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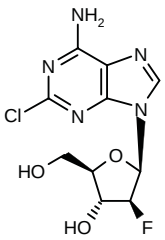
Clofarabine

USAN

Treatment of Acute Leukemia

2-Cl-2'-F-araA
CAFdA
Clofarex™

2-Chloro-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine



$C_{10}H_{11}ClFN_5O_3$
Mol wt: 303.6799
CAS: 123318-82-1
EN: 154861

Abstract

Clofarabine (Clofarex™, 2-Cl-2'-F-araA, CAFdA) is a novel anticancer agent shown to be particularly effective in acute leukemia therapy. It is a second-generation purine nucleoside analogue that works through incorporation into the DNA molecule and inhibition of further DNA synthesis via a number of different mechanisms. Clofarabine is effective in many different cancers, and has proven efficacy in phase I studies in patients with solid tumors. However, clofarabine has demonstrated the most promise within the hematological setting. Results from a number of phase I/II studies indicated potent anticancer activity, particularly in the treatment of acute leukemias. Clofarabine is most often administered as a short 30-min i.v. infusion over 5 days per cycle, meaning that it can be delivered in an outpatient setting rather than requiring hospitalization (as opposed to first-generation analogues). Furthermore, children with resistant or refractory leukemia have exhibited total response rates of 28-44%. Clofarabine is well tolerated in this population, the most commonly reported side effects being nausea and vomiting, reversible hepatotoxicity and myelosuppression. Promising results from phase I and II studies have led to the initiation of phase III studies in both adults and children with acute leukemia.

Synthesis

Reaction of 1,2:5,6-di-*O*-isopropylidene-3-*O*-tosyl-α-D-allofuranose (I) with KF in acetamide at 210 °C gives 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (II), which is treated with a 1:1 mixture of methanol and 0.7% aqueous H_2SO_4 to yield 3-deoxy-3-fluoro-1,2-isopropylidene-α-D-glucofuranose (III). Selective acylation of the sugar (III) with benzoyl chloride in pyridine affords the 6-*O*-benzoyl derivative (IV), which is treated with Amberlite IR-100 (H^+) ion-exchange resin in hot dioxane to provide 6-*O*-benzoyl-3-deoxy-3-fluoro-D-glucofuranose (V). The oxidative cleavage of glucofuranose (V) by means of KIO_4 in water results in rearrangement to give 5-*O*-benzoyl-2-deoxy-2-fluoro-3-*O*-formyl-D-arabinofuranose (VI), which is deformylated by means of NaOMe in methanol to provide 5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose (VII). Acylation of the arabinofuranose (VII) with acetic anhydride in pyridine affords the 1,3-di-*O*-acetyl derivative (VIII), which is treated with HBr in $AcOH/CH_2Cl_2$ to yield 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide (IX) (1). Condensation of compound (IX) with 2-chloroadenine (X) by means of potassium *tert*-butoxide in different solvents gives the acylated 2-chloroadenosine derivative (XI), which is finally deacylated by means of NaOMe in methanol (2). Scheme 1.

Alternatively, condensation of the arabinofuranose (IX) with 2,6-dichloropurine (XII) by means of NaH in acetonitrile gives the dichloropurine nucleoside (XIII), which by treatment with NaOMe in methanol provides the 2-chloro-6-methoxypurine nucleoside (XIV). Finally, this compound is treated with ammonia in hot ethanol in a pressure vessel (3). Moreover, the reaction of the dichloropurine nucleoside (XIII) with ammonia in hot ethanol as before yields directly clofarabine (4, 5). Scheme 1.

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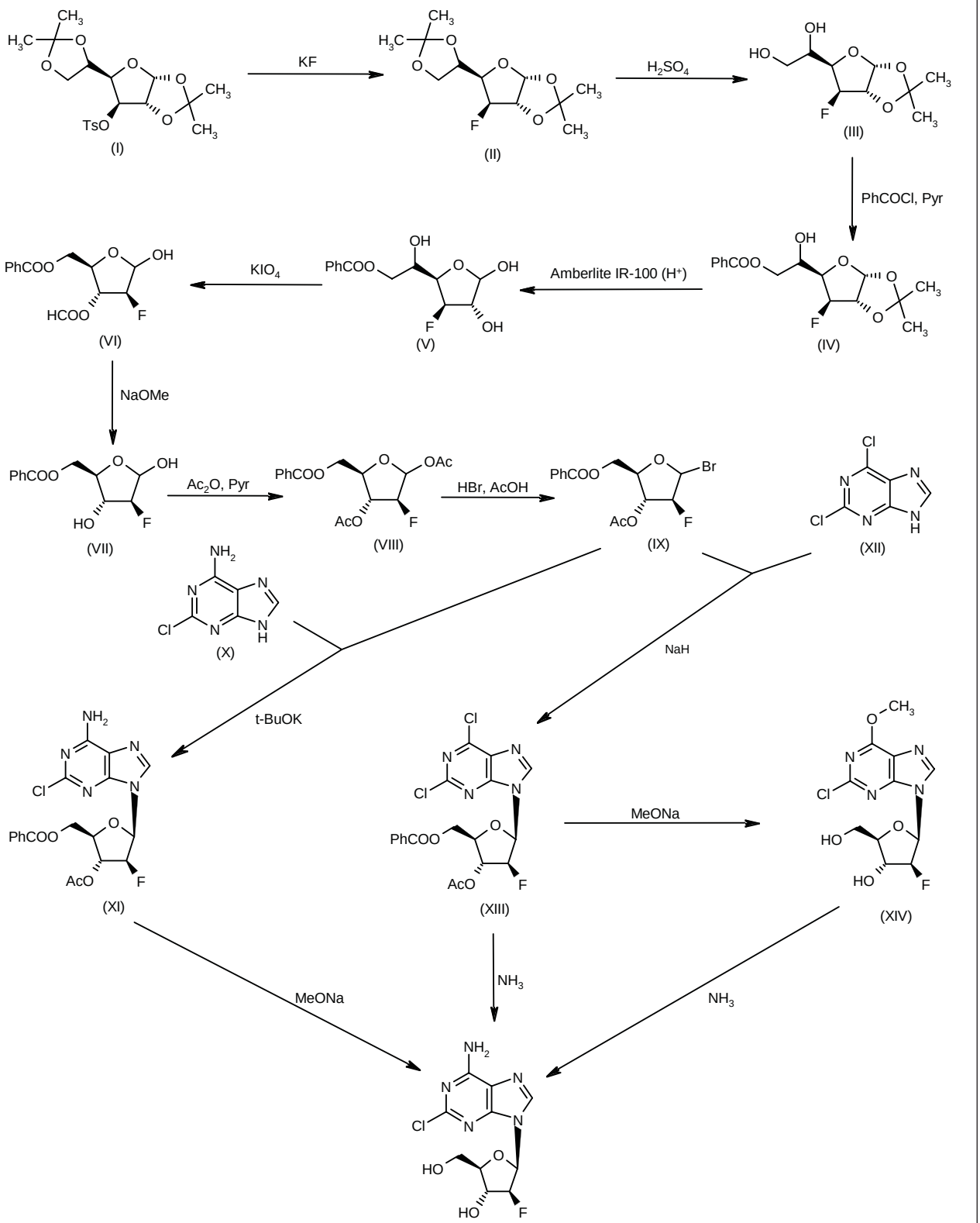
Scheme 1: Synthesis of Clofarabine



Introduction

Nucleoside analogues are widely used in the treatment of cancer and viral diseases. The adenine (or purine) nucleoside analogues fludarabine (Fludara®; Schering AG/Berlex) and cladribine (Leustatin®; Ortho Biotech) are currently available for the treatment of hematological malignancies, fludarabine being the drug of choice for chronic lymphocytic leukemia (CLL) and cladribine for hairy cell leukemia (6-11). The cytotoxicity of both fludarabine and cladribine involves inhibition of DNA synthesis via inhibition of DNA polymerase α and ribonucleotide reductase, although cladribine is more potent against ribonucleotide reductase than DNA polymerase (12-19). These compounds, however, also have certain disadvantages for clinical use, including dose-limiting toxicity, low oral bioavailability and/or solubility problems, which appear to be due to their acidic and/or enzymatic degradation (5, 20-22). In order to overcome these limitations, a group at the Southern Research Institute synthesized a series of 2-halo-2'-halo-2'-deoxy-arabinofuranosyladenine analogues, and initial screening led to the selection of clofarabine as the best compound (5).

Clofarabine shows greater structural similarity to cladribine due to the chloro group at the 2-position of adenine. Substitution of a fluorine at the C-2'-position while retaining the arabino configuration results in reduced susceptibility of clofarabine to the phosphorolytic cleavage by purine nucleoside phosphorylases seen with fludarabine, as well as increased acid stability relative to cladribine. The increased acid stability of clofarabine suggests potentially improved oral bioavailability (5). Moreover clofarabine compares favorably to both fludarabine and cladribine in terms of inhibition of DNA polymerase and ribonucleotide reductase (12).

This second-generation purine nucleoside analogue was thus rationally designed to possess improved efficacy and a better tolerability profile compared to its predecessors. Currently in phase I and II studies in pediatric and adult leukemias, phase III trials as monotherapy or in combination are under consideration (23, 24).

Pharmacological Actions

Initial screening of clofarabine and related compounds demonstrated particularly potent cytotoxic activity for clofarabine, with IC_{50} values against the human cell lines HEp-2, CCRF-CEM and K-562 of 0.012, 0.05 and 0.003 μM, respectively, and an IC_{50} of 0.23 μM against the murine leukemia cell line L1210. Clofarabine was also the most effective compound in mice bearing P388 leukemia and extensive preclinical testing was therefore initiated (5).

The mechanism of action of clofarabine resembles that of cladribine and fludarabine. Recent studies using several human and murine leukemia cells demonstrated that, following phosphorylation by deoxycytidine kinase to the triphosphate, DNA synthesis is preferentially inhibited due to potent inhibition of ribonucleotide reductase and of chain elongation by DNA polymerase α. Clofarabine appears to combine the best properties of cladribine and fludarabine, with inhibitory potency comparable to the former against ribonucleotide reductase and to the latter against DNA polymerase α. It was also more efficiently phosphorylated by deoxycytidine kinase and eliminated more slowly compared to cladribine in human leukemia cells. These characteristics have been suggested to account for tis superior cytotoxicity (IC_{50} = 5 nM against K-562 cells *vs.* 16 and 460 nM, respectively, for cladribine and fludarabine) (12, 25-30).

Other experiments have demonstrated an apoptotic effect for clofarabine in leukemia cell lines *in vitro* and *in vivo*, which appeared to be mediated, at least in part, by downregulation of the Bcl-2 family proteins Bcl-X_L and Mcl-1, and Akt dephosphorylation (25, 31, 32).

Clofarabine, other deoxynucleoside analogues and unrelated anticancer agents such as etoposide were shown to enhance the activity of deoxycytidine kinase (the very enzyme involved in activating the deoxynucleoside analogues) in both normal and transformed human lymphocytes, suggesting pretreatment or combination strategies for improving antitumor efficacy (33). In another study using lymphocytes from patients with CLL, preincubation with clofarabine and fludarabine was found to inhibit the DNA excision repair evoked by the alkylating agent 4-hydroperoxycyclophosphamide, which resulted in more than additive apoptotic cell death. The increase in cytotoxicity was directly related to the extent of repair inhibition (34).

Clofarabine has shown potent anticancer activity in a number of preclinical studies using different cell lines and tumor models. Early results showed clofarabine to have potent submicromolar antiproliferative activity against human CNS, lung and renal cancer, leukemia and melanoma cell lines, as well as *in vivo* efficacy in murine P388 leukemia, colon 36 and mammary 16/C tumor models (35). Clofarabine was generally significaantly more potent than fludarabine against these cell lines, with IC_{50} values of 0.11, 0.29, 0.29, 0.29, 0.67, 0.028 and 0.15 μM, respectively, against renal carcinoma ACHN, renal carcinoma Caki-1, CNS tumor SNB-7, non-small cell lung adenocarcinoma NCI-H23, melanoma SK-MEL-28, chronic myelogenous leukemia K-562 and acute lymphoblastic leukemia CCRF-CEM cells, *versus* respective values of 6.4, 41, 54, 45, 39, 0.58 and 0.29 μM for fludarabine. In the P388 leukemia model, clofarabine exhibited significantly enhanced activity when given on a more intensive schedule, providing ILS values of 200% and 300%, respectively, at doses of 20 mg/kg i.p. every 3 h x 8 on days 1, 5 and 9, or every 4 h x 3 on days 1-9. Antitumor activity after oral administration was less than following i.p. dosing. Clofarabine was curative in mice bearing colon 36 tumors and it also exhibited moderate to excellent efficacy against human colon, non-small cell lung, renal and prostate cancer xenografts in nude or SCID mice, with a broader spectrum of activitiy compared

to fludarabine. Using P388 leukemia cells, some cross-resistance with etoposide and marked cross-resistance with cytarabine was seen, but not with doxorubicin, paclitaxel, methotrexate or melphalan (36).

The *in vitro* cytotoxicity of clofarabine was found to be superior to cladribine using mononuclear cells from leukemia patients. Clofarabine was significantly more cytotoxic than cladribine against cells from patients with CLL, although there was no difference in cytotoxicity in cells from patients with acute myeloid leukemia (AML). Increased sensitivity to clofarabine compared with cladribine was detected in 36 of 52 samples, while 8 were equally sensitive to both drugs. Clofarabine was also phosphorylated more efficiently in these cells than cladribine (37).

The *in vivo* efficacy of clofarabine was compared with cladribine in a severe combined immunodeficiency (SCID) mouse model of CLL. SCID mice were administered oral clofarabine or cladribine 1 mg/ml in the drinking water for 1 week. Clofarabine had superior efficacy in eliminating transferred CLL cells (90% *vs.* 50%) (25, 38).

The dose and schedule dependency of clofarabine were examined in athymic nude mice bearing human non-small cell lung cancer NCI-H460 xenografts. Animals were administered the drug at doses of 8.9, 13.3 and 20 mg/kg i.p. 3 times daily for 9 days 27, 40 and 60 mg/kg/day i.p. over 9 days, 120, 180 and 270 mg/kg i.p. every fourth day x 3, or 120, 180 and 270 mg/kg i.p. once weekly x 3. All treatments were well tolerated. The results indicated that the efficacy of clofarabine is increased with more frequent dosing, the best responses (T/C = 13.6-16.5 days) being obtained on the t.i.d. schedule (39).

In vitro and *in vivo* studies were conducted using human colon tumor cell lines/xenografts. Clofarabine was more active than cladribine and fludarabine in inhibiting the proliferation of human colon tumor cell lines HCT 116, HT-29, DLD-1 and WiDr, with respective log mean IC_{50} values of 0.26, 0.35 and 19 μM. Potent activity was also reported against human colon tumor xenografts following daily i.p. dosing for 5 days. Oral administration was superior to i.v. administration and the therapeutic efficacy appeared to increase with more frequent dosing. Finally, clofarabine was able to completely suppress the formation of liver micrometastases from human colon tumors at a dose of 100 mg/kg/day p.o. (40, 41).

Recently published preclinical data have shown a potential synergism between clofarabine and cytarabine. Clofarabine increased the accumulation of cytarabine triphosphate in human myeloid leukemia K-562 cells when added prior to cytarabine. This was seen at concentrations (1-2 μM) reached in plasma in phase I trials following administration of the MTD of clofarabine. The most effective schedule involved incubation of cells with clofarabine 4 h before cytarabine, and a clinical protocol for combination therapy in acute leukemia was designed based on these findings (42).

In combination therapy studies in athymic nude mice, clofarabine + CPT-11 (irinotecan) was tested against human colorectal tumor HT-29 xenografts (43) and clofarabine + docetaxel against human prostate PC-3 tumor xenografts (44). Clofarabine was more active than CPT-11 in the former study when given alone, and combinations of the drugs resulted in even greater delays in tumor growth than clofarabine alone. In contrast, no increase in toxicity was observed with combination therapy (43). All doses of clofarabine (2.5, 5 and 10 mg/kg t.i.d. i.p. x 9 days) produced significant growth delays in the PC-3 xenograft model, whereas docetaxel was only active at the highest dose (5 mg/kg i.v. every fourth day). The combination of the agents at all but the lowest doses (2.5 mg/kg clofarabine + 1.25 mg/kg docetaxel) was more effective than either drug alone in terms of tumor growth delay. All doses of clofarabine and most combinations were well tolerated as regards weight loss (< 20%) (44).

Pharmacokinetics and Metabolism

Assays have been developed for the quantitation of clofarabine in plasma (45-47).

Clofarabine is metabolized to its mono-, di- and triphosphate forms by the enzyme deoxycytidine kinase in cells, the mono- and triphosphate forms predominating. In cultured human CLL cells, peak levels of the active triphosphate metabolite occurred at 2 h, with a plateau being observed at 2-4 h (12, 27, 48).

The pharmacokinetic profile of clofarabine 10 and 25 mg/kg i.v. was tested in adult male Sprague-Dawley rats. Clearance rates decreased from 2.1 l/h/kg after 10 mg/kg to 1.5 l/h/kg after 25 mg/kg, possibly due to saturated metabolism, and the $t_{1/2}$ values were 1.35 and 1.84 h. The volume of distribution was similar at both doses (3.6 and 3.2 l/kg, respectively, at 10 and 25 mg/kg). Oral bioavailability was estimated to be around 50% (47).

The elimination rate of clofarabine was significantly longer than that of cladribine in a study using isolated rat liver tissue. This indicated increased stability to hepatic enzymes for the clofarabine molecule. The compounds showed similar first-pass metabolism (approximately 50%). Biliary excretion of unchanged clofarabine or cladribine accounted for < 1% of dose, < 0.1% and < 1%, respectively, in the form of the metabolite 2-chloroadenine (49).

Clinical Studies

Pilot studies demonstrated that clofarabine administration in patients with solid tumors was associated with significant myelosuppression compared to patients with hematological malignancies. A phase I study was therefore carried out in order to determine the appropriate dosing regimen in patients with solid tumors. Twelve patients with solid tumors of the lung, colon, pancreas, prostate, bladder, bile duct and larynx were administered clofarabine 4 mg/m^2 i.v. on days 1, 8 and 15 of a 28-day cycle. This dose could be escalated to 6, 10, 14, 18 or 22 mg/m^2 in subsequent cohorts. At the time of publication,

only 1 patient had experienced a best response of stable disease after 4 cycles of treatment. Two patients were removed from the study due to disease progression. No toxicities had been reported at up to 14 mg/m^2. The maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) were therefore not identified (50).

The pharmacological profile of clofarabine was assessed in a phase I study in 31 adult patients with refractory AML, acute lymphoblastic leukemia (ALL) and CML. Clofarabine 4-55 mg/m^2 was administered via 1-h i.v. infusion for 5 days in this study. Twelve patients treated at the 40 mg/m^2 dose level achieved a median 97% reduction in peripheral blasts after the 5-day treatment period. Six patients responded to 5 days of therapy, suggesting clofarabine's therapeutic superiority compared with its predecessors (51). Pharmacodynamic analysis of 26 evaluable patients revealed a linear relationship between the triphosphate metabolite of clofarabine and the number of blast cells between 4 and 22.5 mg/m^2, with a triphosphate peak being noted at a median of 19 μM. Triphosphate levels were also related to the degree of inhibition of DNA synthesis, but did not appear to be related to leukemia subtype. Investigators also noted that patients treated with doses below 40 mg/m^2 recovered their blast ability to synthesize DNA prior to their next daily dose of clofarabine. This recovery rate was reduced at the higher doses (51).

A phase I dose-finding study comparing patients with refractory solid tumors and hematological disease found MTD and DLT values to be significantly different between the groups. The first phase of the study determined the MTD and DLT in patients with solid tumors, and a 3+3 dose escalation was then performed in acute leukemia patients in order to determine the MTD and DLT within this population. Clofarabine administration was initiated at 15 mg/m^2/day for 5 days, delivered by a 1-h i.v. infusion each day, repeated every 28 days. The 15 mg/m^2 dose was found to be myelosuppressive in patients with solid tumors, and was subsequently de-escalated to 7.5, 4 and 2 mg/m^2/day. The 2 mg/m^2 dose was the MTD recommended for use within this population Thirty-two patients with acute leukemia were then administered 7.5 mg/m^2/day following the aforementioned dosing regimen, which could then be escalated to 11.25, 15, 22.5, 30, 40 and 55 mg/m^2/day. The MTD in this population was 40 mg/m^2, with a DLT of severe yet reversible hepatotoxicity (manifested by elevations in transaminase and bilirubin). The DLT occurred at 55 mg/m^2 in this analysis. A response to treatment was obtained in 16% of patients with acute leukemia: 2 patients experienced a CR and 3 had a CR without platelet recovery (CRp; hematological improvement) (52-54).

A recent phase II study included 62 patients with relapsed or refractory AML, myelodysplastic syndrome (MDS), CML or ALL. Patients received a daily 1-h i.v. infusion of clofarabine 40 mg/m^2 for 5 days every 3-6 weeks (depending on treatment response). This dose regimen could be reduced in the presence of extramedullary toxicity. Complete response was defined as normalization of bone marrow aspirate and peripheral blood, PR was defined as a CR with blasts between 6% and 25% in the bone marrow, and hematological improvement was defined as CR without platelet recovery (CRp). The overall response rate in these patients was 48%; a CR was obtained in 20 patients (32%) with previously relapsed or refractory disease, 9 patients (15%) achieved a CRp and 1 patient achieved a PR. Responses were recorded in 55%, 50%, 64% and 17% of AML, MDS, CML and ALL patients, respectively. Responders were shown to accumulate more clofarabine within their blast cells compared with nonresponders (median uptake = 18 μM *vs.* 10 μM). These encouraging efficacy results were complemented by a favorable tolerability profile. Transient, reversible grade 3-4 liver dysfunction was noted in up to 25% of patients, with rash and hand-and-foot syndrome seen in 10-15% of patients. Myelosuppression occurred at rates expected for an anticancer therapy, with fever and infection in 81% and 50% of patients, respectively. No deaths were related to drug toxicity (55, 56).

Preliminary results from a multicenter, open-label phase II study currently under way in a group of adults with refractory or relapsed AML were reported. Treatment was delivered via daily 1-h i.v. infusion of 40 mg/m^2/day for 5 days every 28 days during the induction phase. After 1-2 cycles, responders were administered clofarabine 30 mg/m^2/day over 5 days for 2-3 cycles, in order to consolidate induction therapy. Complete response was defined as a 4-week history of: 1) absence of blasts from peripheral blood; 2) 5% or less blasts and absence of Auer rods in bone marrow; 3) maturation of all cell lines; 4) platelet count of 100 billion/l or more; and 5) absence of extramedullary disease; partial response was defined as CR with either blasts < 25% or the presence of Auer rods. Only 1 CR was detected in this group of patients. Side effects included febrile neutropenia, rash and transient transaminitis (57, 58).

A synergistic interaction between clofarabine and cytarabine (ara-C) was described in a phase I/II study in patients with acute leukemia (first relapse or primary refractory), high-risk MDS (10% or more blasts) and CML (blastic phase) (59). In the dose-finding phase of the study, 12 patients were administered a 1-h i.v. infusion of clofarabine 15-40 mg/m^2/day in combination with a 2-h i.v. infusion of cytarabine 1 g/m^2. Each drug was given for 5 consecutive days within each treatment cycle. The aim of the study was to determine the MTD and DLT for the phase II part of the analysis. The overall response rate was 34%: 2 patients achieved CR at clofarabine doses of 15 and 40 mg/m^2 and 2 others achieved CRp at the doses of 22.5 and 30 mg/m^2. Dose-limiting toxicity was not observed at any of the doses studied. The recommended dose for the second phase was therefore 40 mg/m^2. In the phase II part of the study, 20 patients were administered clofarabine 40 mg/m^2/day plus cytarabine 1 g/m^2. A total of 33% of patients responded to treatment (5 CRs and 1 CRp), all after 1 cycle of therapy. The most commonly reported adverse events were nausea and vomiting, skin rash, diarrhea, hand-and-foot syndrome,

mucositis and neurological signs. Two deaths occurred during the study due to sepsis and pulmonary hemorrhage (59).

Results have shown that clofarabine is both effective and well tolerated in children with ALL and AML, and it is particularly useful in patients with refractory or relapsed disease. This is important in children, as resistant disease forms are a leading cause of death in these patients.

A dose-finding study was recently carried out in a group of 25 pediatric patients with advanced leukemia, *i.e.*, refractory or relapsed ALL (n=17) or AML (n=8). They were administered clofarabine as a daily 1-h i.v. infusion of 11.25-70 mg/m^2/day for 5 days, repeated every 2-6 weeks depending on patient response and adverse events. A modified 3+3 trial design was undertaken, with a 30% dose escalation being employed depending on treatment outcome. Most patients were heavily pretreated and 36% had previously undergone stem cell transplantation. This population had very limited options, as well as a very poor prognosis. The pediatric MTD was calculated to be 52 mg/m^2/day, with the DLT being reversible hepatotoxicity and skin rash at 70 mg/m^2/day. The overall response rate was 32% in this population, with 80% of responses lasting over 1 year. Five children achieved a CR and 3 had a PR; 4 others achieved a reduction in their leukemic cell count sufficient to allow bone marrow transplantation (BMT). Leukemia blasts were reduced to 6-19 mM at the MTD, which implies complete and sustained DNA inhibition at this maximal dose. The most commonly reported side effects were hepatic dysfunction, nausea and vomiting, diarrhea, mucositis, rash and myalgia. Significant hepatic dysfunction was not maintained with repeated cycles of clofarabine administration. Clofarabine was therefore judged to be effective in this population of previously refractory patients with advanced acute leukemia (60, 61).

Clofarabine was reported to be effective and well tolerated in another phase I study in 13 pediatric patients with refractory AML or ALL. Eleven patients had failed 5-11 (median of 10) chemotherapeutic regimens prior to study onset, and 4 had undergone previous BMT. Clofarabine 11.25-52 mg/m^2/day was delivered via 1-h infusion for 5 days on an every-28-day cycle. A 3+3 dose-escalation design was employed. At the time of publication, 9 of the 13 patients were evaluable. Of these patients, 3 had achieved CR and 1 a marrow CR. Responses were recorded in 3 patients with ALL and 1 with AML. Grade 3-4 toxicities were present at these doses, with episodes of fever (n=5), liver dysfunction (n=4), nausea and vomiting (n=3), diarrhea (n=1) and hyperuricemia (n=1) being reported (52).

Two multicenter, open-label phase II studies are assessing the efficacy and tolerability of clofarabine within the pediatric population. Investigators are employing the same dosing regimen for both studies, *i.e.*, a 2-h i.v. infusion of clofarabine 52 mg/m^2 for 5 days, repeated every 2-6 weeks. The studies were designed to assess this dosing regimen in patients with refractory/relapsed ALL and AML. Of 19 evaluable AML patients treated with clofarabine, 1 had a CRp and 5 a PR, for a total response rate of 32%. Furthermore, 5 patients went on to receive BMT. Drug-related adverse events included febrile neutropenia, rash, nausea and vomiting and transient liver transaminase elevations (53, 55). Of 25 evaluable patients with relapsed or refractory ALL, 1 had a CR, 2 a CRp and 4 a PR, for an overall response rate of 28%. Three of the responding patients have received a BMT. Clofarabine was well tolerated in this study, with the most common side effects reported being fever, myelosuppression, nausea and vomiting, skin rash, hand-and-foot syndrome and a rise in liver enzymes indicative of hepatotoxicity. The rise in liver enzymes was reversible, however, and returned to baseline levels following treatment discontinuation (64, 65).

Sources

Southern Research Institute, Birmingham, AL (US); licensed to Bioenvision, Inc. (GB) and codeveloped with Ilex Oncology, Inc. (US).

References

1. Reichman, U., Watanabe, K.A., Fox, J.J. *A practical synthesis of 2-deoxy-2-fluoro-D-arabinofuranose derivatives.* Carbohydr Res 1975, 42: 233-40.

2. Bauta, W.E., Schulmeier, B.E., Cantrell, W.R. Jr., Lovett, D., Puente, J. (Ilex Oncology, Inc.). *Process for preparing purine nucleosides.* US 2003114663, WO 0311877.

3. Montgomery, J.A., Fowler, A.T., Secrist, J.A. III. (Southern Research Institute). *Methods for synthesizing 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purin-6-amine.* CA 2400470, EP 1261350, WO 0160383.

4. Montgomery, J.A., Secrist, J.A. III. (Southern Research Institute). *2-Halo-9-(2-deoxy-2-fluoro-B-D-arabinofuranosyl) adenine nucleoside derivs.* JP 1993502014, US 5034518, WO 9014352.

5. Montgomery, J.A., Shortnacy-Fowler, A.T., Clayton, S.D., Riordan, J.M., Secrist, J.A. III. *Synthesis and biologic activity of 2'-fluoro-2-halo derivatives of 9-β-D-arabinofuranosyladenine.* J Med Chem 1992, 35: 397-401.

6. Beutler, E., Carson, D.A. *2-Chlorodeoxyadenosine: Hairy cell leukemia takes a surprising turn.* Blood Cells 1993,19: 569-72.

7. Piro, L.D., Ellison, D.J., Saven, J. *The Scripps Clinic experience with 2-chlorodeoxyadenosine in the treatment of hairy cell leukemia.* Leuk Lymphoma 1994, 14: 121-5.

8. Keating, M., O'Brien, S., Plunkett, W., Robertson, L., Gandhi, V., Estey, E., Dimopoulos, M., Cabanillas, F., Kemena, A., Kantarjian, H. *Fludarabine phosphate: A new active agent in hematologic malignancies.* Semin Hematol 1994, 31:28-39.

9. Piro, L.D., Carrera, C.J., Carson, D.A., Beutler, E. *Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine.* New Engl J Med 1990, 322: 1117-21.

10. Cheson, B.D. *New antimetabolites in the treatment of human malignancies.* Semin Oncol 1992, 19: 695-706.

11. Keating, M.J., Kantarjian, H., Talpaz, M., Redman, J., Koller, C., Barlogie, B., Velasquez, W., Plunkett, W., Freireich, E.J., McCredie, K.B. *Fludarabine: A new agent with major activity against chronic lymphocytic leukemia.* Blood 1989, 74: 19-25.

12. Parker, W.B., Shaddix, S.C., Chang, C.H., White, E.L., Rose, L.M., Brockman, R.W., Shortnacy, A.T., Montgomery, J.A., Secrist, J.A. III, Bennett, L.L. Jr. *Effects of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5'-triphosphate.* Cancer Res 1991, 51: 2386-94.

13. Plunkett, W., Saunders, P.P. *Metabolism and action of purine nucleoside analogs.* Pharmacol Ther 1991, 49: 239-68.

14. White, E.L., Shaddix, S.C., Brockman, R.W., Bennett, L.L. Jr. *Comparison of the actions of 9-β-D-arabinofuranosyl-2-fluoroadenine and 9-β-D-arabinofuranosyladenine on target enzymes from mouse tumor cells.* Cancer Res 1982, 42: 2260-4.

15. Tseng, W.-C., Derse, D., Cheng, Y.-C., Brockman, R.W., Bennett, L.L. Jr. *In vitro biological activity of 9-β-D-arabinofuranosyl-2-fluoroadenine and the biochemical actions of its triphosphate on DNA polymerases and ribonucleotide reductase from HeLa cells.* Mol Pharmacol 1982, 21: 474-7.

16. Parker, W.B., Bapat, A.R., Shen, Y.-X., Townsend,A.J:,Cheng, Y.-C. *Interaction of 2-halogenated dATP analogs (F, Cl, and Br) with human DNA polymerases, DNA primase and ribonucleotide reductase.* Mol Pharmacol 1988, 34: 485-91.

17. Huang, P., Chubb, S., Plunkett, W. *Termination of DNA synthesis by 9-β-D-arabinofuranosyl-2-fluoroadenine. A mechanism for cytotoxicity.* J Biol Chem 1990, 265: 16617-25.

18. Griffig, J., Koob, R., Blakley, R.L. *Mechanisms of inhibition of DNA synthesis by 2-chlorodeoxyadenosine in human lymphoblastic cells.* Cancer Res 1989, 49: 6923-8.

19. Hentosh, P., Koob, R., Blakley, R.L. *Incorporation of 2-halogeno-2'-deoxyadenosine 5'-triphosphate into DNA during replication by human polymerases α and β.* J Biol Chem 1990, 265: 4033-40.

20. Liliemark, J., Albertioni, F., Hassan, M., Juliusson, G. *On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: Alternative routes of administration.* J Clin Oncol 1992, 10: 1514-8.

21. Cheson, B.D., Vena, D.A., Foss, F.M., Sorensen, J.M. *Neurotoxicity of purine analogs: A review.* J Clin Oncol 1994, 12: 2216-28.

22. Huang, P., Plunkett, W. *Phosphorolytic cleavage of 2-fluoroadenine from 9-β-D-arabinofuranosyl-2-fluoroadenine by Escherichia coli.* Biochem Pharmacol 1987, 36: 2945-50.

23. Litzow, M.R. *PNAs for AML.* Blood 2003, 102: 2315.

24. Kantarjian, H.M. *Clofarabine - A new nucleoside analogue with significant activity in leukemia.* Cancer Invest 2003, 21(Suppl. 1): Abst 3.

25. Carson, D.A., Wasson, D.B., Esparza, L.M., Carrera, C.J., Kipps, T.J., Cottam, H.B. *Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine.* Proc Natl Acad Sci USA 1992, 89: 2970-4.

26. Parker, W.B., Shaddix, S.C., Rose, L.M., Shewach, D.S., Hertel, L.W., Secrist, J.A. III, Montgomery, J.A., Bennett, L.L. Jr. *Comparison of the mechanism of cytotoxicity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine, 2-chloro-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine, and 2-chloro-9-(2-deoxy-2,2-difluoro-β-D-ribofuranosyl)adenine in CEM cells.* Mol Pharmacol 1999, 55: 515-20.

27. Xie, C., Plunkett, W. *Metabolism and actions of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine in human lymphoblastoid cells.* Cancer Res 1995, 55: 2847-52.

28. Xie, K.C., Plunkett, W. *Deoxynucleotide pool depletion and sustained inhibition of ribonucleotide reductase and DNA synthesis after treatment of human lymphoblastoid cells with 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine.* Cancer Res 1996, 56: 3030-7.

29. Parker, W.B., Shaddix, S.C. *Effect of 2-Cl-2'-F-2'-deoxyarabinofuranosyladenine on natural nucleotide pools and its triphosphate on human DNA polymerases*. Proc Am Assoc Cancer Res 1990, 31: Abst 2504.

30. Lotfi, K., Mansson, E., Spasokoukotskaja, T., Pettersson, B., Liliemark, J., Peterson, C., Eriksson, S., Albertioni, F. *Biochemical pharmacology and resistance to 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine, a novel analogue of cladribine in human leukemic cells.* Clin Cancer Res 1999, 5: 2438-44.

31. Takahashi, T., Shimizu, M., Akinaga, S. *Mechanisms of the apoptotic activity of Cl-F-araA in a human T-ALL cell line, CCRF CEM.* Cancer Chemother Pharmacol 2002, 50: 193-201.

32. Xie, C., Plunkett, W. *Mechanism of cytotoxicity of 2-chloro-2'-fluoro-arabinosyladenine in CEM cells.* Proc Am Assoc Cancer Res 1994, 35: Abst 2308.

33. Spasokoukotskaja, T., Sasvári-Székely, M., Keszler, G., Albertioni, F., Eriksson, S., Staub, M. *Treatment of normal and malignant cells with nucleoside analogues and etoposide enhances deoxycytidine kinase activity.* Eur J Cancer 1999, 35: 1862-7.

34. Yamauchi, T., Nowak, B.J., Keating, M.J., Plunkett, W. *DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine.* Clin Cancer Res 2001, 7: 3580-9.

35. Waud, W.R., Schmid, S.M., Harrison, S.D. Jr., Secrist, J.A. III, Montgomery, J.A. *Preclinical antitumor activity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine.* Proc Am Assoc Cancer Res 1992, 33: Abst 3257.

36. Waud, W.R., Schmid, S.M., Montgomery, J.A., Secrist, J.A. III. *Preclinical antitumor activity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (Cl-F-ara-A).* Nucleosides Nucleotides Nucleic Acids 2000, 19: 447-60.

37. Lindemalm, S., Liliemark, J., Gruber, A., Eriksson, S., Karlsson, M.O., Wang, Y., Albertioni, F. *Comparison of cytotoxicity of 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine (clofarabine) with cladribine in mononuclear cells from patients with acute myeloid and chronic lymphocytic leukemia.* Haematologica 2003, 88: 324-32.

38. Carrera, C.J., Cottam, H.B., Wasson, D.B., Kipps, T.J., Saven, A., Piro, L.D., Beutler, E., Carson, D.A. *Stability and oral activity of 2-chloro-2'-ara-fluoro-2'-deoxyadenosine (CAFdA) in a SCID mouse model of chronic lymphocytic leukemia (CLL).* Proc Am Soc Clin Oncol 1992, 11: Abst 268.

39. Stephenson, K., Wilson, S.T., Schmid, S.M., Waud, W.R., Craig, A., Weitman, S.D. *Correlation between frequency of administration and efficacy of clofarabine in the H460 human non-small cell lung tumor xenograft model.* Proc Am Assoc Cancer Res 2003, 44(2nd ed): Abst 814.

40. Takahashi, T., Kanzawa, J., Okabe, M., Akinaga, S., Tamaoki, T. *Antitumor activity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine, a novel deoxyadenosine analog, against human colon tumor xenografts by oral administration.* 10th NCI-EORTC Symp New Drugs Cancer Ther (June 16-19, Amsterdam) 1998, Abst 579.

41. Takahashi, T., Kanazawa, J., Akinaga, S., Tamaoki, T., Okabe, M. *Antitumor activity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine, a novel deoxyadenosine analog, against human colon tumor xenografts by oral administration.* Cancer Chemother Pharmacol 1999, 43: 233-40.

42. Cooper, T., Nowak, B., Gandhi, V. *Biochemical modulation of cytarabine triphosphate by clofarabine.* Proc Am Assoc Cancer Res 2003, 44(2nd ed): Abst 621.

43. Weitman, S.D., Wilson, S.T., Stephenson, K., Waud, W.R., Craig, A., Schmid, S.M. *Antitumor effect of combination treatment with clofarabine and CPT-11 in the HT-29 human colorectal tumor xenograft model.* Proc Am Assoc Cancer Res 2003, 44(2nd ed): Abst 820.

44. Stephenson, K., Wilson, S.T., Schmid, S.M., Waud, W.R., Craig, A., Weitman, S.D. *Antitumor effect of combination treatment with clofarabine and Taxotere in the PC-3 human prostate tumor xenograft model.* Proc Am Assoc Cancer Res 2003, 44(2nd ed): Abst 813.

45. Lindemalm, S., Albertioni, F., Liliemark, J. *Stability and analysis of 2-chloro-2'-deoxyadenosine, 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine and 2-chloroadenine in human blood plasma.* Anti-Cancer Drugs 1997, 8: 445-53.

46. Reichelova, V., Liliemark, J., Albertioni, F. *Structure-activity relationships of 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine and related analogues: Protein binding, lipophilicity, and retention in reversed-phase LC.* J Liq Chromatogr 1995, 18: 1123-35.

47. Qian, M., Wang, X., Shanmuganathan, K., Chu, C.K., Gallo, J.M. *Pharmacokinetics of the anticancer agent 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine in rats.* Cancer Chemother Pharmacol 1994, 33: 484-8.

48. Gandhi, V., Robertson, L.E., Plunkett, W. *2-Chloro-2'-fluoro-arabinosyladenine: Pharmacokinetics and action in chronic lymphocytic leukemia cells.* Proc Am Assoc Cancer Res 1993, 34: Abst 2471.

49. Albertioni, F., Hassan, M., Silberring, J., Liliemark, J. *Kinetics and metabolism of 2-chloro-2'-deoxyadenosine and 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine in the isolated perfused rat liver.* Eur J Drug Metab Pharmacokinet 1995, 20: 225-32.

50. Cunningham, C.C., Nemunaitis, J., Senzer, N., Vukelja, S., Weiss, J., Ferrier, A., Craig, A.R. *A phase I dose-finding and safety study of clofarabine in patients with advanced solid malignancies.* Proc Am Soc Clin Oncol 2003, 22: Abst 605.

51. Gandhi, V., Kantarjian, H.M., Faderl, S. et al. *Cellular pharmacokinetics and pharmacodynamics of clofarabine (2-chloro-2'-fluoro-arabinosyladenine) in acute leukemia blasts during a phase I clinical trial.* Proc Am Assoc Cancer Res 2002, Suppl.: Abst LB-124.

52. Kantarjian, H.M., Gandhi, V., Kozuch, P. et al. *Phase I clinical and pharmacology study of clofarabine in patients with solid and hematologic cancers.* J Clin Oncol 2003, 21: 1167-73.

53. Kantarjian, H.M., Gandhi, V.V., O'Brien, S. et al. *Phase I and pharmacology study of clofarabine (2-Cl-2'-F-deoxy-9-D-arabinofuranosyladenine) in solid and hematologic cancers.* Blood 2001, 98(11, Part 2): Abst 4568.

54. Kozuch, P., Ibrahim, N., Khuri, F. et al. *Phase I clinical and pharmacological study of clofarabine.* Blood 1999, 94(10, Suppl. 1, Part 1): 127a.

55. Kantarjian, H., Gandhi, V., Cortes, J. et al. *Phase 2 clinical and pharmacologic study of clofarabine in patients with refractory or relapsed acute leukemia.* Blood 2003, 102: 2379-86.

56. Cortes, J.E., Gandhi, V., Plunkett, W. et al. *Clofarabine [2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine] is active for patients with refractory or relapsed acute leukemias, myelodysplastic syndromes (MDS) and chronic myeloid leukemia in blast phase (CML-BP).* Blood 2002, 100(11, Part 1): Abst 739.

57. Foran, J.M., Faderl, S., Wetzler, M., Dipersio, J., Rizzieri, D., Kantarjian, H., Craig, A., Weiss, J., Douer, D. *A phase II, open-label study of clofarabine in adult patients with refractory or relapsed acute myelogenous leukemia.* Proc Am Soc Clin Oncol 2003, 22: Abst 2360.

58. Foran, J., Wetzler, M., Kantarjian, H.M., Faderl, S., Abramson, N., Rizzieri, D.A., Craig, A., Weiss, J., Douer, D. *A phase II, open-label study of Clofarex™ in adult patients with refractory or relapsed acute myelogenous leukemia.* Blood 2002, 100(11, Part 2): Abst 4613.

59. Faderl, S., Gandhi, V., Garcia-Manero, G. et al. *Clofarabine is active in combination with cytarabine (ara-C) in adult patients (pts) in first relapsed and primary refractory acute leukemia and high-risk myelodysplastic syndrome (MDS).* Blood 2003, 102(11, Part 1): Abst 2271.

60. Jeha, S., Gandhi, V., Chan, K.W. et al. *Clofarabine, a novel nucleoside analog, is active in pediatric patients with advanced leukemia.* Blood 2004, 103: 784-9.

61. Jeha, S., Gandhi, V., Chan, K.W., McDonald, L., Ramirez, I., Rytting, M.E., Madden, R., Keating, M.J., Plunkett, W. Jr., Kantarjian, H. *Phase I study of clofarabine (Clofarex™) in pediatric leukemia.* Blood 2002, 100(11, Part 1): Abst 315.

62. Jeha, S.C., McDonald, L.R., Chan, K., Tubergen, D., Ramirez, I., Kantarjian, H. *Clofarabine (Clofarex) is an active agent in pediatric leukemias.* Proc Am Soc Clin Oncol 2002, 21(Part 1): Abst 1585.

63. Jeha, S.C., Steinherz, P., Razzouk, B., Kadota, R., Rheingold, S., Gaynon, P., Bomgaars, L., Craig, A., Bishop, R., Arceci, R. *A phase II, open-label study of clofarabine in pediatric patients with refractory or relapsed acute-myelogenous or lymphoblastic leukemia.* Proc Am Soc Clin Oncol 2003, 22: Abst 3251.

64. Jeha, S., Steinherz, P., Gaynon, P. et al. *A phase II, open-label study of clofarabine in pediatric patients with refractory or relapsed acute myelogenous leukemia.* Blood 2003, 102(11, Part 1): Abst 2278.

65. Jeha, S., Gaynon, P., 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(11, Part 1): Abst 3277.

Additional References

Mansson, E., Flordal, E., Liliemark, J., Spasokoukotskaja, T., Elford, H., Lagercrantz, S., Eriksson, S., Albertioni, F. *Down-regulation of deoxycytidine kinase in human leukemic cell lines resistant to cladribine and clofarabine and increased ribonucleotide reductase activity contributes to fludarabine resistance.* Biochem Pharmacol 2003, 65: 237-47.

Jeha, S., Steinherz, P., Altman, A., Rheingold, S., Gaynon, P., Goldman, S., Craig, A., Weiss, J., Arceci, R. *A phase II, open-label study of Clofarex™ in pediatric patients with refractory or relapsed acute-myelogenous or lymphoblastic leukemia.* Blood 2002, 100(11, Part 2): Abst 4616.

Sjöberg, A.H., Wang, L., Eriksson, S. *Substrate specificity of human recombinant mitochondrial deoxyguanosine kinase with cytostatic and antiviral purine and pyrimidine analogs.* Mol Pharmacol 1998, 53: 270-3.

King, K.M., Vickers, M.F., Damaraju, V., Mowles, D.A., Lang, T., Young, J.D., Cass, C.E. *Nucleoside transporter proteins are determinants of cytotoxicity of clofarabine (Clofarex™) in cultured leukemic cell lines.* Blood 2002, 100(11, Part 1): Abst 1247.

Chiao, N., Bumgardner, A., Duvic, M. *Clofarabine-induced acral erythema during the treatment of patients with myelodysplasia and acute leukemia: Report of two cases.* Leuk Lymphoma 2003, 44: 1405-7.