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Article

Experience with Autologous Stem Cell Transplantation in Patients with Acute Myeloid Leukemia

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Abstract

Objectives: Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults, and consolidation with autologous hematopoietic stem cell transplantation (HSCT) in AML patients represents an alternative therapeutic option in the absence of related or unrelated donors, in the elderly or in patients with good or standard risk. In this retrospective analysis, the data were evaluated from a total of 47 AML patients who underwent autologous hematopoietic stem cell transplantation between November 2012 and March 2023 at the Bone Marrow Transplantation Unit of Medicalpark Izmir Hospital. The present study also investigates the factors affecting overall survival (OS) and progression-free survival (PFS). **Methods:** This study is a retrospective evaluation of the data obtained from 47 patients with AML who underwent an autologous HSCT. **Results:** 24 patients were female, and 23 patients were male. The median age at diagnosis was 39 years (range: 18-68 y). The mean OS from diagnosis to the last follow-up or death was 26 months (4-116 months), and the PFS was 20 months (3-69 months). An assessment of the factors that influenced OS and PFS showed no significant association of NPM positivity, gender, risk group, response to first-line chemotherapy, transplantation at CR (Complete remission) 1 or CR2, LDH (lactate dehydrogenase), CD34 count, and the day of neutrophil engraftment with OS or PFS. In patients with FLT3 (fms benzeri tirozin kinaz 3) positivity, OS was significantly shorter ($p < 0.05$), while PFS was not significantly different ($p=0.21$). **Conclusions:** Consolidation with auto-HSCT in AML patients can be preferred in subjects with good or intermediate 1 risk category according to ELN (European leukemia net) criteria, or in subjects with intermediate 2 or poor-risk category who have no related or unrelated donor.

Keywords: acute myeloid leukemia; autologous stem cell transplantation

1. Introduction

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults, with a reported incidence of 3-5 per 100,000 individuals. The major determinants of prognosis include age, performance status, comorbid conditions, and cytogenetic and molecular characteristics of the leukemic clone. Following standard induction therapy, complete remission (CR) can be achieved in 50-75% of patients with AML, except for those with AML-M3 [1]. Most patients with CR recur unless intensive chemotherapy, autologous transplantation, or allogeneic transplantation is administered for consolidation [2]. Allogeneic stem cell transplantation (allo-HSCT) is the most effective therapeutic approach for patients after remission, consolidation with autologous hematopoietic stem cell transplantation represents an alternative therapeutic option in the absence of related or unrelated donors, elderly patients or patients with good or standard risk. Although autologous transplantation offers certain advantages, such as easy harvesting of the graft, practicality in elderly patients, absence

of graft versus host disease (GVHD), and lower morbidity and mortality, it is also associated with a higher risk of relapse due to the lack of the graft versus leukemia effect [3].

Later 1970s saw the introduction, auto-HSCT was introduced in AML CR1 and CR2 patients with no sibling donors; it was initially performed using bone marrow and subsequently with peripheral stem cells. In the absence of fully matched sibling donors for intermediate risk AML CR1 patients, a comparison of consolidation therapy with autologous transplantation or chemotherapy showed longer leukemia-free survival (LFS) than the former therapeutic approach [4,5]. The use of peripheral blood for autologous transplantation as the source of stem cells resulted in a decreased transplant-related mortality (TRM) rate, which fell from 15-20% to 5-10% [6].

In our center, consolidation with auto-HSCT in AML patients is performed in subjects with good or intermediate 1 risk categories according to ELN criteria or in subjects with intermediate 2 or poor risk categories who have no related or unrelated donor. [7]

This retrospective analysis evaluated data from 47 AML patients who underwent autologous hematopoietic stem cell transplantation between November 2012 and March 2023 at the Bone Marrow Transplantation Unit of Medicalpark Izmir Hospital. Factors affecting OS and PFS are also examined.

Evaluation and Definitions

PFS was defined as the time from transplantation to relapse or death from any cause, and OS was defined as the date of diagnosis to death or the last follow-up. Engraftment was demonstrated by increased neutrophil and platelet counts unsupported by transfusions. Neutrophil engraftment after transplantation was defined as an absolute neutrophil count (ANC) exceeding 500/mL for three consecutive days. The first of these 3 consecutive days was considered the day of engraftment. Platelet recovery was defined as the time after transplantation needed to achieve a blood platelet count exceeding 20,000/mL without transfusion support for two consecutive days.

2. Materials and Methods

This study was a retrospective evaluation of data from 47 patients with AML who underwent autologous hematopoietic stem cell transplantation at Izmir Medicalpark Hospital between November 2012 and March 2023.

Inclusion criteria for the study were: absence of secondary AML, ECOG performance status of 0-1, all patients in the good risk group according to ELN criteria, and those in the moderate and poor risk groups but without related, unrelated, or haploidentical stem cell donors for allogeneic transplantation, normal liver and kidney function tests, echocardiography, and respiratory function tests, and being in remission before transplantation. Patients with pre-existing severe coronary artery disease, other cancers, uncontrolled diabetes, or advanced chronic obstructive pulmonary disease were excluded from the study.

All patients whose molecular markers were found to be positive by PCR (polymerase chain reaction) at the time of diagnosis received consolidation therapy after achieving remission. MRD (minimal residual disease) was tested in the patients by PCR, and autologous stem cells were collected from the patients after the MRD became negative.

All patients received a regimen including busulfan and cyclophosphamide at myeloablative doses (busulfan 3.2 mg/kg/day for 4 days, cyclophosphamide 60 mg/kg/day for 2 days) and autologous peripheral hematopoietic cells harvested from peripheral blood and stored at -80 C were administered via a central venous line after thawing.

As an infection prophylaxis strategy, all patients were admitted to isolated HEPA filter rooms with visitor restriction in the bone marrow transplant unit. All subjects received prophylaxis with levofloxacin 500 mg per oral, acyclovir 400 mg per oral 3 times daily, and fluconazole 400 mg per oral, before the onset of fever.

2.1. Cytogenetic Analysis

Bone marrow aspiration material or peripheral blood was collected into 5 cc heparinized tubes for the purposes of the study. Bone marrow aspiration samples were studied by applying the 24-hour or overnight culture method. Peripheral blood samples were studied by modifying the 72-hour culture method developed by Moorehead et al. [8].

2.2. Statistical Analysis

Data are expressed as mean \pm SD for normally distributed continuous variables, median (minimum-maximum) for skew-distributed continuous variables, and frequencies for categorical variables. Pearson's chi-squared test was used to compare categorical variables. The means of normally distributed continuous variables were compared using analysis of variance. Skew-distributed continuous variables were compared using the Mann-Whitney U-test. OS was calculated as the time from the date of diagnosis to the date of the last contact or death. LFS was calculated from diagnosis until the last follow-up or until leukemic progression. The Statistical Package for Social Sciences (SPSS) for Windows version 15.0 (SPSS Inc., Chicago) was used for the analysis, and a two-sided p value of <0.05 was considered significant.

3. Results

Of the study subjects, 24 (51.1%) were female and 23 (48.9%) were male. The median age at diagnosis was 39 years (range: 18-68 y). The median hemoglobin level, leukocyte count, and bone marrow blast percentage at the time of diagnosis were 8 g/dl (3.5-12.3 g/dl), 36700/mm³ (2000-270.000/mm³), and 76% (23-97%), respectively (Table 1).

Table 1. General characteristics of the patients.

Patients	47 (Number)	Percentage (%)
Female	24	51.1%
Male	23	48.9%
Median age at diagnosis	39	(18-68)
Median leukocyte count	36700/mm ³	(2000-270000)
Median Hb	8	(3.5-12.3)
LDH	471	(220-1477)
BM blast at diagnosis	76	(23-97)
Apheresis, days	2	(1-5 days)
Mean CD34 product count	10,14	(4.56-53.34)
Time from diagnosis to transplant	7.94 months	(2-90 months)
Neutrophil engraftment	11	(9-19)
Platelet engraftment	21.17	(9-110)
Need for RBC transfusion	2,7	(0-1)1
Need for platelets	4,74	(0-23)
Undergoing NPA	41	(87.2%)
Possible IPA	3	(6.4%)
TRM	1	(2.1%)
100-day mortality	1	(2.1%)
Total duration of follow up	26 months	(4-116 months)
PFS	20 months	(3-69 months)
Follow up after transplant	18.08 months (2.5-51 months)	
Final status	Living patients	30(63.8%)
	Exitus patients	17(36.2%)

Hb:hemoglobin, LDH: Lactate dehydrogenase, RBC :red blood cell, NPA : neutropenic fever, IPA:invasive pulmonary aspergillosis, TRM: Transplant-related mortality, PFS: Progression-free survival.

ECOG (Eastern Cooperative Oncology Group) performance score at the time of diagnosis was 0 in seven patients (14.9%), 1 in 37 patients (78.7%), and 2 in three patients (6.4%). With regard to ELN risk scores, cytogenetic and molecular analyses were performed in 44 patients at the time of diagnosis, and 22 (50%), 14 (31.8%), and 8 (18.2%) patients had good, intermediate, and poor risk statuses, respectively.

The patient population consisted of patients in the good-risk group and those in the moderate-risk group who had no related unrelated or haploidentical allogeneic donors. Autologous transplantation was performed, and all the patients were in remission.

In terms of molecular characteristics, 10 patients were negative for in molecular markers, while 11 patients presented NPM (nucleophosmin) positivity only, 6 patients had FLT3-ITD positivity only, 4 patients had combined NPM and FLT3-ITD positivity, 4 had t(8,21) positivity, 4 had inversion 16 positivity, and 2 had 11q23 positivity (Table 2).

Table 2. Molecular characteristics of the patients.

Molecular	Frequency	Percentage
Not performed	6	12.8
Negative	10	21.1
NPM	11	23.2
FLT3-ITD	6	12.8
FLT3-ITD-NPM	4	8.6
t(8,21)	4	8.6
inv16	4	8.6
11q23	2	4.3
Total	47	100

FLT3: Fms like tyrosine kinase 3, NPM: Nucleophosmin.

Thirty-five of the 47 patients (74.5%) responded to first-line remission induction therapy consisting of 7+3 cytosine arabinoside (ara-C) 200 mg/m²/day/ 7 days and daunorubicin 60 mg/m²/day/3 days.

Midostaurin was added to remission induction therapies for FLT3 inhibitor-positive patients in 2020 upon its arrival in our country; it was not used in consolidation or maintenance therapies, and other FLT3 inhibitors were not used.

In 12 patients, CR1 was achieved with second-line remission induction. As second-line remission induction therapy, EMA (etoposide-mitoxantrone- cytosine arabinoside) was administered to eight patients, and four patients received 7+3 ara-C-daunorubicin treatment again.

Auto-HSCT was performed in 45 patients with CR1 and two patients with CR2.

Until transplantation, 19 (40.4%) patients received 2 cycles, 12 (25.5%) patients received 3 cycles, and 12 (25.5%) patients received 4 cycles of treatment. As a mobilization regimen, 19 patients (40.4%) received 6+3 high-dose ara C-daunorubicin, 13 patients (27.7%) received etoposide (375 mg/m² per day for/2 days), 9 patients (19.1%) received high-dose ara-C, 4 patients (8.5%) received 5+2 ara C-idarubicin, and 2 patients (4.3%) received the EMA regimen. The patients were mobilized using consolidation treatment. We failed to collect sufficient stem cells in 17 patients; we administered etoposide to 13 patients, 5+2 ara C-idarubicin to 2 patients, and HIDAC (high dose cytosine arabinoside) to 2 patients as mobilization regimens.

After chemotherapy, all patients received filgrastim 0.5 MU/kg/day during a collection of stem cells. Apheresis was performed for a median of 2 days (1-5 days).

The mean CD34 count in the administered product was 10.14x10⁶/kg (4.56-53.34x10⁶).

All patients whose molecular markers were found to be positive by PCR (polymerase chain reaction) at the time of diagnosis received consolidation therapy after achieving remission. MRD (minimal residual disease) was tested in the patients by PCR, and autologous stem cells were collected from the patients after the MRD became negative.

The average time between diagnosis and transplantation was 7.94 months (2-90 months).

All patients received busulfan (3.2 mg/kg/day for /4 days IV), and cyclophosphamide (60 mg/kg/day for /2 days) as a preparative regimens. At the time of transplantation, 3 patients (6.4%) were free of mucositis, and 25 (53.2%) and 19 (41.4%) patients had grade 1-2 and grade 3-4 oral mucositis. After transplantation, neutrophil engraftment occurred at a median duration of 11 days (9-19 days), and platelet engraftment occurred at 21 days (9-110 days). During transplantation, patients received 2.7 units of erythrocytes (0-11 U) and 4.74 units of apheresis platelets (0-23 U) on average.

Forty-one patients (87.2%) experienced neutropenic fever during transplantation. Catheter infection, pneumonia, urinary tract infection, and possible invasive pulmonary aspergillosis were detected in 19, 7, 8, and 3 patients, respectively. In the first 100-day period, there was only one death, due to a gram-negative infection in a subject with no neutrophil engraftment.

The mean OS from diagnosis to the last follow-up or death was 26 months (4-116 months), and the PFS was 20 months (3-69 months) (Figure 1).

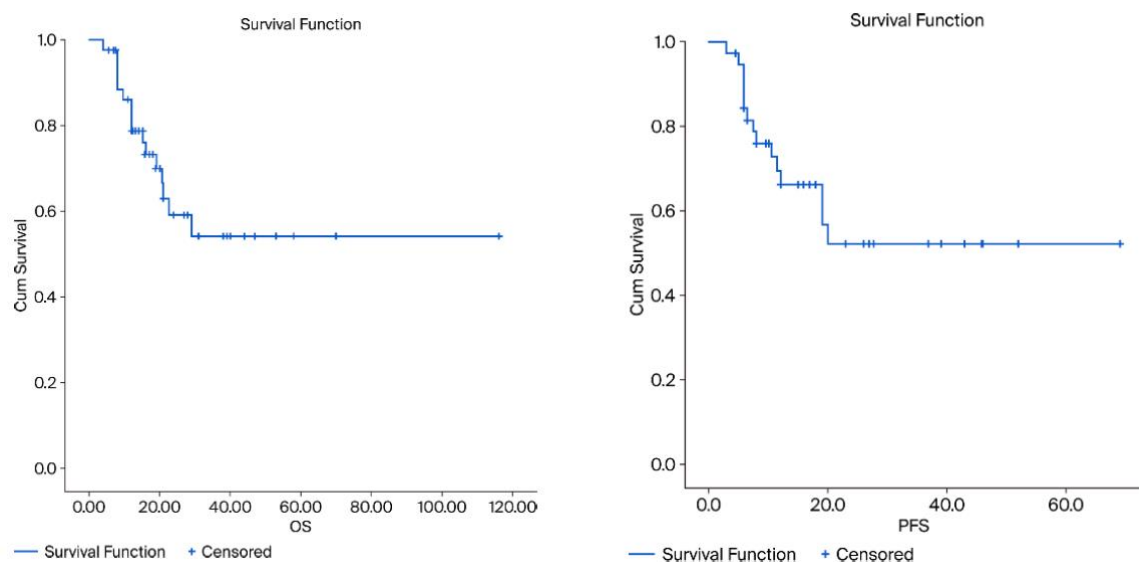


Figure 1. OAS and PFS in all of the patients.

45 patients underwent transplantation with CR1, and 17 of the 45 patients died during follow-up. The OAS and PFS graphics of patients undergoing transplantation in CR1 are shown in Figure 2.

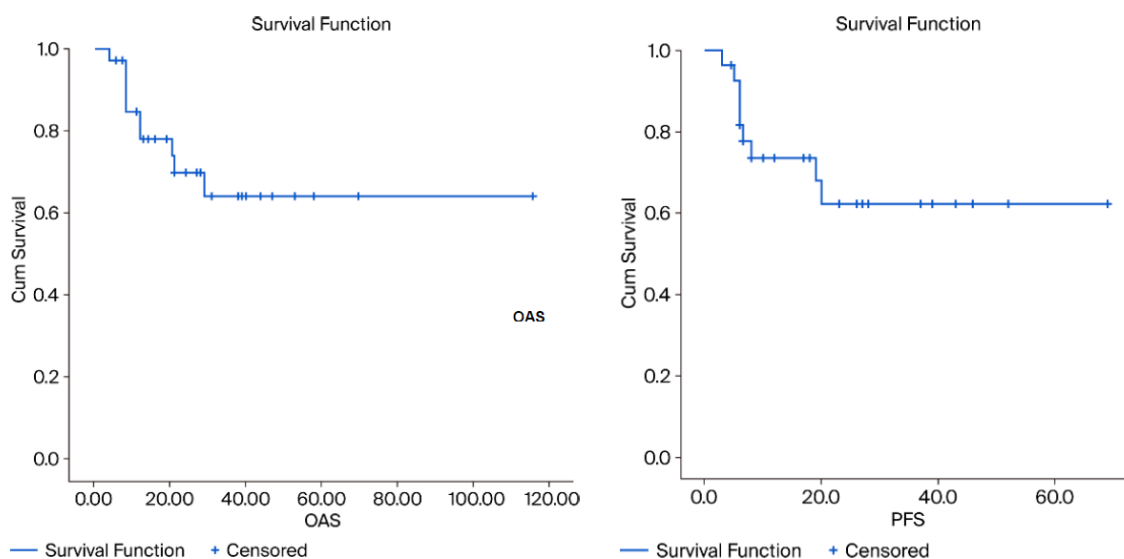


Figure 2. OAS and PFS in CR1 patients.

An assessment of the factors that influenced OS and PFS showed no significant association of NPM positivity, sex risk group, response to the first chemotherapy, transplantation at CR1 or CR2, LDH, CD34 count, and day of neutrophil engraftment with OS or PFS. In patients with FLT3 positivity, OS was significantly shorter ($p < 0.05$), whereas PFS was not significantly different ($p: 0.21$).

OS was longer in those with earlier platelet engraftment ($p: 0.004$), whereas genetic risk groups had no statistically significant effect on OS and PFS ($p: 0.093$, $p: 0.57$).

In the last evaluation, 17 of the 47 patients died (36.2%), and 30 (63.8%) were alive. Two FLT3-positive patients and all FLT3-NPM-positive patients (four patients) died during follow-up.

The causes of death were as follows: infection in one patient without neutrophil engraftment during transplantation, acute hepatitis B infection in one patient, autoimmune disease and pneumonia in one patient after transplantation, AML relapse and central nervous system bleeding in one patient, AML relapse and lung cancer in one patient, and disease progression and infection during chemotherapy with relapse in another eight patients. Four patients had MDS (myelodysplastic syndrome) after transplantation, and one these patients received unrelated allogeneic bone marrow transplantation. After transplantation, this patient died of lung infection, while two other patients died of post-chemotherapy infection. One patient remains alive. In one patient with recurrent disease, allogeneic transplantation from a fully -matched sibling donor was performed twice; however the patient died due to disease progression. The reason for not performing allogeneic transplantation in this patient was t(8,21)-positivity in the good-risk group.

4. Discussion

While consolidation therapy with allogeneic stem cell transplantation at CR1 is recommended by the ELN for AML patients in the intermediate and poor risk groups, allo-SCT is not recommended for AML patients with good risk [7]. The incidence of relapse in patients with good and intermediate risk status may be as high as 30-40% and 50- 60%, respectively, after consolidation with chemotherapy.

Most patients undergoing consolidation with high-dose chemotherapy relapse within 2-3 years of treatment [9]. In the last two decades, patients with good risk status, as well as those with no related or unrelated donors, have undergone consolidation therapy with auto-HSCT [10,11].

In a meta-analysis published in 2004 involving 1044 AML-CR1 patients, a comparison of consolidation therapy with either auto-SCT or chemotherapy revealed a lower relapse rate, better leukemia-free survival, and similar OS in the former group of patients [12]. In the absence of fully-

matched sibling donors in intermediate-risk AML CR1 patients, autologous transplantation and chemotherapy were compared as consolidation regimens, and patients who underwent autologous transplantation had a longer LFS. The use of peripheral blood as a source of stem cells for autologous transplantation is associated with a decrease in TRM from 15-20 to % to 5-10% [4,6,13].

Heini et al. found that elderly AML patients with ASCT had longer PFS (PFS: 16.3 vs. 5.1 months, $P = 0.0166$) and OS (OS: n.r. vs. 8.2 months; $P = 0.0255$) than elderly AML patients without ASCT consolidation. In addition, elderly AML patients undergoing ASCT had comparable PFS ($P = 0.9462$) and OS ($P = 0.7867$) to AML patients aged < 65 years who received ASCT consolidation in CR1. Their data suggest that ASCT is an option for elderly patients with AML who appear to benefit from autologous consolidation in a similar way to younger patients with AML [14].

A comparison of auto-SCT with allogeneic transplantation from fully a matched sibling donor transplantation (MSD-SCT) showed similar OS for both regimens, although the relapse rate was higher and TRM was lower with auto-SCT, while MSD-SCT was associated with an LFS advantage despite having a higher TRM [15,16].

In a phase III prospective randomized trial by HOVON and the Swiss group, consolidation with auto-SCT and high-dose chemotherapy was compared in AML CR1 patients, showing lower relapse rates (58% vs. 70%, $p=0.02$) and higher 5-year LFS (38% vs. 29%, $p=0.065$) in the auto-SCT group [17].

In a retrospective analysis by (Center for International Blood and Marrow Transplantation Research (CIBMTR), the 3-year LFS in AML patients undergoing auto-SCT at CR1 and CR2 was 50% and 30%, respectively. The CIBMTR The Center for International Blood and Marrow Transplant Researc) recommends auto-SCT suitable for consolidation at CR1 in the absence of a fully-matched sibling donor [18–20].

In our center, consolidation therapy for all AML patients in the good risk group is performed via autologous transplantation, therefore there is no control group receiving consolidation therapy with HIDAC. However, when compared to the results of other studies that included consolidation therapy with HIDAC, our patient group had a lower number of patients in the intermediate and poor-risk groups, yet a 3-year OS rate of 55% and a 3-year PFS rate of 52% were observed.

Patients were given a sufficient number of stem cells with a product containing $10.14 \times 10^6/\text{kg}$ CD34. Engraftment was achieved in all patients after transplantation. There was no engraftment failure.

In our patient group, after a mean follow-up of 18.08 months (2.5-51 months) 14 patients died due to relapse (29.78%) and 3 patients (3.68%) died due to infection without a relapse, while 30 patients (63.8%) are still in remission. Again 1-year, 2-year and 3-year OS rates were 86.2%, 60%, and 55%, respectively, whereas the corresponding PFS rates were 74.2%, 55%, and 52%, respectively.

The mean OS from diagnosis to the last follow-up or death was 26 months (4-116 months), and the PFS was 20 months (3-69 months)

OS and PFS did not differ significantly according to NPM positivity, cytogenetic risk group, response to the first chemotherapy, transplantation at CR1 or CR2, CD34 count in the product, or the day of neutrophil engraftment. FLT3-positive patients had a significantly shorter OS ($p < 0.05$), whereas PFS was not affected by FLT3 positivity ($p = 0.21$).

In the last evaluation, 17 of the 47 patients died (36.2%), and 30 (63.8%) were alive.

Although neutropenic fever developed in 87.2% of patients admitted for autologous transplantation, only one patient died from gram-negative infection in a subject with no neutrophil engraftment. (100 days TRM 2.1%)

Of the 17 patients who died, only 3 died from infection without relapse. The other 14 patients experienced relapse and died from disease progression and other causes. Two FLT3-positive patients and all FLT3-NPM-positive patients (four patients) died during follow-up.

In a study by Nagler et al., who retrospectively analyzed EBMT(European bone marrow transplantation) data in 952 AML patients, the 2-year OS, LFS, relapse incidence (RI), and NRM among patients receiving IV busulfan prior to auto-SCT were 67%, 53%, 40%, and 7%, respectively; however, there was no significant difference in 2-year LFS and RI between 815 patients receiving

transplantation at CR1 (%52 and %40, respectively) and 137 patients receiving transplantation at CR2 (58% and 35%, respectively). A comparison of cytogenetic groups showed a 2-year LFS of 63%, 52%, and 37% in patients with good, intermediate, and poor risk status, respectively ($p=0.01$) [21].

In AML patients with normal cytogenetics and intermediate risk status, allogeneic stem cell transplantation is the best therapeutic option in the presence of a fully -matched sibling donor. In a study by Mirzutani et al. involving patients receiving high-dose therapy supported by autologous stem cell transplantation in the absence of sibling donors, as well as patients undergoing transplantation from a fully EBMT(European bone marrow transplantation), LFS after auto-SCT was significantly shorter with increasing numbers of chemotherapy courses until remission in comparison with MUD-SCT. (match unrelated stem cell transplantation) There was no significant difference in OS and LFS between the other subgroups in other arm of the study [22].

Conclusions; In light of all this data, autologous stem cell transplantation can be performed in AML patients at good risk group or in elderly intermediate 1 risk AML patients who do not have a suitable donor for allogeneic transplantation, after achieving MRD negativity following remission induction and/or one course of consolidation therapy. In autologous transplantation, the lower TRM and absence of GVHD compared to allogeneic stem cell transplantation are still significant advantages.

Today, the effectiveness of remission induction therapy is increased by adding FLT3 inhibitors, bcl-2 inhibitors, and hypomethylating agents to the treatment. We believe that the use of these new agents in maintenance therapy after autologous transplantation in this patient group will reduce post-transplant relapse rates.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: S. Kahraman conducted. She activities of designing and planning the study, designing the article, making statistics, and writing the article. S.Cagirgan found patients in the outpatient clinic, obtained blood samples, and evaluated the results.

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Informed Consent Statement: All participants provided informed consent to participate in the study.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AML	Acute myeloid leukemia
OS	Overall survival
PFS	Progression-free survival
LFS	Leukemia-free survival
TRM	Transplant-related mortality
HSCT	Hematopoietic stem cell transplantation
allo-HSCT	Allogeneic stem cell transplantation
MSD-SCT	Matched sibling donor transplantation
MUD-SCT	Match unrelated stem cell transplantation)
CR	Complete remission
LDH	Lactate dehydrogenase
FLT3	Fms like tyrosine kinase 3
ELN	European leukemia net

GVHD	Graft versus host disease
ANC	Absolute neutrophil count
SPSS	The Statistical Package for Social Sciences
ECOG	Eastern Cooperative Oncology Group
NPM	Nucleophosmin
Ara-C	Cytosine arabinoside
EMA	Etoposide-mitoxantrone- cytosine arabinoside
HIDAC	High dose cytosine arabinoside
MDS	Myelodysplastic syndrome
CIBMTR	The Center for International Blood and Marrow Transplant Research
EBMT	European bone marrow transplantation
MRD	Minimal residual disease

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