Clofarabine as a Novel Nucleoside Analogue Approved to Treat Patients with Haematological Malignancies: Mechanism of Action and Clinical Activity

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Abstract: Clofarabine is a second generation of purine nucleoside analogues designed to combine the most favorable pharmacokinetic properties of fludarabine and cladribine. Clofarabine acts by inhibiting DNA polymerases and ribonucleotide reductase as well as by inducing apoptosis in cycling and non-cycling cells. Phase I/II clinical studies revealed its efficacy in hematological malignancies, and in 2004 clofarabine was approved by the United States Food and Drug Administration for the treatment of pediatric relapsed or refractory acute lymphoblastic leukemia after at least two prior chemotherapy regimens. The mechanism of action, pharmacology and clinical activity of clofarabine is the subject of this review.

Key Words: Clofarabine, purine nucleoside analogues, mechanism of action, clinical application, acute leukemia.

INTRODUCTION

For the last few years purine nucleoside analogues (PNAs), such as cladribine (2-CdA) and fludarabine (FA) (Fig. **1**), have been approved by United States Food and Drug Administration (FDA) for the treatment of hematological malignancies [1-3]. These drugs have chemical structure similar to adenosine (Ado) or deoxyadenosine (dAdo) and are made up a purine base which is linked to a deoxyribose sugar through a glycosidic bond [4]. Deactivation of PNAs occurs through deamination by adenosine deaminase (ADA) and by cleavage of the glycosidic bond by the enzyme purine nucleoside phosphorylase (PNP) [4-6]. Since these drugs were susceptible to glycosidic bond cleavage, with fludarabine subject to some phosphorylase cleavage and cladribine subject to hydrolytic and enzymatic cleavage, a search for compounds overcame this limitation have developed [7, 8]. Clofarabine (CAFdA) (Fig. **1**) is a second generation of PNAs which has been designed to combine the most favorable pharmacokinetic properties of fludarabine and cladribine. The structure of CAFdA, similarly to 2-CdA, consists of deoxyadenosine with substitution of hydrogen by chlorine at the 2-position of the adenine ring causing electronic changes that make the amino group resistant to deamination by ADA [9]. Substitution of a fluorine atom at the arabinosyl configuration at the 2'-position of the carbohydrate decreased the susceptibility of CAFdA to phosphorolytic cleavage by PNP (Fig. **1**). Additionally, it makes CAFdA more acid stable and leads to an increase of its oral bioavailability [10]. Clofarabine shows significant efficacy in pediatric acute lymphoblastic leukemia (ALL) patients and was approved by the US FDA in December 2004 (Clolar™, Genzyme) and by the European Commission in May 2006 (Evoltra®, Bioenvision) for the treatment of relapsed or refractory ALL in children after at least two prior chemotherapy regimens. In adults, clofarabine is also active in acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and chronic myelogenous leukemia in blastic phase (CML-BP), both as a single agent and in combination with other anticancer drugs [8, 11-14]. In June 2008 Evoltra® was approved in Europe for the treatment of AML in elderly patients who have one or more of the following: adverse cytogenetics, secondary AML, greater than or equal to 70 years old or significant co-morbidities and are therefore not considered suitable for intensive chemotherapy.

This review article summarizes current knowledge about mechanism of action, pharmacokinetics, pharmacological properties, clinical activity and toxicity of clofarabine.

MECHANISM OF ACTION

The mechanism of action of clofarabine is well described and is similar to that of cladribine and fludarabine [15-17]. Clofarabine is a slightly lipophilic prodrug entering into cells *via* active nucleoside-specific membrane transporters (NTs), and at higher concentration and longer exposure time, by passive diffusion across lipid membranes [18]. Recently, a few different NTs have been identified [18, 19]. Three of them were defined as human equilibrative nucleoside transporters (hENTs) which are sodium-independent-facilitated system and transport the nucleoside down the concentration gradient. Other three NTs known as human concentrative nucleoside transporters (hCNTs) are sodium-dependent and can transport nucleosides against the concentration gradient [20]. hENT1 and hENT2 are found in plasma membranes and hENT1 is also present on mitochondrial membranes. hENT3 is associated with lysosomal/endosomal membranes

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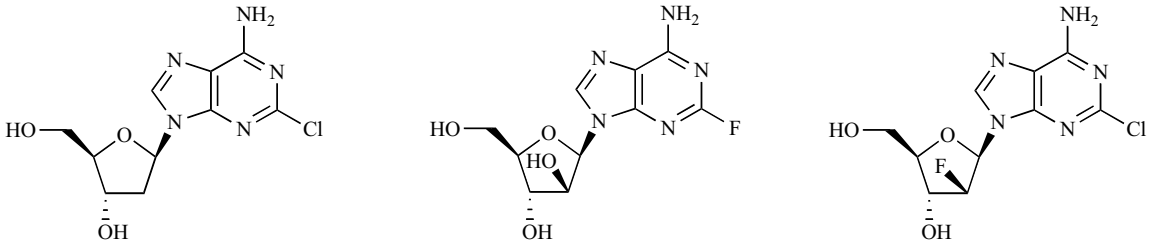

Cladribine
[2-chloro-9-(2-deoxy-β-D-ribofuranosyl)-9H-purin-6-yl]amine
(2-CdA)

Fludarabine
[2-fluoro-9-(β-D-arabinofuranosyl)-9H-purin-6-yl]amine
(FA)

Clofarabine
[2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purin-6-yl]amine
(CAFdA)

Fig. (1). Structure of cladribine, fludarabine and clofarabine.

and its nucleoside transport activity is broadly selective and proton-dependent [21]. The hCNTs exhibit selective transport activity for pyrimidine nucleosides (hCNT1), purine nucleosides (hCNT2), and both pyrimidine and purine nucleosides (hCNT3) [18]. NTs are integral membrane proteins responsible for mediating nucleosides and nucleobasis across the cellular membrane and they accept only dephosphorylated forms of nucleosides. CAFdA is transported into the intracellular compartments by hENT1, hENT2 and hCNT3 and with low activity by hCNT2 [18, 20]. The importance of membrane NTs was suggested by results in cultured human leukaemic cells in which the rate of clofarabine intake into cells was about 10-fold higher in transport-competent than in transport-deficient cells [8, 18]. Additionally, clofarabine was more cytotoxic to cells having the hENT1 transport process than either 2-CdA or FA [18].

Like other PNAs, CAFdA, after the uptake into the cells, is converted by deoxycytidine kinase (dCK) to the 5'-monophosphate metabolite, and then by mono- and diphosphokinases to the active 5'-triphosphate form (CAFdA-TP). Human dCK can phosphorylate the pyrimidine nucleoside deoxycytidine (dCy) and the purine nucleosides deoxyadenosine (dAdo) and deoxyguanosine (dGuo), with a much higher activity for dC [22]. dCK phosphorylates CAFdA with a 50% lower Michaelis-Menten constant (K_M) but a 4-fold higher maximum velocity (V_{max}) than the physiological substrate dCy [23, 24]. Additionally, clofarabine was reported to be more efficient substrate for purified recombinant dCK than FA and 2-CdA [17, 23]. In lymphoid tissues, a high ratio of dCK to 5'-nucleotidase (5'-NT) activity favours the accumulation of phosphorylated metabolites, however a substantial concentration of dCK was also revealed in non-lymphoid tissues [25]. Malignant cells were reported to have higher dCK concentrations than their normal counterparts [26]. It is known that dCK activity is augmented by DNA-synthesis inhibitors and ribonucleotide reductase (RR) inhibitors, which reduce deoxynucleotide pools of deoxycytidine mono-, di-, and triphosphate (dCMP, dCDP, dCTP) and cytidine triphosphate (CTP) that are feedback inhibitors of dCK [25, 26]. Since CAFdA inhibits RR, it has been suggested that this drug may increase cellular dCK activity and enhance its own activation [8]. Deoxycytidine kinase is known to be rate-limiting enzyme for many of adenosine analogues, but is not rate limiting with clofarabine and clofarabine 5'-monophosphate that serve as a reservoir for the formation of 5'-triphosphate metabolites [27-29]. Clofarabine is also a good substrate of deoxyguanine kinase (dGK), which may contribute to its role in mitochondrial damage [24, 30]. The 5'-mono-, di-, and triphosphate forms of CAFdA must be dephosphorylated by 5'-NT for transport out of the cell. The cellular elimination kinetics of the phosphorylated metabolites of CAFdA showed triphasic kinetics with a β-half life of 8-24 hours and a γ-half life lasting more than 24 hours which indicates prolonged cellular retention [27]. Additionally, the cellular retention of phosphorylated CAFdA metabolites was longer than that of FA and 2-CdA [16, 23].

Clofarabine acts *via* four mechanism of action: incorporation into DNA and inhibition of DNA polymerases, inhibition of RR, as well as induction of apoptosis (Fig. **2**) The clofarabine triphosphate form (CAFdA-TP) is required for its cytotoxicity. CAFdA, in contrast to other anticancer drugs but similarly to the whole class of PNAs, is active both in mitotic and quiescent cell cycle phase [8, 31]. The cytotoxicity of CAFdA-TP in the dividing cells is mainly due to the inhibition of either DNA polymerases or RR, leading to disequilibrium in deoxynucleotide triphosphates pool and *via* endonuclease activation results in DNA strand breaks [16, 32]. The CAFdA-TP inhibits DNA polymerases, mainly polymerases α and ε, whereas DNA polymerases β (involved in DNA repair process) and polymerase γ (mitochondrial) are much less sensitive to CAFdA [8, 32]. The incorporation of the monophosphate of CAFdA into DNA by DNA polymerase α results in chain termination and strand breakage. Clofarabine triphosphate inhibits RR, presumably by binding to the allosteric site on the regulatory subunit [8, 16]. The inhibition of RR in consequence reduces the amount of intracellular deoxynucleoside triphosphates (dNTP) available for DNA synthesis, mainly dCTP and deoxyadenosine triphosphate (dATP) but not deoxythymidine triphosphate (dTTP) [16]. The depletion of the dCTP pool is sufficient to limit DNA synthesis, and reduction in dATP concentration allows CAFdA-TP to compete with dATP for incorporation into DNA [8, 16]. When the ratio of CAFdA-TP to dATP is more than 1, clofarabine monophosphate is preferentially inserted into the end of the DNA chain resulting in termination of chain elongation [16, 27]. If this ratio is less than 1, clofarabine monophosphate is incorporated into the middle of

the DNA structure leading to the inhibition of DNA repair [16, 27]. In comparison with FA and 2-CdA, CAFdA inhibits more completely both RR and DNA polymerases [17, 23].

Clofarabine can also induce the apoptosis, and both P53-dependent and P53-independent cytotoxicity mechanisms are suggested [33]. It is known that P53 influences Bax and other proapoptotic proteins leading to mitochondrial changes typical for apoptosis, resulting in secretion of cytochrome c from mitochondria to cytosol and its binding with pro-caspase-9 and APAF-1 (apoptotic protease activating factor-1). Cytochrome c release is a critical point during apoptosis, however the mechanism by which it exits mitochondria is not well understood. APAF-1, cytochrome c and procaspase-9 form a complex termed apoptosome which after binding with CAFdA-TP influences the activation of caspase-9 cascade and *via* DNA condensation and fragmentation leads to apoptosis (Fig. **2**) [30, 34]. Additionally, CAFdA-TP similarly to 2-CdA can induce apoptosis by binding directly to proteins located in mitochondrial membrane. On this way CAFdA-TP *via* mitochondrial permeability transition pore (MPT) results in the release of either cytochrome c and formation of apoptosome, or apoptosis-inducing factor (AIF) leading to chromatine condensation and DNA fragmentation without caspase-9 cascade activation [9, 30]. Thus, the direct mitochondrial effects of CAFdA and 2-CdA may explain why these drugs are toxic to CLL cells at concentrations 5-10-fold lower than FA [9, 30]. Apoptosis induced by CAFdA-TP is mainly mediated on the intrinsic pathway described above, nevertheless the role of the extrinsic pathway should be also taken into consideration. However, it is still little known about the induction of apoptosis *via* the death receptor Fas/CD95 [35].

PHARMACOKINETICS AND PHARMACODYNAMICS

The pharmacokinetics of CAFdA were evaluated both in adult and pediatric patients in the phase I studies [11, 28, 36]. During the initial phase I clinical studies conducted in patients with hematological malignancies and refractory solid tumours the maximum tolerated doses (MTD) and the dose-limiting toxicities (DLT) were assessed [28, 36, 37]. CAFdA was given as 1-hour infusion daily for 5 days, every 3 to 6 weeks. Dose-escalation studies in acute leukemias started at the dose of 7.5mg/m^2, with escalation to 55mg/m^2 daily for 5 days. Since the DLT was a reversible hepatotoxicity at the dose of 55mg/m^2, therefore the recommended dose for phase II studies was 40mg/m^2 intravenously (i.v.) for 5 days in patients with acute leukemias, MDS and CML-BP [28, 36]. In patients with chronic lymphoproliferative disorders (LPDs) and solid tumors, the DLT was myelosuppression at the dose of 15mg/m^2, therefore CAFdA at the doses of 2mg/m^2 or 4mg/m^2 i.v. daily for 5 days were recommended for phase II clinical trials in these patients [37]. Based on phase I studies conducted in pediatric patients with relapsed and refractory ALL and AML, CAFdA at a dose of 52mg/m^2 i.v. over 2 hours for 5 consecutive days was recommended [11]. The DLT was a reversible hepatotoxicity and skin rash at 70mg/m^2 i.v. daily for 5 days [38].

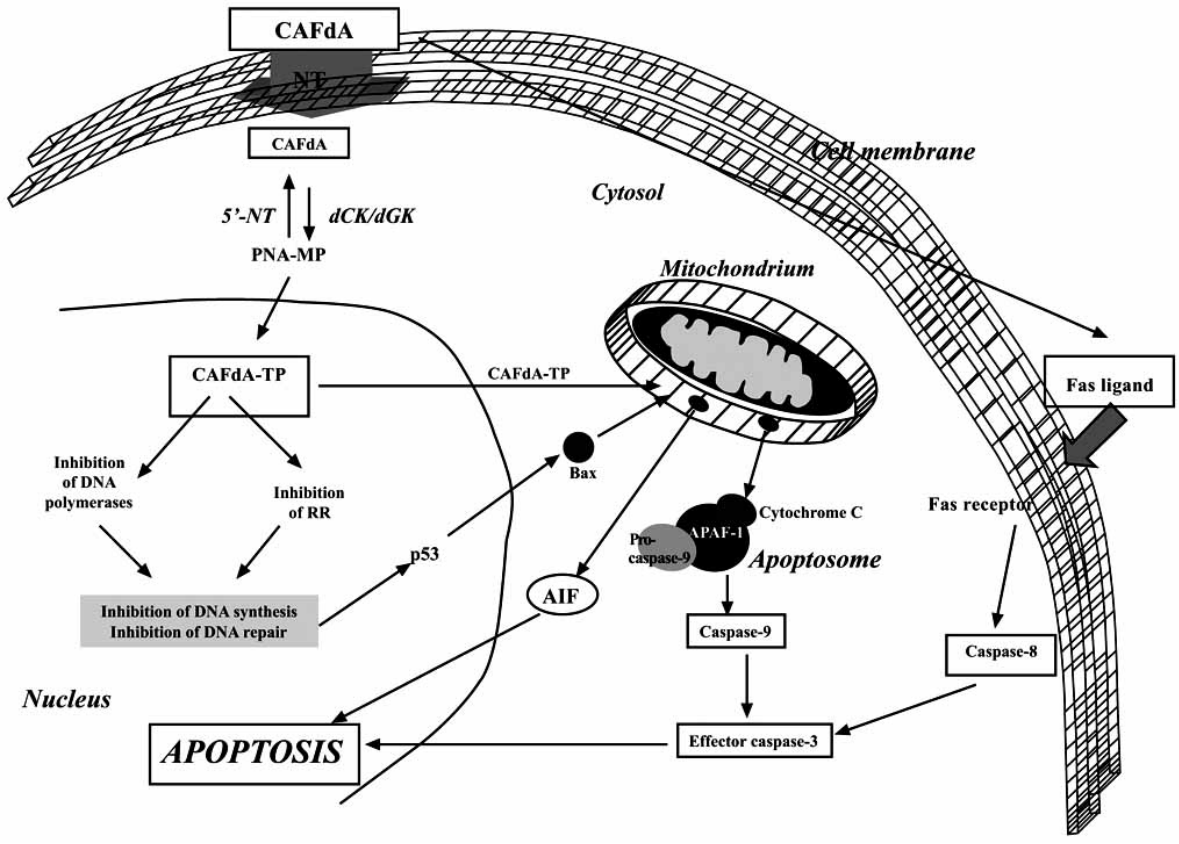

Fig. (2). Mechanism of action of clofarabine.

After administration of CAFdA at a dose of 40mg/m^2 as a 1-hour i.v. infusion, the median plasma level of CAFdA was 1.0 μM (range 0.26-1.94μM) [36]. The peak level of CAFdA in plasma occurred at the end of the infusion and, despite heterogeneity among patients, a linear increase in plasma CAFdA concentration with increasing doses was observed [28]. Clofarabine was only 47% bound to plasma proteins, predominantly to albumin and showed extensive tissue distribution, having a volume of distribution at steady state (V_{dss}) of 179 litre for a 40-kg patient (about 4.5 l per kg) [8, 28, 29]. CAFdA appeared to be eliminated in a biphasic manner with faster kinetics during the first 6 hours followed by slower kinetics up to 24 hours [28, 36]. The elimination half-lives were not calculated in the studies, however there was a measurable plasma CAFdA concentration of 0.038 mM (range, 0.013 mM-0.11 mM) at 24 hours. The pathway of elimination is primarily *via* renal excretion, and the renal clearance was 10.8 l/h/m^2 with 57% of the dose excreted unchanged in the urine [8, 28, 29]. The total systemic clearance for clofarabine was 28.8 l/h/m^2. Compared with pediatric patients, adults showed lower clearance and smaller volume of distribution [8].

Similar to plasma CAFdA levels, the concentration of intracellular CAFdA-TP measured in circulating leukemic blasts varied among patients and the median value was 15 μM with a range of less than 1 μM to 44 μM. A dose-proportional accumulation was observed at lower doses but this correlation was lost with no further increases for the doses >30mg/m^2 [36]. No differences were observed between myeloid and lymphoid leukemic blasts regarding the retention of intracellular CAFdA-TP [36]. It has been reported that in blast cells of responding patients, the accumulation of CAFdA-TP was greater and retained more than 50% of the initial concentration of CAFdA-TP at 24 hours after first infusion of CAFdA [28, 36]. Additionally, blast cells from patients with AML or ALL treated with clofarabine retain more than a half of the initial concentration of CAFdA-TP for 24 hours, in contrast to the mean half-life time ($T_{1/2}$) of 8-10 hours for FA or 2-CdA in similar patient populations [28, 39]. DNA synthesis was inhibited by 75-90% at the end of infusion with clofarabine at doses ranging from 22.5 to 55 mg/m^2. A partial recovery of DNA synthesis was observed at 24 hours in blasts of patients treated with 22.5 and 30 mg/m^2. When a dose of 40 and 55 mg/m^2 was used, the inhibition of DNA synthesis was maintained for more than 72 hours [12, 39].

CLINICAL TRIALS

Clofarabine is the most promising PNA in current clinical trials in pediatric and adult patients with hematological malignancies. During phase I studies the antileukemic activity at tolerable doses was well established thus several phase II clinical trials with clofarabine alone or in combination with other cytotoxic drugs have been recently conducted[1,2,3].

Clofarabine Clinical Trials in Pediatric Patients

Jeha *et al*. [11] conducted a first pediatric patients trial of clofarabine in relapsed or refractory ALL and AML. In total, 17 patients with ALL and 8 with AML were entered into this study. Clofarabine's doses ranged from 11.25 to 70mg/m^2 i.v. daily for 5 days. Five patients (20%) achieved complete response (CR), and 3 (12%) achieved partial response (PR), for an overall response (OR) rate of 32%. Based on the results of phase I clinical trials with clofarabine in pediatric patients with advanced leukamias, the MTD was established as 52 mg/m^2 daily for 5 days. The DLT was reversible hepatotoxicity and skin rash at 70mg/m^2 i.v. per day for 5 days [11].

A phase II multicenter studies were performed in children with refractory or relapsed ALL or AML. The OR rate was 31% in ALL patients including 6 CR, 4 CR_p (complete response with incomplete platelet recovery), and 5 PR. In AML patients the OR rate was 26% (1 CR_p and 8PR). Median survival was 42 weeks for responding ALL patients and 39 weeks for responding AML patients. The most clofarabine-related adverse events were febrile neutropenia, diarrhea, nausea and vomiting, fever, skin rash, headache, elevation in liver enzymes and bilirubin, infusion-related flushing and anxiety[*1].

In a phase II, multicenter study, 61 children with refractory or relapsed acute ALL received CAFdA at a dose of 52 mg/m^2 i.v. over 2 hours daily for 5 days, every 2 to 6 weeks [40]. The response rate was 30%, consisting of 7 CR, 5 CR_p, and 6 PR. The most common adverse events of grade 3-4 were febrile neutropenia, anorexia, hypotention, and nausea. Based on this study, clofarabine was approved by the US FDA in December 2004 for the treatment of pediatric patients with relapsed or refractory ALL after at least two prior chemotherapy regimens.

Recently Gidwani *et al*. [41] have published the results of the first report of successful remission induction in multiple relapsed ALL in a 9-year-old boy after combined treatment with CAFdA and arabinoside cytosine (ara-C).

Clofarabine Clinical Trials in Adult Patients

In a phase II study, 62 patients with relapsed or refractory AML, ALL, CML-BP, and MDS received clofarabine at a dose of 40 mg/m^2 i.v. over 1 hour infusion daily for 5 days, every 3 to 6 weeks [36]. In all patients, the OR rate was 48%, consisting of 20 CR, 9 CR_p, and 1 PR, however, it differed by diagnosis, salvage status, and duration of first remission. The OR rate was 55% in AML, 50% in MDS, 64% in CML-BP, and 17% in ALL patients. The most frequently observed adverse events included hepatotoxicity, skin rashes and mucositis.

A single institution phase II trial was conducted with CAFdA as first line treatment in 28 elderly patients (> 70 years) with AML who were not suitable for conventional chemotherapy. Clofarabine was given at a dose of 30mg/m^2

[1] Jeha, S.; Razzouk, B.; Rytting, M.E.; Gaynon, P.S.; Kadota, R.; Rheingold, S.; Luchtman-Jones, L.; Shen, V.; Arceci, R.J.; Fernandez, M.; Weitman, S.; Steinherz, P.G. *Blood,* **2004**, *104,* Abstract 684.

[2] Jeha S, Gaynon PS, Steinherz P *et al*. A Phase II, open-label study of clofarabine in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. *Blood,* **2003**, *102,* Abstract 3277.

[3] Jeha S, Steinherz P, Gaynon PS *et al*. A Phase II, open-label study of clofarabine in pediatric patients with refractory or relapsed acute myelogenous leukemia. *Blood,* **2003**, *102,* Abstract 2278.

Table 1. Activity of Clofarabine Alone or in Combination in Adult Patients with Hematological Malignancies

References	Treatment Schedule	Disease	Median Age (Range)	No of Pts.	OR Rate (%)	CR Rate (%)	CR_p Rate (%)
Kantarjian *et al*. 2003 [37]	CAFdA 11.25- 55 mg/ m^2 daily for 5 days	Relapsed/refractory	42 (19-78)				
		AML		16	13	6	6
		ALL		13	15	8	8
		CML-BP		3	33	0	0
Kantarjian *et al.* 2003 [36]	CAFdA 40mg/ m^2 daily for 5 days	Relapsed/refractory	54 (19-82)				
		AML		31	55	42	13
		MDS		8	50	25	25
		ALL		12	17	8	8
		CML-BP		11	64	36	18
Kantarjina *et al*. 2003 [37]	CAFdA 2mg/m^2 daily for 5 days	Solid tumors	48 (19-78)	13	0	NA	NA
		CLL, NHL		6	0	NA	NA
Burnett *et al*. 2004 [*4]	CAFdA 30mg/ m^2 daily for 5 days	Newly diagnosed AML	71 (60-79)	28	59	59	NA
Burnett *et al*. 2006 [*5]	CAFdA 30mg/ m^2 daily for 5 days	Newly diagnosed AML	71 (64-81)	66	48	29	15
Faderl *et al*. 2005 [42]	CAFdA 40mg/m^2 on days 2-6 and Ara-C 1g/m^2 on days 2-5	Relapsed/refractory	63 (18-84)				
		AML		25	40	28	12
		high-risk MDS		4	50	0	50
		ALL		2	0	NA	NA
		CML-BP		1	0	NA	NA
Faderl *et al*. 2006 [44]	CAFdA 40mg/m^2 on days 2-6 and Ara-C 1g/m^2 on days 2-5	Newly diagnosed AML	61 (50-74)	60	60	52	8
Faderl *et al*. 2008 [45]	CAFdA 30mg/m^2 daily for 5 days with or without Ara-C 20mg/m^2 s.c. daily for 14 days	Newly diagnosed AML and high-risk MDS	71 (60-83)	70	59	56	3
Uy *et al*. 2008 [49]	CAFdA 4mg/m^2 daily for 5 days	Refractory MM	49 (42-66)	8	0	NA	NA

Abbreviations: CAFdA – clofarabine, ALL – acute lymphoblastic leukaemia, AML – acute myeloid leukaemia, CML-BP- chronic myeloid leukemia in blastic phase, MDS - myelodysplastic syndrome, CLL – chronic lymphocytic leukemia, NHL – non-Hodgkin lymphoma, MM – myeloma multiplex, OR – overall response, CR – complete response, CR_p - CR with incomplete platelets recovery.

i.v. daily for 5 days every 4 weeks[4]. Seventeen patients (59%) achieved CR. Hepatotoxicity of grade 3-4 was observed in 39% of patients. In another multicenter phase II study in elderly patients with newly diagnosed AML who were not eligible to standard chemotherapy, the OR rate achieved 44% (21% CR, 23% CR_p). The response rate was even higher (47%) in patients with adverse cytogenetics and in patients aged more than 70 years (59%)[5].

Recently, the results of combined therapy of clofarabine with other antileukemic drugs have also been published [42-46]. The combined phase I and II study was performed in 32 patients with relapsed acute leukaemia consisting of 25 AML, 2 ALL, 1 CML-BP, and 4 high-risk MDS patients [42]. In phase I trial, the optimal dose of clofarabine was established when combined with ara-C. The optimal increase of ara-CTP levels was demonstrated when clofarabine was dosed 4 hours prior to ara-C exposure [43]. In a phase II trial, CAFdA was given at a dose of 40mg/m^2 i.v. daily on days 2-6 followed 4 hours later by ara-C at a dose of 1g/m^2 i.v. over 2-3 hours daily for on days 1-5. The OR rate was 40% in AML patients (28% CR, 12% CR_p) and 50% in patients with MDS. No response was observed in ALL or CML-BP patients [42].

[4] Burnett A.K.; Russell N.; Kell J.W.; Milligan D.; Culligan D. A phase 2 evaluation of single agent clofarabine as first line treatment for older patients with AML who are not considered fit for intensive chemotherapy *Blood,* **2004**, *104,* Abstract 869.

[5] Burnett AK, Baccarani M, Johnson P, Yin J, Saunders A, Russell N, Hills R. Effectiveness of Clofarabine in Elderly AML Patients with Adverse Cytogenetics Unfit for Intensive Chemotherapy. *Blood,* **2006**, *108,* Abstract 1985.

Another phase II study, with combination of clofarabine and ara-C, was performed in patients aged 50 years and older with previously untreated AML [44]. Clofarabine was given at a dose of 40mg/m^2 as a 1-hour iv infusion for 5 days (days 2 to 6) followed 4 hours later by ara-C at a dose of 1g/m^2 as a 2-hour i.v. infusion for 5 days (days 1 to 5). Of note, 48% had secondary AML, 50% had abnormal karyotypes, and 21% showed FLT3 abnormalities. The OR rate was 60% including 52% CR and 8% CR_p. Toxicity observed in this trial was acceptable.

In a randomized study clofarabine versus clofarabine plus low-dose ara-C as front line therapy was assessed in patients aged 60 years and older with AML and high-risk MDS [45]. Patients received 30mg/m^2 clofarabine i.v. daily for 5 days with or without 20mg/m^2 ara-C subcutaneously (s.c.) daily for 14 days as induction. Consolidation regimen consisted of 3 days of CAFdA with or without 7 days of ara-C.

For the both treatment groups the OR rate was 59% and 56% of patients achieved CR. However the OR and CR rates were significantly higher in patients treated with the combined regimen than compared to those receiving clofarabine alone (67% *vs* 31%, p=0.012, and 63% *vs* 31%, p=0.025, respectively). The toxicity was comparable in both groups of patients.

Clofarabine was also used in combination with alkylating agents based on the results of preliminary studies showing synergistic cytotoxicity and inhibition of DNA repair for combining regimen. Eighteen patients with relapsed acute leukemias were treated with cyclophosphamide (CY) alone at the dose of 200mg/m^2 on day 0, and with CAFdA at the dose of either 20mg/m^2 or 10mg/m^2 plus CY on day 1 [46]. Pharmacodynamic end points along with clinical results suggest usefulness of this combination strategy, whereas toxicity data suggest reduction of chemotherapeutic intensity.

Clofarabine Clinical Trials in Lymphoproliferative Disorders and Solid Tumors

Clinical trials with clofarabine in patients with LPDs and solid tumors are limited [5]. In the phase I trial in patients with chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL), a significantly myelosuppression was observed which led to the decreasing of CAFdA dose to 2mg/m^2 i.v. daily for 5 days. The evidence of its activity, including decrease in lymphadenopathy and peripheral lymphocytosis, was seen in these patients even at low doses of CAFdA, however, no objective response was observed [37]. The role of CAFdA and its optimal dose schedule in LPDs and solid tumors patients needs to be defined [5]. A recent study was performed in 8 patients with refractory multiple myeloma (MM). Clofarabine was given at a dose of 4mg/m^2 i.v. on days 1 to 5 of a 28-day cycle [47]. No objective evidence of antineoplastic activity of such regimen was observed. All patients experienced grade 3-4 neutropenia and a greater than 50% decrease in platelets counts during treatment. Phase I/II trials with clofarabine in adult patients are listed in Table **1**.

TOXICITY

The common toxicities associated with clofarabine treatment concern haematopoiesis, skin, gastrointestinal tract, and central nervous system. Myelosuppression associated with febrile neutropenia is observed in more then 81% of patients treated with clofarabine [36, 37]. Other side effects recorded after clofarabine administration include transient headache, infusion-related irritability, acral erythema, infusion-related facial flushing, nausea, vomiting, diarrhoea, stomatitis, hyperbilirubinemia, transaminitis, and myalgia [12, 36, 37].

CONCLUSIONS

Clofarabine is the most promising purine nucleoside analogue in current clinical development. This agent is active in pediatric patients with refractory or relapsed ALL as well as demonstrates efficacy alone or in combination with other cytotoxic drugs in adults with AML. The toxicity of clofarabine is acceptable. An oral formulation of clofarabine is also being developed.

ABBREVIATIONS

2-CdA	=	2-chlorodeoxyadenosine; cladribine
5'-NT	=	5'-nucleotidase
ADA	=	Adenosine deaminase
Ado	=	Adenosine
ALL	=	Acute lymphoblastic leukemia
AML	=	Acute myeloid leukemia
Ara-C	=	Arabinoside cytosine; cytarabine
ATP	=	Adenosine triphosphate
CAFdA	=	2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine; clofarabine
CAFdA-TP	=	Clofarabine triphosphate
CLL	=	Chronic lymphocytic leukemia
CML	=	Chronic myelogenous leukemia
CML-BP	=	CML in blastic phase
CR	=	Complete response
CTP	=	Cytidine triphosphate
CY	=	Cyclophosphamide
dAdo	=	Deoxyadenosine
dATP	=	Deoxyadenosine triphosphate
dCK	=	Deoxycytidine kinase
dCTP	=	Deoxycytidine triphosphate
dCy	=	Deoxycytidine
dGK	=	Deoxyguanosine kinase
dGTP	=	Deoxyguanosine triphosphate
dGuo	=	Deoxyguanosine
DLT	=	Dose limiting toxicity
dTTP	=	Deoxythymidine triphosphate
FA	=	9-β-D-arabinosyl-2-fluoroadenine; fludarabine

FA-MP = Fludarabine monophosphate, soluble form of fludarabine

hCNT = Human concentrative nucleoside transporter

hENT = Human equilibrative nucleoside transporter

i.v. = Intravenously

K_M = Michaelis-Menten constant

LPDs = Lymphoproliferative disorders

MDS = Myelodysplastic syndrome

MM = Multiple myeloma

MTD = Maximum tolerated dose

NHL = Non-Hodgkin lymphoma

NT = Nucleoside transporter

OR = Overall response

PNA = Purine nucleoside analogue

PNA-TP = Triphosphate forms of purine nucleoside analogue

PNP = Purine nucleoside phosphorylase

PR = Partial response

RR = Ribonucleotide reductase

s.c = Subcutaneously

$T_{1/2}$ = Half life time

V_{max} = Maximum velocity

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