Stratton Lecture, 1982), Blood 62 (1) (1983) 1–13.
[2] D. Pulte, L. Jansen, F.A. Castro, A. Krilaviciute, A. Katalinic, B. Barnes, M. Ressing,

B. Holleczek, S. Luttmann, H. Brenner, Survival in patients with acute myeloblastic leukemia in Germany and the United States: major differences in survival in young adults, Int. J. Cancer 139 (6) (2016) 1289–1296, https://doi.org/10.1002/ijc.30186.

[3] D.A. Breems, W.L.J. van Putten, P.C. Huijgens, G.J. Ossenkoppele, G.E.G. Verhoef, L.F. Verdonck, E. Vellenga, G.E. de Greef, E. Jacky, J. van der Lelie, M.A. Boogaerts, B. Lowenberg, Prognostic index for adult patients with acute myeloid leukemia in first relapse, J. Clin. Oncol. 23 (9) (2005) 1969–1978, https://doi.org/10.1200/JCO.2005.06.027.

[4] G. Juliusson, P. Antunovic, A. Derolf, S. Lehmann, L. Möllgård, D. Stockelberg, U. Tidefelt, A. Wahlin, M. Höglund, Age and acute myeloid leukemia: Real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry, Blood 113 (18) (2009) 4179–4187, https://doi.org/10.1182/blood-2008-07-172007.

[5] W. Hiddemann, W. Kern, C. Schoch, C. Fonatsch, A. Heinecke, B. Wörmann, T. Büchner, Management of acute myeloid leukemia in elderly patients, J. Clin. Oncol. 17 (11) (1999) 3569–3576, https://doi.org/10.1200/JCO.1999.17.11.3569.

[6] F.R. Appelbaum, H. Gundacker, D.R. Head, M.L. Slovak, C.L. Willman, J.E. Godwin, J.E. Anderson, S.H. Petersdorf, Age and acute myeloid leukemia, Blood 107 (9) (2006) 3481–3485, https://doi.org/10.1182/blood-2005-09-3724.

[7] B. Löwenberg, G.J. Ossenkoppele, W. van Putten, H.C. Schouten, C. Graux, A. Ferrant, P. Sonneveld, J. Maertens, M. Jongen-Lavrencic, M. von Lilienfeld-Toal, B.J. Biemond, E. Vellenga, M. van Marwijk Kooy, L.F. Verdonck, J. Beck, H. Döhner, A. Gratwohl, T. Pabst, G. Verhoef, High-dose daunorubicin in older patients with acute myeloid leukemia, N. Engl. J. Med. 361 (13) (2009) 1235–1248, https://doi.org/10.1056/NEJMoa0901409.

[8] M. Stahl, M. DeVeaux, P. Montesinos, R. Itzykson, E.K. Ritchie, M.A. Sekeres, J.D. Barnard, N.A. Podoltsev, A.M. Brunner, R.S. Komrokji, V.R. Bhatt, A. Al-Kali, T. Cluzeau, V. Santini, A.T. Fathi, G.J. Roboz, P. Fenaux, M.R. Litzow, S. Perreault, T.K. Kim, T. Prebet, N. Vey, V. Verma, U. Germing, J.M. Bergua, J. Serrano, S.D. Gore, A.M. Zeidan, Hypomethylating agents in relapsed and refractory AML: outcomes and their predictors in a large international patient cohort, Blood Adv. 2 (8) (2018) 923–932, https://doi.org/10.1182/bloodadvances.2018016121.

[9] E. Omoto, S. Deguchi, S. Takaba, K. Kojima, T. Yano, Y. Katayama, K. Sunami, M. Takeuchi, F. Kimura, M. Harada, I. Kimura, Low-dose melphalan for treatment of high-risk myelodysplastic syndromes, Leukemia 10 (4) (1996) 609–614.

[10] R. Kerr, J. Cunningham, D.T. Bowen, Low-dose melphalan in elderly acute myeloid leukaemia: complete remissions but resistant relapse with therapy-related karyotypes, Leukemia 14 (5) (2000) 953.

[11] Y. Ontachi, H. Yamauchi, A. Takami, H. Asakura, S. Nakao, Low dose melphalan therapy was effective in an elderly patient with MDS-AML, Nihon Ronen Igakkai Zasshi 38 (3) (2001) 405–408.

[12] K. Anargyrou, G. Vaiopoulos, E. Terpos, M. Tsironi, K. Konstantopoulos, M. Samarkos, J. Meletis, Low dose melphalan is a treatment option in elderly patients with high risk myelodysplastic syndrome or secondary acute myeloblastic leukaemia, Haematologia (Budap) 32 (2) (2002) 169–173.

[13] R. Gologan, D. Ostroveanu, L. Gioada, V. Iacob, Remission induction in elderly acute myeloid leukemia by low-dose melphalan, Rom. J. Intern. Med. 41 (1) (2003) 67–73.

[14] A.M. Whittle, S. Feyler, D.T. Bowen, Durable second complete remissions with oral melphalan in hypocellular Acute Myeloid Leukemia and Refractory Anemia with Excess Blast with normal karyotype relapsing after intensive chemotherapy, Leuk. Res. Rep. 2 (1) (2013) 9–11, https://doi.org/10.1016/j.lrr.2012.10.001.

[15] C. Denzlinger, D. Bowen, D. Benz, K. Gelly, W. Brugger, L. Kanz, Low-dose melphalan induces favourable responses in elderly patients with high-risk myelodysplastic syndromes or secondary acute myeloid leukaemia, Br. J. Haematol. 108 (1) (2000) 93–95.

[16] T. Robak, A. Szmigielska-Kaplon, H. Urbanska-Rys, K. Chojnowski, A. Wrzesien-Kus, Efficacy and toxicity of low-dose melphalan in myelodysplastic syndromes and acute myeloid leukemia with multilineage dysplasia, Neoplasma 50 (3) (2003) 172–175.

[17] H. Döhner, E. Estey, D. Grimwade, S. Amadori, F.R. Appelbaum, T. Büchner, H. Dombret, B.L. Ebert, P. Fenaux, R.A. Larson, R.L. Levine, F. Lo-Coco, T. Naoe, D. Niederwieser, G.J. Ossenkoppele, M. Sanz, J. Sierra, M.S. Tallman, H.-F. Tien, A.H. Wei, B. Löwenberg, C.D. Bloomfield, Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel, Blood 129 (4) (2017) 424–447, https://doi.org/10.1182/blood-2016-08-733196.

[18] B.D. Cheson, J.M. Bennett, K.J. Kopecky, T. Buchner, C.L. Willman, E.H. Estey, C.A. Schiffer, H. Doehner, M.S. Tallman, T.A. Lister, F. Lo-Coco, R. Willemze, A. Biondi, W. Hiddemann, R.A. Larson, B. Lowenberg, M.A. Sanz, D.R. Head, R. Ohno, C.D. Bloomfield, Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia, J. Clin. Oncol. 21 (24) (2003) 4642–4649, https://doi.org/10.1200/JCO.2003.04.036.

[19] P.M. Grambsch, T.M. Therneau, Proportional hazards tests and diagnostics based on weighted residuals, Biometrika 81 (3) (1994) 515–526, https://doi.org/10.1093/biomet/81.3.515.

[20] T.M. Therneau, P.M. Grambsch, Modeling Survival Data: Extending the Cox Model, Springer, New York, NY, 2000.

[21] P. Chevallier, M. Labopin, P. Turlure, T. Prebet, A. Pigneux, M. Hunault, K. Filanovsky, P. Cornillet-Lefebvre, I. Luquet, L. Lode, S. Richebourg, O. Blanchet, N. Gachard, N. Vey, N. Ifrah, N. Milpied, J.-L. Harousseau, M.-C. Bene, M. Mohty, J. Delaunay, A new Leukemia Prognostic Scoring System for refractory/relapsed adult acute myelogeneous leukaemia patients: a GOELAMS study, Leukemia 25 (6) (2011) 939–944, https://doi.org/10.1038/leu.2011.25.

[22] V.H. Duong, B. Bhatnagar, D.P. Zandberg, E.J. Vannorsdall, M.L. Tidwell, Q. Chen, M.R. Baer, Lack of objective response of myelodysplastic syndromes and acute myeloid leukemia to decitabine after failure of azacitidine, Leuk. Lymphoma 56 (6) (2015) 1718–1722, https://doi.org/10.3109/10428194.2014.966708.

[23] R. Nanah, K. McCullough, W. Hogan, K. Begna, M. Patnaik, M. Elliott, M. Litzow, A. Al-Kali, Outcome of elderly patients after failure to hypomethylating agents given as frontline therapy for acute myeloid leukemia: single institution experience, Am. J. Hematol. 92 (9) (2017) 866–871, https://doi.org/10.1002/ajh.24780.

[24] S. Surget, E. Lemieux-Blanchard, S. Maïga, G. Descamps, S. Le Gouill, P. Moreau, M. Amiot, C. Pellat-Deceunynck, Bendamustine and melphalan kill myeloma cells similarly through reactive oxygen species production and activation of the p53 pathway and do not overcome resistance to each other, Leuk. Lymphoma 55 (9) (2014) 2165–2173, https://doi.org/10.3109/10428194.2013.871277.

[25] J. Cao, G. Lin, Y. Gong, P. Pan, Y. Ma, P. Huang, M. Ying, T. Hou, Q. He, B. Yang, DNA-PKcs, a novel functional target of acriflavine, mediates acriflavine's p53-dependent synergistic anti-tumor efficiency with melphalan, Cancer Lett. 383 (1) (2016) 115–124, https://doi.org/10.1016/j.canlet.2016.09.029.

[26] A. Merlat, J.L. Lai, Y. Sterkers, J.L. Demory, F. Bauters, C. Preudhomme, P. Fenaux, Therapy-related myelodysplastic syndrome and acute myeloid leukemia with 17p deletion. A report on 25 cases, Leukemia 13 (2) (1999) 250–257.

[27] C. Haferlach, F. Dicker, H. Herholz, S. Schnittger, W. Kern, T. Haferlach, Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype, Leukemia 22 (8) (2008) 1539–1541, https://doi.org/10.1038/leu.2008.143.

[28] D. Bowen, M.J. Groves, A.K. Burnett, Y. Patel, C. Allen, C. Green, R.E. Gale, R. Hills, D.C. Linch, TP53 gene mutation is frequent in patients with acute myeloid leukemia and complex karyotype, and is associated with very poor prognosis, Leukemia 23 (1) (2009) 203–206, https://doi.org/10.1038/leu.2008.173.

[29] F.G. Rücker, R.F. Schlenk, L. Bullinger, S. Kayser, V. Teleanu, H. Kett, M. Habdank, C.-M. Kugler, K. Holzmann, V.I. Gaidzik, P. Paschka, G. Held, M. von Lilienfeld-Toal, M. Lübbert, S. Fröhling, T. Zenz, J. Krauter, B. Schlegelberger, A. Ganser, P. Lichter, K. Döhner, H. Döhner, TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome, Blood 119 (9) (2012) 2114–2121, https://doi.org/10.1182/blood-2011-08-375758.

[30] M. Lübbert, B.H. Rüter, R. Claus, C. Schmoor, M. Schmid, U. Germing, A. Kuendgen, V. Rethwisch, A. Ganser, U. Platzbecker, O. Galm, W. Brugger, G. Heil, B. Hackanson, B. Deschler, K. Döhner, A. Hagemeijer, P.W. Wijermans, H. Döhner, A multicenter phase II trial of decitabine as first-line treatment for older patients with acute myeloid leukemia judged unfit for induction chemotherapy, Haematologica 97 (3) (2012) 393–401, https://doi.org/10.3324/haematol.2011.048231.

[31] J.S. Welch, A.A. Petti, C.A. Miller, C.C. Fronick, M. O'Laughlin, R.S. Fulton, R.K. Wilson, J.D. Baty, E.J. Duncavage, B. Tandon, Y.S. Lee, L.D. Wartman, G.L. Uy, A. Ghobadi, M.H. Tomasson, I. Pusic, R. Romee, T.A. Fehniger, K.E. Stockerl-Goldstein, R. Vij, S.T. Oh, C.N. Abboud, A.F. Cashen, M.A. Schroeder, M.A. Jacoby, S.E. Heath, K. Luber, A. Janke, N. Hantel, M.J. Khan, R.W. Sukhanova, W. Knoebel, T.A. Stock, M.J. Graubert, P. Walter, D.C. Westervelt, J.F. Link, T.J. DiPersio, Ley, TP53 and Decitabine in Acute Myeloid Leukemia and Myelodysplastic Syndromes, N. Engl. J. Med. 375 (21) (2016) 2023–2036, https://doi.org/10.1056/NEJMoa1605949.

[32] G.L. Uy, E.J. Duncavage, G.S. Chang, M.A. Jacoby, C.A. Miller, J. Shao, S. Heath, K. Elliott, T. Reineck, R.S. Fulton, C.C. Fronick, M. O'Laughlin, L. Ganel, C.N. Abboud, A.F. Cashen, J.F. DiPersio, R.K. Wilson, D.C. Link, J.S. Welch, T.J. Ley, T.A. Graubert, P. Westervelt, M.J. Walter, Dynamic changes in the clonal structure of MDS and AML in response to epigenetic therapy, Leukemia 31 (4) (2017) 872–881, https://doi.org/10.1038/leu.2016.282.

[33] J. Frikeche, A. Clavert, J. Delaunay, E. Brissot, M. Grégoire, B. Gaugler, M. Mohty, Impact of the hypomethylating agent 5-azacytidine on dendritic cells function, Exp. Hematol. 39 (11) (2011) 1056–1063, https://doi.org/10.1016/j.exphem.2011.08.004.

[34] M. Scurr, T. Pembroke, A. Bloom, D. Roberts, A. Thomson, K. Smart, H. Bridgeman, R. Adams, A. Brewster, R. Jones, S. Gwynne, D. Blount, R. Harrop, R. Hills, A. Gallimore, A. Godkin, Low-dose cyclophosphamide induces antitumor T-Cell responses, which associate with survival in metastatic colorectal Cancer, Clin. Cancer Res. 23 (22) (2017) 6771–6780, https://doi.org/10.1158/1078-0432.CCR-17-0895.

[35] E. Poplin, H. Smith, B. Behrens, B. Redman, L. Flaherty, J. Neidhart, D. Alberts, SWOG 8825: melphalan GM-CSF: a phase I study, Gynecol. Oncol. 44 (1) (1992) 66–70.

[36] S. Jones, E. Winer, C. Vogel, L. Laufman, L. Hutchins, M. O'Rourke, B. Lembersky, D. Budman, J. Bigley, J. Hohneker, Randomized comparison of vinorelbine and melphalan in anthracycline-refractory advanced breast cancer, J. Clin. Oncol. 13 (10) (1995) 2567–2574, https://doi.org/10.1200/JCO.1995.13.10.2567.

[37] A. Palumbo, A. Bertola, P. Musto, T. Caravita, V. Callea, M. Nunzi, M. Grasso, P. Falco, C. Cangialosi, M. Boccadoro, Oral melphalan, prednisone, and thalidomide for newly diagnosed patients with myeloma, Cancer 104 (7) (2005) 1428–1433, https://doi.org/10.1002/cncr.21342.

[38] P. Mougenot, M. Fabbro, F. Bressolle, D. Pouessel, S. Culine, F. Pinguet, Phase II study of melphalan as a single-agent infused over a 24-hour period with individual adapted dosing in patients with recurrent epithelial ovarian cancer, Oncol. Rep. 15 (1) (2006) 237–241.

[39] S. Cesaro, G. Meloni, C. Messina, M. Pillon, A. Proglia, E. Lanino, M. Caniggia, S. Bagnulo, A. Pession, F. Locatelli, High-dose melphalan with autologous hematopoietic stem cell transplantation for acute myeloid leukemia: results of a retrospective analysis of the Italian Pediatric Group for Bone Marrow Transplantation, Bone Marrow Transplant. 28 (2) (2001) 131–136, https://doi.org/10.1038/sj.bmt.

1703122.
[40] G. Bug, J. Atta, S.A. Klein, B. Hertenstein, L. Bergmann, S. Boehrer, S. Mousset, D. Hoelzer, H. Martin, High-dose melphalan is an effective salvage therapy in acute myeloid leukaemia patients with refractory relapse and relapse after autologous stem cell transplantation, Ann. Hematol. 84 (11) (2005) 748–754, https://doi.org/10.1007/s00277-005-1075-8.
[41] J. Gullbo, E. Lindhagen, S. Bashir-Hassan, M. Tullberg, H. Ehrsson, R. Lewensohn, P. Nygren, M. de La Torre, K. Luthman, R. Larsson, Antitumor efficacy and acute toxicity of the novel dipeptide melphalanyl-p-L-fluorophenylalanine ethyl ester (J1) in vivo, Invest. New Drugs 22 (4) (2004) 411–420, https://doi.org/10.1023/B:DRUG.0000036683.10945.bb.
[42] S. Strese, S.B. Hassan, E. Velander, C. Haglund, M. Hoglund, R. Larsson, J. Gullbo, In vitro and in vivo anti-leukemic activity of the peptidase-potentiated alkylator melflufen in acute myeloid leukemia, Oncotarget (2016), https://doi.org/10.18632/oncotarget.13856.
[43] M. Piedfer, D. Dauzonne, R. Tang, J. N'Guyen, C. Billard, B. Bauvois, Aminopeptidase-N/CD13 is a potential proapoptotic target in human myeloid tumor cells, FASEB J. 25 (8) (2011) 2831–2842, https://doi.org/10.1096/fj.11-181396.
[44] Å. Berglund, A. Ullén, A. Lisyanskaya, S. Orlov, H. Hagberg, B. Tholander, R. Lewensohn, P. Nygren, J. Spira, J. Harmenberg, M. Jerling, C. Alvfors, M. Ringbom, E. Nordström, K. Söderlind, J. Gullbo, First-in-human, phase I/IIa clinical study of the peptidase potentiated alkylator melflufen administered every three weeks to patients with advanced solid tumor malignancies, Invest. New Drugs 33 (6) (2015) 1232–1241, https://doi.org/10.1007/s10637-015-0299-2.