Treatment of molecular relapse in patients with acute myeloid leukemia using clofarabine monotherapy

Zdenek Racil,[1]* Martina Toskova,[1] Dana Dvorakova,[1] Ivana Jeziskova,[1] Filip Razga,[1] Lucie Buresova,[2] Shira Timilsina,[1] and Jiri Mayer[1,3]

Few studies have examined the treatment of molecular relapse in patients with acute myeloid leukemia (AML) using different treatment regimens. We describe for the first time in the literature experiences with administration of clofarabine monotherapy in the treatment of eight patients with AML with molecular relapse of the disease.

A substantial proportion of patients with AML who initially respond to treatment will relapse on the current options available. In patients with AML with detectable molecular markers (i.e., fusion genes or mutated genes), quantitative real-time polymerase chain reaction (RQ-PCR) provides a sensitive monitoring technique for measuring minimal residual disease (MRD) as well as the early detection of relapse prior to an overt hematological relapse [1]. Several studies have already proven the benefit of early intervention at the stage of molecular relapse in patients with acute promyelocytic leukemia (APL) [2]. However, only limited data related to early intervention in patients with non-APL AML have been reported to-date [3–5], particularly in regards to the beneficial effect of this approach.

Clofarabine, a novel nucleoside analog, has demonstrated efficacy with a good toxicity profile in primary therapy of elderly patients with AML as well as in the salvage treatment of relapsed/refractory AML patients with or without additional allogeneic stem cell transplantation [6,7]. However, so far, this drug has not been used in the early treatment of molecular relapse. Therefore, the aim of this study was to evaluate the efficacy and feasibility of using clofarabine monotherapy for the treatment of molecular relapse in patients with non-APL AML.

All patients with AML treated at our institution who were monitored for MRD and who had a molecular relapse between April 2009 and August 2010 were included in this study. All patients signed an informed consent form for participation in the study, and the study protocol was approved by the IRB of the University Hospital Brno, Brno, Czech Republic.

Peripheral blood (PB) and bone marrow (BM) samples were used to monitor MRD during all phases of initial therapy of AML. After the end of this initial treatment, samples were obtained every 2–3 months for the first two years or more frequently in unstable cases. Moreover, any new reappearance of the molecular marker was confirmed by additional sampling within 2 weeks. After clofarabine therapy, samples for MRD evaluation (PB and BM) were obtained after each cycle (if clofarabine was administered repeatedly), before and after an allogeneic hematopoietic stem cell transplantation (HSCT) (if performed after clofarabine treatment), and every 2–3 months thereafter.

Quantitative reverse-transcription polymerase chain reaction (RQ RT-PCR) and real-time PCR (RQ-PCR) were used to measure fusion transcripts (*RUNX1/RUNX1T1*, *CBFB/MYH11*, and the fusion transcript of the *MLL* gene) and the mutated *NPM1* gene, respectively, in order to monitor MRD as previously described [3,8]. The sensitivity of RQ-PCR assays range from 1:10,000 to 1:10,00,000. All samples were analyzed in duplicate.

Molecular relapse was defined as the reappearance of the molecular marker in PB or BM samples, or a 10-fold increase if detected repeatedly, when the simultaneously assessed BM morphology, immunophenotype, and cytogenetics remained normal [3]. After clofarabine therapy, complete molecular remission (CMoR) was defined as the reduction of the particular molecular marker to a value of 0 (i.e., undetected level) in all monitored compartments. Partial molecular remission (PMoR) was defined as a one order of magnitude reduction of the molecular marker level in the monitored compartment together with complete cytogenetic and hematological remission.

The clofarabine regimen for the treatment of molecular relapse consisted of one cycle of a 40 mg/m^2 intravenous infusion of clofarabine for 5 days. Any additional therapy for patients who exhibited a response differed by patient and is shown in Table I. If a second cycle of clofarabine therapy was administered, the dosage was identical to the first cycle. All patients received prophylaxis treatment with posaconazole and co-trimoxazole. The Common Terminology Criteria for Adverse Events (CTCAE), version 4.03 (National Cancer Institute, Bethesda, MD), were used for the classification of adverse events.

During the study period, eight patients with AML exhibited a molecular relapse and were treated with clofarabine monotherapy. Table I shows a summary of the baseline patient characteristics. The median age of patients at the time of molecular relapse was 51 years. Primary therapy of AML consisted of induction 3+7 in all patients, followed by post-remission therapy using conventional chemotherapy in five patients (62.5%), autologous BM transplantation in one patient (12.5%), and allogeneic HSCT in two patients (25%). One patient (no. 7) was treated with clofarabine after relapsing from a previously treated molecular relapse that had occurred after an allogeneic HSCT. Seven patients (87.5%) fulfilled criteria for the reappearance of the molecular marker, and one patient (12.5%) had persistent detection of the marker and fulfilled the criterion of a 10-fold increase. The median time from the end of the last treatment to molecular relapse was 5.7 months (range 2.4–11.8 months).

The efficacy of clofarabine for reinduction as well as additional post-remission treatment is shown in Table I. After one cycle of clofarabine reinduction, all patients had a sustainable complete hematological remission. A molecular response was achieved in 7 of 8 patients (87.5%), 6 patients (75%) achieved CMoR, and 1 patient (12.5%) achieved a PMoR. In one case, a progressive increase in the molecular marker occurred and the patient relapsed hematologically within one month despite clofarabine therapy. Post-remission therapy in patients achieving a CMoR or PMoR included an allogeneic HSCT in three patients (Table I).

During the follow-up period, a new molecular relapse occurred at a median of 151 days (range 42–169 days) in 4 of 7 patients (57%) who exhibited a treatment response to clofarabine. Three of the four patients who did not receive a transplant after the initial treatment for molecular relapse with clofarabine developed second molecular relapse. In contrast, only 1 of 3 patients who underwent an allogeneic HSCT after clofarabine treatment for molecular relapse had a recurrence of the disease during the follow-up period. Moreover, the patient that relapsed after receiving an allogeneic HSCT only achieved a PMoR with clofarabine, and therefore received the transplant when MRD was still detectable (patient SM in Table I).

The 6-month overall survival (OS) rate for the evaluated group of AML patients was 100%, and the 6-month event-free survival (EFS) as well as disease-free survival (DFS) was 75% (95% CI: 50.3–100%), respectively.

Table I shows the associated individual toxicities with the clofarabine regimen used in this study. All patients experienced hematological toxicity (Table II). Also similar frequency and length of myelosupression were reported in previously published phase 2 study [9], these patients were treated for manifested disease whereas in our study they received therapy only for relapse at a molecular level, being otherwise without any clinical manifestation of the AML. Recently published studies showed efficacy of clofarabine in the treatment of newly diagnosed AML with reduction of myelosuppression when lower doses were used [6,10]. Thus, because worsening of quality of life caused by cytopenia-related complications is an important issue in patients treated for molecular relapse of AML, in future trials the dose of clofarabine might be further reduced possibly with maintenance of its efficacy. Non-hematological toxicity was substantially less frequent. Infection occurred in four patients (50%), but these events were uncomplicated febrile neutropenia without clinically or microbiologically documented infection. In one patient,

TABLE I. Baseline Characteristics, Efficacy of Treatment of Molecular Relapse and Associated Toxicities, and Follow-Up of Patients Involved in the Study

Baseline characteristics				Initial therapy of AML						CLOFARABINE reinduction and post-reinduction treatment			CLOFARABINE toxicity					Follow-up			
Initials	Sex	Age at relapse	Molecular marker	Primary therapy	Effect of primary/ previous therapy	First/repeated molecular relapse	Time to relapse from the end of previous therapy (months)	Magnitude of molecular marker at the time of relapse—peripheral blood[a]	Magnitude of molecular marker at the time of relapse—bone marrow[a]	Effect of reinduction therapy with CLO[b]	Postremission therapy	Type of postremission therapy	Neutrophils $< 1.0 \times$ 10.9/l (days)	Lymphocytes $< 1.0 \times$ 10.9./l (days)	Thrombocytes $< 50 \times$ 10.9/l (days)	Non-hematological toxicity	Type of toxicity	Further molecular relapse	Time to further molecular relapse (months)	Death in follow up period	Cause of death
1	F	30	*RUNX1/ RUNX1T1*	I, C	CMoR	first	6.8	0.17%	0.13%	CMoR	yes	1× CLO & allo HSCT	25	33	7	yes	febrile neutropenia	no	NA	yes	TRM
2	M	53	*CBFB/ MYH11*	I, C	CMoR	first	2.4	13.36%	10.43%	PMoR	yes	allo HSCT	21	33	11	yes	febrile neutropenia, hepatopathy, palmar-plantar erythema	yes	5.6	no	NA
3	F	47	*NPM1* mutation	I, auto BMT	CMoR	first	5.0	467.8	1568	progression	NA	NA	70	NA	68	no	NA	NA	NA	no	NA
4	F	54	*CBFB/ MYH11*	I,C	CMoR	first	5.0	5.14%	6.80%	CMoR	no	NA	19	38	12	no	NA	yes	4.5	no	NA
5	F	23	*CBFB/ MYH11*	I, C	CMoR	first	11.8	0.20%	0.23%	CMoR	yes	allo HSCT	3	15	8	no	NA	no	NA	yes	TRM
6	M	48	*NPM1* mutation	I, allo HSCT	CMoR	first	2.8	1640	45635	CMoR	yes	DLI	18	24	23	no	NA	no	NA	no	NA
7	M	56	*MLL-ELL* fusion gene	I, allo HSCT	PMoR	repeated	7.8	17.89%	NA	CMoR	yes	1 x CLO & interferon	24	47	27	yes	febrile neutropenia	yes	1.4	yes	progression
8	M	66	*NPM1* mutation	I, C	CMoR	first	6.4	3517	32928	CMoR	no	NA	37	41	66	yes	febrile neutropenia	yes	5.6	no	NA

F, female; M, male; I, induction 3+7; C, consolidation [usually high dose cytosinarabinoside]; CMoR, complete molecular remission; PMoR, partial molecular remission; CLO, clofarabine; DLI, donor lymphocyte infusion; alo HSCT, allogeneic hematopoietic stem cell transplantation; TRM, transplant related mortality; NA, not applicable.

[a]Mutant *NPM1* normalized copy number for *NPM1* mutation; % of fusion gene/abl for *RUNX1/RUN1T1*, *CBFB/MYH11* and *MLL-ELL*.

[b]Bone marrow assessment was used for evaluation of CLO therapy effect.

TABLE II. Hematological Toxicity of Clofarabine Regimen in the Treatment of Molecular Relapse

	Neutropenia	Lymphopenia	Thrombocytopenia
% of patients with toxicity CTCAE grade ≥ 3	100%	100%	100%
No. of days with toxicity CTCAE grade ≥ 3—median (range)	22 (3–70)	31 (7–46)	18 (7–68)
Lowest detected count ($\times 10^9$/l)—median (range)	0.03 (0.01–0.21)	0.03 (0.01–0.18)	7.5 (2–45)

CTCAE, The Common Terminology Criteria for Adverse Events, version 4.03; National Cancer Institute, Bethesda, MD.

palmar-plantar erythrodysesthesia syndrome (grade 2) and elevated liver enzymes (grade 3) occurred after clofarabine administration, but these were reversible non-hematological toxicities.

The data presented in this study can be primarily compared to a study published by our group several years ago on a cohort of AML patients with preemptive therapy for molecular relapse [3]. In this historical cohort, we treated 21 patients with molecular relapse and obtained a 62% response rate; however, only half of the responding patients achieved a CMoR (32%) [3]. The rate of complete molecular response was similar among the regimens used for treatment—conventional chemotherapy "5+2" – 25% (n = 8), gemtuzumab ozogomycin—29% (n = 7), and immunomodulation after allogeneic HSCT—43% (n = 7). Moreover, Doubek et al. showed that of the 71% of patients successfully treated for relapse in their study, a new molecular relapse occurred during the course of follow-up monitoring with a median progression-free duration of 119 days. In consideration of these data, the present study that used clofarabine as a monotherapy achieved a higher rate of complete molecular response compared to previously used strategies at our institution (75% vs. 32%, respectively) [5]. Very recently, a small cohort of AML patients with NPM1 mutation that were treated for molecular relapse using several courses of azacytidine was published. At least temporal molecular response was observed in 7 out of 10 (70%) treated patients. However data about the depth of this response were not provided and thus cannot be compared to our cohort [5]. Rubnitz et al. showed in a childhood AML study that the persistence of significant MRD positivity after induction is an adverse prognostic factor for both EFS and OS [11]. Therefore, if this approach is used for the treatment of molecular relapse, then clofarabine seems to be superior in this situation compared to a "5+2" type chemotherapy or gemtuzumab ozogamycin, at least in patients without a history of allogeneic HSCT.

Regardless of the therapy used for molecular relapse treatment, our present results as well as our historical data [3] clearly showed that without allogeneic HSCT, the further reappearance of the disease after treatment of molecular relapse is highly probable, especially in patients that do not achieve a complete molecular response. In consideration of these findings, clofarabine monotherapy, which has the highest frequency of complete molecular responses and a toxicity profile similar to a conventional "5+2" chemotherapy or gemtuzumab ozogamycin, could represent an ideal bridge for AML patients with molecular relapse to cover the period of time until an allogeneic HSCT. Therefore, we believe that a prospective trial addressing the role of clofarabine in the treatment of molecular relapse in AML is warranted.

[1]*Department of Internal Medicine—Hemato-Oncology, Masaryk University and University Hospital Brno, Brno, Czech Republic* [2]*Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic* [3]*CEITEC—Central European Institute of Technology, Masaryk University Brno, Brno, Czech Republic*
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*Correspondence to: Zdenek Racil, M.D., Ph.D.; Department of Internal Medicine—Hematology and Oncology, University Hospital Brno; Jihlavska 20; 625 00 Brno; Czech Republic. Tel.: +420-532233642. Fax: +420-532233603. E-mail: zracil@fnbrno.cz
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