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Clofarabine and Adult Acute Myeloid Leukemia

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Abstract

Acute myeloid leukemia (AML) is a clonal, malignant disease of hematopoietic tissues that is characterized by accumulation of abnormal blast cells, principally in the marrow and impaired production of normal blood cells. The unsatisfactory clinical outcomes of AML patients urged the development of new therapy strategies, one of which includes the implementation of new nucleoside analogs. Clofarabine has offered new promising perspectives within induction and consolidation therapies. This chapter will evaluate the efficacy and tolerability of clofarabine as a single agent and in combination therapy, including hematopoietic stem cell transplantation, for AML patients.

Keywords: Clofarabine, adult acute myeloid leukemia

1. Introduction

Acute myeloid leukemia (AML) is defined as a clonal disorder characterized by the uncontrolled proliferation and survival of immature myeloid progenitors that undergo a differentiation block at various maturation steps, leading to accumulation of leukemic cells in bone marrow and inhibition of normal hematopoiesis [1].

The treatment of AML patients includes both induction and consolidation chemotherapy. The overall goal of induction is to provide complete remission (CR) – <5% blast cells in the bone marrow, absolute neutrophils count >1,000/ μ l and platelet count >100,000/ μ l. Consolidation or post-remission chemotherapy, with or without hematopoietic stem cell transplantation (HSCT), further improves the outcome by decreasing relapses.

Unfortunately, the therapy of AML patients has shown only partial progress over the past 20 years. For many years, the conventional induction chemotherapy for AML patients has consisted of cytarabine plus an anthracycline (7+3 regimen; i.e., 7 days of treatment with

cytarabine and 3 days of treatment with an anthracycline) [2]. Although the overall response rate (ORR) to this combination is 70–80%, only 30–50% of them remains alive for more than 5 years since most patients will relapse and die from their disease or associated complications [3]. Numerous modifications of this combination (using different anthracyclines, adding a third agent such as mitoxantrone or etoposide, extending the number of days of cytarabine, priming of leukemia blasts with hematopoietic growth factors) failed to improve response rates and overall survival (OS) [4–8].

Some 15–25% de novo AML patients fail to achieve CR because of resistance to treatment and 40% of CR patients will relapse within 2 years. Although several different salvage chemotherapy combinations have been administered to patients with refractory/relapsed AML, the prognosis in this subset of patients is very poor, with a CR rate ranging from 30 to 50% [9]. The goal of salvage chemotherapy is to provide a bridge to HSCT aimed at prolonged OS.

Older AML patients do very poorly [10]. Standard induction therapy with 7+3 in older patients has resulted in CR rates ranging from 30 to 50% and induction mortality rates of 10–35% [11,12]. Remissions are usually transient and rarely last more than 12 months. The median OS is 5–10 months, with less than 10% of patients remaining in remission at 3 years [13].

The unsatisfactory clinical outcomes of patients with AML urged the development of new nucleoside analogs. Over the past years, a series of deoxyadenosine analogs have been synthesized with the aim to improve drug efficacy [14]. Several of these compounds, such as fludarabine, cladribine, nelarabine, or gemcitabine, have been introduced into the clinical practice for hematological malignancies. Clofarabine has progressively gained some attention as a possible new weapon available for patients with AML [15] since this drug could be implemented in induction and consolidation therapy including HSCT.

2. Clofarabine pharmacology

Clofarabine (2-chloro-2'-fluoro-deoxy-9- β -D-arabinofuranosyladenine) is a second-generation nucleoside analog synthesized in the 1990s. The rationale behind its design was to combine the most favorable pharmacokinetic properties of fludarabine and cladribine. Like cladribine and fludarabine, clofarabine is toxic to both non-proliferating human lymphocytes and rapidly proliferating cells. Clofarabine is believed to enter cells by both facilitated and active nucleoside transport mechanisms as well as, at higher concentrations and upon longer exposure, by passive diffusion across lipid membranes [16]. Once inside the cell, clofarabine is phosphorylated to its active triphosphate form by cellular enzymes, including deoxycytidine and deoxyguanosine kinases [17]. Clofarabine metabolites are retained by cells to a greater extent than are the metabolites of cladribine. This could lead to more pronounced anti-tumoral effect and higher hematological toxicity of the former [18]. The anti-cancer activity of clofarabine involves three major mechanisms: inhibition of deoxyribonucleic acid (DNA) synthesis, inhibition of ribonucleotide reductase, and direct induction of apoptosis [19,20]. Clonogenicity assays showed a strong inverse correlation between cell survival and incorporation of clofarabine monophosphate into DNA, thus suggesting this latter mechanism as a critical step for the cytotoxic potential of this drug [18].

Given its mechanisms of action, clofarabine was predicted to work separately or synergistically with other chemotherapeutic agents such as other purine nucleoside analogs and DNA-damaging or cross-linking agents such as anthracyclines and platinum agents [21]. Therefore, it is not a big surprise that clofarabine has gained so much interest in the past years. A lot of clinical trials had been performed and relevant English language studies are summarized in Table 1.

Reference	Study phase	AML study population	Patients, N	Median age	ORR %	CR %	Median OS (weeks)	Induction mortality %
Combination with cytarabine								
Faderl et al. [27]	2	Untreated	60	61	60	52	41	7
Becker et al. [28]	2	Untreated	50	53	82	76	97	2
Faderl et al. [30]	3	Untreated	70	71	67	63	46	16
Faderl et al. [32]	2	Untreated	60	70	66	58	51	7
Martínez-Cuadrón et al. [31]	2	Untreated	11	74	27	27	Not reported	73
Becker et al. [40]	1–2	Relapsed/refractory	46	53	61	46	36	11
Tse et al. [42]	2	Relapsed/refractory	21	45		43	Not reported	9.5
Scappini et al. [43]	2	Relapsed/refractory	47	50.5	61.5	51	28	13
Faderl et al. [38]	2	Relapsed/refractory	25	63	40	28	23	3
Faderl et al. [39]	3	Relapsed/refractory	163	67	47	35	26	16
Combination with idarubicin and cytarabine								
Willemze et al. [23]	1	Untreated	25	55.9	84	76	Not reported	16
Selleslag et al. [24]	1/2	Untreated	62	50	84	74	74% at 1 year	
Nazha et al. [29]	2	Untreated	59	48	79	74	Not reached	4
Combination with daunorubicin								
Vigil et al. [34]	2	Untreated	21	69	38	28.6	45	14
Monotherapy								
Kantarjian et al. [35]	2	Untreated	112	71	46	38	41	16
Burnett et al. [36]	2	Untreated	106	71	48	32	19	18
Burnett et al. [37]	3	Untreated	203	74	38	22	13% at 2 years	32
Kantarjian et al. [26]	2	Relapsed/refractory	31	54	55	42	Not reported	5

List of abbreviations:
AML – acute myeloid leukemia; ORR – overall response rate; CR – complete remission; OS – overall survival

Table 1. Studies evaluating clinical responses of patients with acute myeloid leukemia to clofarabine combination and monotherapy

According to data from phase 1 trial, the maximum tolerated dose (MTD) of clofarabine, given as 1-h infusion daily for 5 days in patients with hematological malignancies, was determined to be 40 mg/m²/day. The dose-limiting toxicity (DLT) was hepatotoxicity. Among 16 AML patients 16% ORR was registered, as two of them achieved CR and three had CR with incomplete platelet recovery (CRi) [22].

In order to determine the MTD of clofarabine combined with 3+10 induction regimen (idarubicin + cytarabine) in adults with untreated intermediate and unfavorable risk AML or high-risk myelodysplastic syndrome (MDS), a phase 1 of the EORTC/Gimema AML-14A study was carried out [23]. The study included 25 patients with median age of 55.9 years that received clofarabine 1-h infusion (arm A) or push injection (arm B) for 5 days at the dose level of 5 × 10 mg/m²/day or 5 × 15 mg/m²/day in an algorithmic dose escalation 3+3 design. Patients in CR were planned to receive a consolidation course (intermediate dose cytarabine, idarubicin). Primary endpoint was safety and tolerance as measured by dose-limiting toxicity (DLT). The clofarabine dose of 5 × 15 mg/m²/day resulted in four DLTs and three patients' withdrawals due to adverse events not classified as DLT while clofarabine dose of 5 × 10 mg/m²/day was associated with one DLT and no treatment withdrawals. The latter clofarabine dose was considered by the authors as MTD. CR and CRi were achieved in 21 patients (11/12 (92%) receiving clofarabine 10 mg/m²/day; 10/13 (77%) receiving clofarabine 15 mg/m²/day).

Selleslag et al. reported the final results of the combined phase 1 and 2 parts of the EORTC/Gimema AML-14A study that explored the antitumor activity of clofarabine containing induction combination regimens at the aforementioned phase 1 selected dosage schedules [24]. Patients 18–60 years old (*n* = 57) with previously untreated intermediate- and bad risk AML or high risk MDS (*n* = 5) were included in the study. Clofarabine was administered as 1-h infusion (Arm A) or push injection (Arm B) at 10 mg/m² on days 2, 4, 6, 8, and 10 in combination with cytarabine (100 mg/m²/day on days 1–10) and idarubicin (10 mg/m²/day, on days 1, 3, and 5). One cycle of consolidation including cytarabine (500 mg/m² every 12 h on days 1–6) and idarubicin (10 mg/m²/day on days 4, 5, and 6) was administered in patients who achieved a CR/CRi. The two tested clofarabine (5×10 mg/m²) containing regimens yielded an impressive (84%) CR/CRi rate, whereas OS of both regimens was 74% at 1 year.

2.1. Clofarabine in AML induction therapy

2.1.1. Newly diagnosed AML patients

2.1.1.1. Clofarabine combination therapy

The benefit of combining clofarabine with cytarabine, was hypothesized given in vitro data showing increased conversion of cytarabine to its active triphosphate form (ara-CTP) via deoxycytidine kinase when cytarabine was given after clofarabine, and this drug combination produced synergistic killing of myeloid leukemia cells [25]. The infusion of cytarabine was begun 4 h after starting clofarabine as previous studies demonstrated that this was the time of maximum clofarabine triphosphate accumulation in leukemia blasts [26].

The combination of clofarabine and cytarabine was studied as an induction therapy by Faderl and colleagues [27]. They had enrolled 60 patients with newly diagnosed AML or high-risk

MDS patients. The median age of the study group was 61 years (range, 50–74 years). The induction therapy consisted of intravenous clofarabine 40 mg/m² for 5 days (days 2 to 6) followed 4 h later by intravenous cytarabine 1 g/m² on days 1 to 5. On day 1, only cytarabine was administered; on day 6, only clofarabine was administered. This cytarabine infusion rate generated cytarabine concentrations in plasma that maximize the rate of ara-CTP accumulation in leukemic blasts. Cycles were repeated every 4 to 6 weeks depending on leukemia response, recovery of normal hematopoiesis, or occurrence of treatment-related toxicities. Patients were allowed to receive a maximum of three induction cycles or until a CR, CRi or partial response (PR) was achieved. The maintenance therapy given as a consolidation in responding patients consisted of up to six additional courses with clofarabine 40 mg/m² daily followed 4 h later by cytarabine 1 g/m² daily for three consecutive days. Of 60 patients, 48% had secondary AML, 50% had abnormal karyotypes, and 21% showed *FLT3* gene abnormalities. The ORR was 60% with 52% of CR and 8% of CRi. Induction death was observed in 7% of the patients. Despite the good CR rate, OS did not appear to be improved compared with other induction regimens.

In order to improve the clinical outcomes of previously untreated AML patients, combination therapy of clofarabine and cytarabine was tested with granulocyte colony-stimulating factor (G-CSF) priming [28]. This therapy regimen was named GCLAC and 50 newly diagnosed patients with AML or advanced MDS or advanced myeloproliferative neoplasm (MPN) were included in the study. The median age was 53 years (range, 22–64 years). The treatment consisted of intravenous clofarabine (30 mg/m²/day) followed 4 h later by intravenous cytarabine (2 g/m²/day) for five consecutive days. Patients received daily subcutaneous G-CSF priming from the day before chemotherapy until neutrophil recovery. A second induction cycle was administered if a patient had >5% marrow blasts 21 days post induction. Patients in remission received up to three post remission cycles with reduced dosages. Twenty-six percent of the patients had unfavorable, 64% intermediate, and 8% favorable cytogenetics. The ORR was 82% with 76% of CR and 6% of CRi. For patients with an antecedent hematologic disorder (AHD), the CR rate was 65%, compared to 85% for those without an AHD. Seventy percent of AML patients with the internal tandem duplication of fms-like tyrosine 3 (*FLT3*-ITD) achieved CR after treatment with GCLAC without the inclusion of a *FLT3* inhibitor. The 60-day mortality was 2%. Median overall survival was 24.3 months and at a median follow-up of 15 months, 32 patients were alive of whom 21 were in remission. The authors concluded that front-line GCLAC is a well-tolerated, effective induction regimen for AML and advanced myelodysplastic or myeloproliferative disorders although GCLAC was not compared with other induction regimens.

The combination of clofarabine, cytarabine, and idarubicin was applied in phase 2 study with newly diagnosed AML patients less than 60-years-old. The study included 59 patients with median age of 48 years (range, 19–60 years). The therapy consisted of clofarabine (20 mg/m²) and cytarabine (1 g/m²) both for 5 days and idarubicin (10 mg/m²) for 3 days, followed in patients in remission by up to six consolidation cycles with reduced dosages. The ORR was 79%. With a median follow-up of 10.9 months, the median OS was not reached and the median event-free survival (EFS) was 13.5 months. Four-week and eight-week mortality were 2% and 4%, respectively. Forty-two percent of the patients proceeded with allogeneic SCT in first remission. The authors performed a retrospective comparison with patients treated with idarubicin and cytarabine, and concluded that the addition of clofarabine showed an apparent

improvement in both OS and EFS. According to authors, patients ≤ 40 years and those with unfavorable karyotype benefited most of triple combination therapy [29].

Several different clofarabine combinations were tested in previously untreated older AML patients to find more effective and less toxic treatments.

The combination of clofarabine and low-dose cytarabine (LDAC) was tested as front-line therapy in previously untreated older AML patients aged 60 years and older. The study included 70 patients with newly diagnosed AML and median age was 71 years (range, 60–83 years). The patients were randomized to treatment with clofarabine alone versus clofarabine plus LDAC [30]. Fifty percent of the patients had secondary AML/MDS, 50% had abnormal karyotypes, and 11% showed *FLT3* abnormalities. Clofarabine (30 mg/m²/day) was given intravenously over 1 h (days 1 through 5) and in combination arm cytarabine was added (20 mg/m²/day) given by subcutaneous injection daily for 14 days (days 1 through 14), but beginning 4 h after the start of clofarabine. On days 1 to 5, administration of clofarabine preceded the injection of cytarabine by approximately 4 h. A second induction cycle was permitted for stable disease, partial response, or hematologic improvement after the first induction cycle. Patients with at least a CRi could receive up to 12 consolidation cycles. During the consolidation, clofarabine (30 mg/m²/day) was given on days 1 through 3 with or without 7 days of cytarabine. Overall, 56% achieved CR and CR rate was significantly higher with the combination. Induction mortality was not significantly different in both treatment arms (19% with the combination versus 31% with clofarabine alone). The combination showed better EFS but not OS.

Similar clinical study was performed on much smaller group of patients [31]. The study included 11 patients with median age of 74 years (range, 63–87 years). Clofarabine (20 mg/m²/day) was given intravenously over 1 h (days 1 through 5) in combination with LDAC (20 mg/m²/day) given by subcutaneous injection daily for 14 days (days 1 through 14). Patients in CR could receive a maximum of 10 consolidation courses with intravenous clofarabine (15 mg/m²/day) during five consecutive days and subcutaneous cytarabine (20 mg/m²/day) during seven consecutive days. CR was achieved in 27%. The mortality rates at 4 and 8 weeks were 46% and 73%, respectively. Due to this unacceptable early mortality rate, the study was prematurely discontinued. High mortality rate could be in part explained by inadequate supportive care of the patients. The authors concluded that tight patients' clinical monitoring, follow-up, and intensive supportive care seem crucial to achieve at least acceptable clinical outcomes in elderly AML patients receiving clofarabine plus LDAC.

The combination of clofarabine and LDAC was evaluated as an induction and consolidation therapy, latter alternating with decitabine [32]. Decitabine is an important drug in induction of global and gene-specific DNA hypomethylation [33]. Sixty patients with a median age of 70 years (range, 60–81 years) with newly diagnosed AML were included in the study. Induction therapy consisted of clofarabine 20 mg/m² by intravenous infusion daily (days 1 through 5) plus cytarabine 20 mg subcutaneously twice daily (days 1 through 10). On days 1 through 5, clofarabine preceded the cytarabine injections by about 3 to 4 h. Patients who did not achieve a CR could receive one reinduction cycle at the same dose and schedule. In the case of persistent disease after reinduction, patients could proceed with decitabine 20 mg/m² as a 1- to 2-h

intravenous infusion daily for five consecutive days. Responding patients received consolidation therapy consisting of same clofarabine and cytarabine scheme alternating with decitabine 20 mg/m² for 5 days up to 17 cycles. Overall, 66% achieved CR and CRi. Notably, all of the seven patients with an FLT3-ITD responded, which included CR in six of them (86%). Induction mortality was low (7% at 8 weeks) and toxicities manageable. Among the 40 patients who have achieved CR/CRi, the median relapse-free survival (RFS) and OS were 14.1 and 24.2 months, respectively. The median OS of all patients was 12.7 months. However, compared with a historical group of patients who received clofarabine plus low-dose cytarabine with a shorter consolidation, RFS was not statistically different.

The combination of clofarabine and daunorubicin was tested as a first-line therapy in previously untreated older AML patients aged 60 years and older [34]. The median age of the 21 patients was 69 years (range, 60–85 years). Fourteen patients (67%) had unfavorable risk features. Induction mortality was 14%. CR was registered in 28.6% of the patients and the median OS was 11.2 months.

2.1.1.2. Clofarabine monotherapy

Clofarabine monotherapy was tested as an induction therapy predominantly in newly diagnosed older AML patients.

A phase 2 study assessed the efficacy of clofarabine monotherapy in older adults with untreated AML and at least one unfavorable baseline prognostic factor [35]. The study included 112 patients with median age of 71 years (range, 60–88 years). Sixty two percent of patients were ≥70 years old, 41% had intermediate, and 55% had unfavorable cytogenetics. Clofarabine was administered intravenously daily at 30 mg/m²/d during induction (days 1 through 5) and 20 mg/m²/d during reinduction/consolidation (six cycles maximum). The median duration of remission was 56 weeks. The ORR was 46% and according to authors it did not seem to be affected by the presence of multiple unfavorable prognostic factors. Noteworthy, five patients in remission proceeded to HSCT. The all-cause 30-day mortality rate was 9.8% and the all-cause 60-day mortality rate was 16%. Median OS was 41 weeks for all patients, 59 weeks for patients who achieved CR or CRi, and 72 weeks for patients who achieved a CR.

Another two consecutive phase 2 studies (UWCM-001 and BIOV-121) assessed the efficacy of clofarabine monotherapy in older adults with untreated AML [36]. UWCM-001 patients were either older than 70 years or 60–69 years of age with cardiac comorbidity or poor performance status (WHO >2) whereas BIOV-121 patients were at least 65 years of age and considered not eligible for intensive chemotherapy. The studies enrolled 106 patients. Clofarabine was administered intravenously daily at 30 mg/m²/d for 5 days and the patients could receive up to four or six courses of this drug. CR+CRi were achieved in 48% of the patients and induction death within 30 days was 18%. Interestingly, response and overall survival were not inferior in the adverse cytogenetic risk group. The authors performed a retrospective comparison with patients treated with LDAC and showed that the rate of CR/CRi with clofarabine was significantly superior (48% vs 17%). They had also demonstrated that OS with clofarabine was significantly superior to LDAC and non-significantly inferior to intensive chemotherapy.

To confirm survival benefit of clofarabine, a randomized comparison of LDAC vs clofarabine was performed in 406 untreated older patients with AML and high-risk MDS [37]. The median age of the patients was 74 years (range, 51–90 years). LDAC was given as a twice-daily 20-mg subcutaneous injection (days 1 through 10) with the aim of delivering four courses at approximately 6-week intervals and clofarabine was given as 20 mg/m² daily (days 1 through 5) by intravenous infusion for four courses approximately 4 to 6 weeks apart. Patients who were considered to be benefiting (CR or stable disease) were permitted to receive additional courses of treatment. The ORR (CR + CRi) was significantly improved in the clofarabine arm (38% vs 19%). However, there was no OS difference between the treatment arms. The authors explained the lack of clofarabine survival benefit by the superior survival of LDAC patients who failed to enter CR or who relapsed from CR.

2.1.2. Refractory or relapsed AML patients

2.1.2.1. Clofarabine combination therapy

Several clinical trials proved the clinical activity of clofarabine combination therapy in refractory or relapsed AML patients. Although many clofarabine combinations had been tested, the combination of clofarabine and cytarabine was largely applied in these patients.

Faderl and colleagues published a phase 2 study of clofarabine in combination with cytarabine to treat patients with relapsed AML [38]. The trial was conducted in 32 patients with relapsed acute leukemia, 25 of which are with AML. The median age of the whole study population was 59 years (range, 18 to 84 years). Clofarabine 40 mg/m² was administered as a 1-h intravenous infusion daily (days 2 through 6) followed 4 h later by an intermediate dose of cytarabine (1 g/m² as a 2-h constant-rate intravenous infusion) once daily (days 1 through 5). On day 1, only cytarabine was administered; on day 6, only clofarabine was administered. Patients were allowed to receive a maximum of two cycles of induction therapy and responding patients could receive up to six additional cycles of maintenance therapy at 75% of the induction doses of both clofarabine and cytarabine. ORR among AML patients was 40%. Only one patient died during induction (within 4 weeks of therapy start), constituting an induction mortality of 3%. The authors demonstrated that the combination of clofarabine with cytarabine is safe and active.

Given the encouraging clinical results of the previous study, a phase 3, randomized, double-blind, placebo-controlled trial based on the administration of clofarabine 40 mg/m² or placebo followed by cytarabine 1 g/m² for five consecutive days was carried out in relapsed or refractory AML patients [39]. The primary end point of the trial was OS. The median age was 67 years and 320 patients were included in the trial. Although OS did not differ between the treatment arms, it was clearly demonstrated that the combination of clofarabine and cytarabine significantly improved response rates and EFS.

The combination regimen of clofarabine and high-dose cytarabine with G-CSF priming (GCLAC) was tested in a phase 1–2 study in patients with relapsed or refractory AML [40]. The median age of the patients was 53 years. Most patients were treated at the maximum dose of clofarabine 25 mg/m² per day and cytarabine 2 g/m² beginning 4 h after the start of clofarabine for 5 days. G-CSF 5 µg/kg was given from the day before chemotherapy until neutrophil

recovery. The 30-day mortality for GCLAC was 0%. CRs were seen in 21 of the 46 evaluable patients (46%) and the ORR was 61%. The authors demonstrated that CR rates were considerably higher among relapsed patients whose first CR durations had exceeded 6 months. Median survival for all 50 patients was 9 months with 17 patients remaining alive after a median follow up of 1.9 years since beginning GCLAC. Thirteen of these 17 received HSCT after treatment with GCLAC. The authors showed that GCLAC is highly active in relapsed and refractory AML.

Given the positive clinical activity of GCLAC in relapsed or refractory AML, this regimen was retrospectively compared to fludarabine at 30 mg/m² and cytarabine 2 g/m² both daily for 5 days with (FLAG) or without (FA) G-CSF priming [41]. It was shown that after accounting for the duration of first complete remission, salvage number, age, and cytogenetics, GCLAC was associated with a higher CR rate and longer OS. Despite the retrospective nature of the analyses, the authors concluded that GCLAC may be superior to FA/FLAG, particularly in patients with short duration of first complete remission or unfavorable cytogenetics.

The combination of clofarabine and high-dose cytarabine was evaluated in 21 AML patients with refractory or relapsed disease [42]. The median age of the patients was 45 years (range, 22–62 years). The treatment comprised intravenous clofarabine (40 mg/m²/day) and intravenous cytarabine (1–2 g/m²/day) starting 4 h after clofarabine infusion for five consecutive days. Patients in CR could receive further consolidation chemotherapy with clofarabine + cytarabine, high-dose cytarabine, or allogeneic HSCT. CR was achieved in 42.9% of the patients. The authors demonstrated that although the small number of the cases, CR could be achieved only in relapsed or refractory AML receiving clofarabine in combination of cytarabine at 2 g/m²/day.

Another dose combination of clofarabine and cytarabine was tested in high-risk AML patients who relapsed or failed to respond to at least two induction therapies [43]. The study was conducted on 47 patients with median age of 50.5 years (range, 21–71 years). The therapy consisted of clofarabine 22.5 mg/m² given i.v., followed after 3 h by intravenous cytarabine at 1 g/m² daily for five consecutive days. Patients achieving CR or partial response (PR) were slated to receive a further consolidation cycle with clofarabine at 22.5 mg/m² and cytarabine at 1 g/m² on days 1 through 4. Induction mortality was 13%. Among all patients, ORR was 61.5% – 24 (51%) achieved a CR and another five patients (10.5%) had a PR. Notably, among the 24 patients, 13 patients underwent allogeneic HSCT. Given the poor prognosis of these patients, combination therapy of clofarabine and cytarabine definitely represented a “bridge” to transplantation.

The combination of clofarabine and high-dose cytarabine was tested in 35 refractory or relapsed AML patients, at a median age of 39.4 years (range, 21–60 years), treated in our institution [44]. Clofarabine was given 40 mg/m² daily i.v. for five consecutive days (days 2–6) followed 4 h later by cytarabine 1 or 2 g/m² administered i.v. for five consecutive days (days 1–5). Eighteen patients (51.4%) achieved CR, whereas thirteen patients (37.1%) had resistant disease, and four (11.4%) died during induction. In patients with CR, 55.6% (10/18) were able to proceed to allogeneic HSCT. The 24-month OS for the whole patients’ cohort was 28.8%. We concluded that clofarabine in combination with cytarabine is effective in refractory and

relapsed AML patients, and it represents a useful remission induction strategy to serve as a bridge to transplantation in these patients.

2.1.2.2. Clofarabine monotherapy

A phase 2 clinical and pharmacologic study of clofarabine in patients with refractory or relapsed acute leukemia was performed [22]. The study included 62 patients of which 50% had AML. Clofarabine was administered intravenously at 40 mg/m² over 1 h daily for 5 days every 3 to 6 weeks. CR was achieved in 42% of the patients whereas ORR (CR+CRi) was 55%. Response rates were higher in patients with longer first CR durations.

2.2. Clofarabine and hematopoietic stem cell transplantation

Over the past few years, the potential role of clofarabine has been studied also as a cytoreduction therapy for patients candidate to transplant and within different conditioning regimens.

2.2.1. Clofarabine as a cytoreductive agent for patients candidate to transplant

The feasibility of a cytoreductive strategy based on clofarabine before allogeneic SCT for refractory AML was specifically explored [45]. Clofarabine 30–40 mg/m² i.v. daily for 5 days was administered in 17 patients with plans to initiate conditioning during the nadir, 14 days later. Bone marrow biopsy 12 days after clofarabine showed effective cytoreduction in 59% of the patients which correlated with higher progression-free survival (PFS) and OS. The toxicity of the regimen was acceptable. Sixteen patients received their hematopoietic stem cell infusion at a median of 22 days after starting clofarabine. Transplant-related mortality at day 100 and 2-year was 6 and 36% respectively, while 1 year PFS and OS were 25 and 38%, respectively. These results showed that clofarabine cytoreduction followed by immediate HSCT is feasible with acceptable toxicity and transplantation-related mortality (TRM).

A retrospective analysis was performed in order to evaluate the antileukemic efficacy and toxicity of clofarabine-based chemotherapy followed by reduced-intensity conditioning (RIC) and allogeneic SCT for high-risk, relapsed, or refractory AML or MDS [46]. A total of 27 patients underwent allogeneic SCT after treatment with clofarabine and cytarabine for 5 days and RIC based on 4 Gy total body irradiation, cyclophosphamide, and anti-thymocyte globulin (ATG). Prophylaxis of graft-versus-host disease (GvHD) consisted of cyclosporine and mycophenolate mofetil. Most transplants were performed from peripheral blood stem cells and unrelated donors. The rates of OS and RFS were 56 and 52% at 2 years, respectively. Clofarabine-based chemotherapy followed by RIC showed good antileukemic efficacy even in patients with high-risk AML or MDS, with engraftment and GvHD-incidence comparable to other RIC regimens.

A retrospective study was performed to evaluate the feasibility and anti-leukemic activity of a sequential therapy using clofarabine for cytoreduction followed by conditioning for haploidentical HSCT in patients with non-remission acute leukemia [47]. Patients received clofarabine ($5 \times 30\text{mg/m}^2$ i.v.) followed by a T cell replete haploidentical transplantation for AML ($n = 15$) or ALL ($n = 3$). Conditioning consisted of fludarabine, cyclophosphamide plus either melphalan, total body irradiation or treosulfan/etoposide. High-dose cyclophosphamide was administered for post-grafting immunosuppression. Neutrophil engraftment was achieved in

83% and complete remission in 78% at day +30. The toxicity of the regimen was acceptable and the non-relapse mortality (NRM) at 1 year was 23%. The estimated OS and RFS at 1 year from haploidentical HSCT were 56 and 39%, respectively. The concept of a sequential therapy using clofarabine for cytoreduction followed by haploidentical HSCT proved to be feasible and allows successful engraftment, while providing an acceptable toxicity profile and anti-leukemic efficacy in patients with advanced acute leukemia.

Very recently, the combination of clofarabine 30 mg/m² and cytarabine 1 g/m² on days 1–5 (CLARA) was tested on 84 patients with relapsed or refractory AML with median age of 61 years (range, 40–75) [48]. Patients with a donor received HSCT in aplasia after first CLARA. In case of a prolonged donor search, HSCT was performed as soon as possible. The conditioning regimen consisted of clofarabine 30 mg/m², day -6 to -3, and melphalan 140 mg/m² on day -2. In patients with partially matched unrelated donors, ATG at a cumulative dose of 4.5 mg/kg was recommended. GvHD prophylaxis consisted of cyclosporine and mycophenolate mofetil. Donors were HLA-identical siblings in eight cases (14%), HLA-compatible unrelated donors in 30 cases (55%), and unrelated donors with one mismatch in 17 cases (31%). ORR assessed at day 15 after start of CLARA was 80% while 31% of patients having less than 5% BM blasts at that time. Treatment success was achieved in 61% of the patients. With a median follow-up of 25 months, OS and leukemia-free survival (LFS) for all enrolled patients at 2 years was 42 and 52%, respectively. At the time of enrollment, 14% of patients had a related donor and 33% had an unrelated donor available. In 46% of the patients, donor search was initiated at the time of enrollment. The OS at 2 years for patients with a related or an unrelated donor available was 75 and 47%, respectively, while it was 29% for patients for whom donor search was initiated at the time of enrollment.

2.2.1.1. Clofarabine in different conditioning regimens

Given the evidence of clinical activity of clofarabine and cytarabine in induction therapy of AML patients, a RIC regimen containing both drugs was explored in AML and MDS patients [49]. Seven patients were enrolled. Their median age was 54 years; three were with MDS and four with AML. The treatment plan consisted of clofarabine 40 mg/m² i.v. and cytarabine 1 g/m² i.v. both on days -6 to -2 and ATG 1 mg/kg on day -4 and 2.5 mg/kg on days -3 and -2. The median duration of neutropenia was 14 days and that of thrombocytopenia was 22 days. No acute GVHD was observed. Enrollment to the trial was halted due to unacceptable high mortality. The high mortality rate was caused by the insufficiently immunosuppressive activity of the regimen to ensure engraftment.

In order to improve the immunosuppressive activity of clofarabine and cytarabine combination as a conditioning regimen, the addition of cyclophosphamide, busulfan, and ATG was performed [50]. A phase 2 prospective multicenter trial aimed to assess the efficacy and safety of a sequential conditioning regimen was conducted. Twenty-seven AML patients in primary induction failure were included. The treatment consisted of clofarabine 30 mg/m²/d for 5 days, cytarabine 1g/m²/d for 5 days, and after a 3 days rest, cyclophosphamide 60 mg/kg/d for 1 day, IV busulfan 3.2 mg/kg/d for 2 days, and ATG 2.5 mg/kg/d for 2 days. For GVHD prophylaxis, patients received cyclosporine alone in case of a family donor, and cyclosporine + mycophenolate mofetil in case of an HLA-matched unrelated donor. The cumulative incidence of

disease progression and NRM at 1 and 2 years were 46, 59, 8, and 13%, respectively. The OS and LFS at 1 and 2 years were 54, 37, 46, and 28%, respectively.

Clofarabine had been tested in many other combination therapies in the context of conditioning regimens. A phase 1 trial was conducted to determine the MTD of clofarabine with high-dose busulfan followed by allogeneic SCT in patients with high-risk and refractory acute leukemia, most of which are with AML [51]. A total of 15 patients, median age 48 (30–58) years were included in the study. Patients received intravenous busulfan 0.8 mg/kg every 6 h on days –6 to –3 and clofarabine 30–60 mg/m² per day on days –6 to –2. Graft-versus-host disease prophylaxis included sirolimus plus tacrolimus. All the patients engrafted, and the MTD was not reached. One-year EFS and OS were 53 and 60%, respectively.

Very recently, clofarabine busulfan 4 (Clo/Bu4) conditioning regimen was compared in AML patients with active disease with standard conditioning regimens (cytotoxin/total body irradiation (Cy/TBI) or fludarabine/busulfan (Flu/Bu). The study included 16 patients whose outcomes were compared to those of 16 historical controls. RFS at 1 year for the Clo/Bu4 and control patients was 79 and 19%, respectively. OS in the Clo/Bu4 and control groups is 81 and 25%, respectively [52]. While more than half of the patients experienced some level of GvHD, most cases remained mild to moderate in both acute and chronic presentations.

The addition of clofarabine to combination of fludarabine and busulfan was tested as pre-transplant conditioning for advanced AML and MDS [53]. Patients were randomized between four different arms combining different dosages of nucleoside analogs followed on each day by busulfan infused to a specific pharmacokinetically targeted daily area under the curve. Fifty-one patients with a median age of 45 years have been enrolled with a minimum follow-up exceeding 100 days. The prophylaxis of GvHD consisted of tacrolimus and mini-methotrexate (MTX) ± low-dose rabbit-ATG. All patients engrafted and despite that forty-one patients had active leukemia at the time of transplant, CR was achieved in 85%. The projected median OS was 23 months.

A phase 1 study combining escalating doses of clofarabine with high-dose melphalan as RIC for allogeneic SCT was conducted in adult AML patients [54]. Sixteen patients with median age of 63 years were included in the study. Two patients died during dose escalation. All other patients demonstrated complete engraftment by day 30 with a median time to absolute neutrophil and platelet count recovery of 14 and 16 days, respectively. Only two patients relapsed and four patients died with a median follow-up of 17 months.

The combination of clofarabine–melphalan–alemtuzumab had been tested as a conditioning regimen for patients with advanced hematologic malignancies phase 1–2 study [55]. No DLT was observed in the phase 1 study and clofarabine 40 mg/m² for 5 days, melphalan 140 mg/m² for 1 day, and alemtuzumab 20 mg for 5 days were adopted for the phase 2 study. Seventy two patients with a median age of 54 years were included in the study of which 44 had AML or MDS. All evaluable patients engrafted. Median neutrophil and platelet recovery time was 10 and 18 days, respectively. At 1 year, the cumulative incidence of TRM, cumulative incidence of relapse, OS, and PFS were 26, 29, 59, and 45%, respectively. The main toxicity in the phase 2 study was rapid-onset renal, which was observed more frequently in older patients and those with baseline decrease in glomerular filtration rate. In 21% of the patients treated at the phase 2 doses was observed grade 3–5 renal toxicity.

3. Conclusion

Clofarabine exhibits efficacy in AML patients as a single agent and in combination with other cytotoxic drugs. Regarding older AML patients, first-line clofarabine monotherapy is associated with similar efficacy and potentially lower induction mortality compared with intensive chemotherapy regimens. Therefore, it may be an appropriate alternative treatment option for older patients with decreased PS or those who are unable to tolerate an anthracycline.

Clofarabine combinations with cytarabine and idarubicine provided encouraging results in untreated younger AML patients. Clofarabine containing induction regimens yielded an impressive CR/CRi rate, low mortality rate, and high OS at 1 year [23,24].

Combinations of clofarabine and cytarabine as a second-line therapy have offered promising results showing ORR ranging between 47% in elderly subjects and 61% in younger patients [32,40,43].

Another important aspect of clofarabine implementation is the transplantation strategy – as a cytoreductive agent for patients candidates to transplant and within conditioning regimens [45–50,52]. Considering that in most studies clofarabine has shown a possible impact on response rates and DFS but not on OS, one of the best possible achievements offered by this drug could be the opportunity to bring more patients to SCT with a good disease control, especially in subjects treated in second line [15].

These data confirm that clofarabine could be a useful option in the induction, reinduction, and transplantation therapy of AML patients.

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