Older and New Formulations of Cladribine. Pharmacology and Clinical Efficacy in Hematological Malignancies

Tadeusz Robak[1,*], Anna Korycka[1] and Ewa Robak[2]

[1]*Department of Hematology, Medical University of Lodz and Copernicus Memorial Hospital, 93-513 Lodz, Pabianicka 62, Poland,* [2]*Department of Dermatology, Medical Uniwersity of Lodz, 94-017 Lodz, Krzemieniecka 5, Poland*

Received: May 25, 2005; Accepted: August 23, 2005; Revised: September 13, 2005

Abstract: The purine nucleoside analog (PNA) - cladribine (2-CdA, 2-chlorodeoxyadenosine) is a cytotoxic agent of high efficacy in lymphoid and myeloid malignancies. This drug was approved by the FDA for treatment of hairy cell leukemia and in some European countries for treatment of refractory/relapsed chronic lymphocytic leukemia. 2-CdA is usually administered as continuous or intermittent intravenous infusion. Recently however, new formulations of this agent has been developed for subcutaneous and oral administration. In contrast to other PNA, 2-CdA is equally cytotoxic to both proliferating and quiescent cells and several pathways may be responsible for the mechanism of its action. In addition, recent data indicate that 2-CdA combined with other cytotoxic agents and monoclonal antibodies show synergistic proapoptotic and cytotoxic activity on lymphoid and myeloid neoplastic cells. This review article summarizes recent achievements in the understanding of 2-CdA mechanism of action, pharmacokinetics of different pharmaceutical formulations and its approved and possible future applications in the treatment of hematological malignancies.

The most important recent patents concerning oral formulations of 2-CdA have been presented.

Keywords: Cladribine, oral, subcutaneous, pharmacokinetics, leukemia, lymphoma, systemic mastocytosis, Langerhans cell histiocytosis, acute myeloid leukemia.

INTRODUCTION

The purine nucleoside analogs (PNA), fludarabine (FA), cladribine (2-chlorodeoxyadenosine, 2-CdA) and pentostatin (2´-deoxycoformycin, DCF) represent a novel group of cytotoxic agents with high activity in lymphoid and myeloid malignancies [1]. These three agents share similar chemical structures and mechanism of action, such as induction of apoptosis. However, they exhibit also significant differences, especially in their interactions with enzymes involved in adenosine and deoxyadenosine metabolism activity.

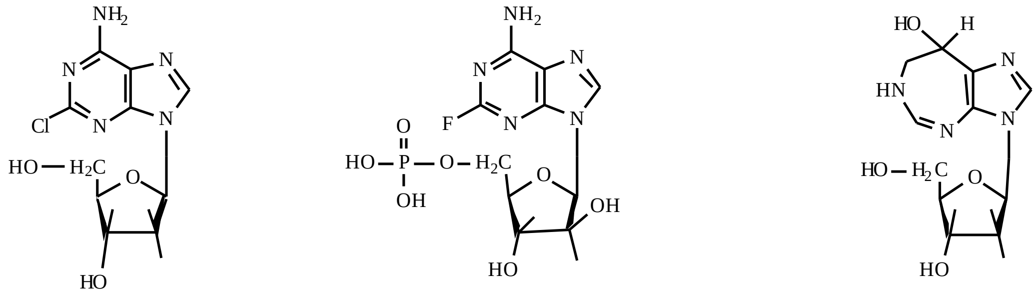
2-CdA is resistant to deamination by adenosine deaminase (ADA) and is selectively accumulated in lymphocytes finally inducing programmed cell death (apoptosis) (Fig. **1**) [2]. It was synthesized in 1980 by Carson *et al*. [3] and more recently by Kazimierczuk *et al*. (4) by a simple substitution of the hydrogen atom by a chlorine atom at position 2 of the purine ring of deoxyadenosine. The rationale for the development of 2-CdA came from the observation that 30% of children with severe combined immunodeficiency disease (SCID) manifested ADA deficiency [5]. Severe lymphocytopenia affecting both B and T cells is observed in SCID. As it was demonstrated that, unlike adenosine, deoxyadenosine is not metabolized by any enzyme other than ADA, deficiency of this enzyme leads to deoxyadenosine accumulation in cells and is associated with lymphopenia evident in the course of SCID [6]. 2-CdA was approved by the FDA for treatment of hairy cell leukemia(HCL) and in some European countries for treatment of refractory/relapsed chronic lymphocytic leukemia (CLL). 2-CdA is usually administered as continuous or intermittent intravenous infusion. Recently however, new formulation of this agent has been developed for subcutaneous and oral administration. In contrast to other PNA, 2-CdA is equally cytotoxic to both proliferating and quiescent cells and several pathways may be responsible for the mechanism of its action. In addition, recent data indicate that 2-CdA combined with other cytotoxic agents and monoclonal antibodies (MoAb) show synergistic proapoptotic and cytotoxic activity on lymphoid and myeloid neoplastic cells. This review article will summarize recent achievements in the understanding of 2-CdA mechanism of action, pharmacokinetics of different pharmaceutical formulations and its both currently approved and future alternative ways of application in the treatment of hematologic malignancies.

*Address correspondence to this author at the Department of Hematology, Medical University of Lodz, 93-513 Lódz, Pabianicka 62, Poland; Tel: +48 42 6895191; Fax: + 48 42 6895192; E-mail: robaktad@csk.umed.lodz.pl

MECHANISM OF ACTION

The uptake of 2-CdA into the cells may significantly modulate the intracellular bioavailability of the drug and, consequently, responsiveness to therapy [7,8]. Two gen families (SLC28 and SLC29), encoding human equilibrative nucleoside transporters (hENT) and human concentrative nucleoside transporters (hCNT), are involved in this process [7]. Up to now, three subtypes of hCNT transporters (hCNT1, hCNT2 and hCNT3) and four members of hENT have been cloned. However, only hENT1 and hENT2 are well-characterized plasma membrane transporters, whereas hENT3 and hENT4 are less well characterized and located inside the cells [7]. Either 2-CdA or other PNA are high affinity substrate for hENT1, hENT2 and hCNT3, while hCNT1 does not recognize them [7,8].

1574-8928/06 $100.00+.00



2-Chloro-2'-deoxyadenosine (2-CdA, cladribine)

9-β-D-Arabinosylo-2'-fluoro-adenosine phosphate (F-ara-AMP, fludarabine monophosphate)

2'-Deoxycoformycin (DCF, pentostatin)

Fig. (1). Chemical structure of cladribine (2-chlorodeoxyadenosine), fludarabine monophosphate and deoxycoformycin (pentostatin).

For cytostatic effect to occur, intracellular phosphorylation of 2-CdA is necessary. The drug is phosphorylated by deoxycytidine kinase (dCK) and acumulates in the cells as chlorodeoxyadenosine triphosphate (2-CdA-TP) [6]. High activity of this enzyme in lymphocytes along with their low 5´-nucleotidase (5´-NT) activity probably explains its relatively high selectivity for lymphoid cells [9-15].

In contrast to other antineoplastic drugs, 2-CdA is cytotoxic to both proliferating and quiescent cells. Several pathways may be responsible for the mechanism of their action. The cytotoxicity of 2-CdA in the proliferating cells is mainly due to either suppression of DNA synthesis via inhibition of DNA polymerases, or incorporation into DNA, or inhibition of DNA repair and accumulation of DNA breaks. In addition, 2-CdA strongly inhibits ribonucleotide reductase (RR) activity leading to imbalance in deoxynucleotide triphosphates pool, and via endonuclease activation leads to DNA strand breaks [11,12]. It is also suggested that the cytotoxic mechanism of 2-CdA may originate not only from the suppression of RR and DNA polymerases, but also from the inhibition of deoxyadenosine deamination and phosphorylation, with subsequent inactivation of 5-adenosylhomocysteine hydrolase (SAHM) as a natural consequence of adenosine accumulation [13]. It is also known that RR and two other enzymes: 5´-NT and purine nucleoside phosphorylase (PNP) participate in catalysing purine nucleoside degradation [16,17].

In quiescent cells 2-CdA-TP interferes with proper repair of DNA and leads to a total disruption of cellular metabolism via accumulation of breaks in DNA strand, which in turn leads to p53 expression and consequently to induction of apoptosis [14,15,18]. In these cells, however, both p53-dependent and p53-independent cytotoxicity mechanisms are suggested to occur [19,20]. DNA breaks activate poly(ADP-ribose) polymerase (PARP), leading to nicotinamide adenine dinucleoside (NAD) and adenosine triphosphate (ATP) depletion and can kill cell along the necrosis pathway [1]. On the other hand, DNA damage induces p53 expression and eventually leads to apoptosis which is the main mechanism of 2-CdA cytotoxicity (Fig. **2**).

Two major death pathways have been identified so far. The pathway that is regulated by p53 and Bcl-2 family proteins involves activation of mitochondria and the second one is connected with death receptor signaling by Fas receptor [14,15,18, 21,22].

Apoptosis induced by 2-CdA can be mediated either via DNA damage and p53 protein expression or directly via mitochondrial permeability transition pore (mtPTP) [19]. Inhibition of DNA repair and accumulation of DNA breaks leads to p53 expression which plays a key role in the control of apoptosis and cell cycle and influences the bcl-2 protein family with antiapoptotic properties as well as bcl-2 like proteins such as bax, bcl-xs and bak which have proapoptotic action [23,24]. The most important however is the relationship between bax and bcl-2. Bax activity leads to mitochondrial changes resulting in the release of cytochrome c from mitochondrium to cytosol and its binding with apoptotic protease activating factor (APAF-1) and activation of procaspase-9. APAF-1, cytochrome c and procaspase-9 form a complex termed apoptosome which after binding with 2-CdA-TP leads in consequence to activation of caspase-9 cascade (Fig. **2**) [25,26]. On the other hand, it is also known that 2-CdA-TP induces apoptosis by direct mitochondrial pathway [20]. It can directly bind to proteins located in mitochondrial membrane resulting in the release of either cytochrome and formation of apoptosome or apoptosis inducing factor (AIF). The AIF protein leads to chromatin condensation and DNA fragmentation without the caspase-9 cascade activation [20].

Induction of apoptosis by 2-CdA *via* the death receptor Fas/CD95 on extrinsic pathway remains controversial (Fig. **2**) [21,22]. Nomura *et al*. [21] have shown that apoptosis of leukemic cells in response to 2-CdA could be mediated through Fas/FASL pathway, however, Klopfer *et al*. [22] have documented that apoptosis induced by 2-CdA is independent of CD95/Fas receptor or of direct effect on APAF-1, and rather follows the mitochondrial signaling pathway of cytochrome and caspase-9 cascade activation.

PHARMACOKINETICS AND ADMINISTRATION OF DIFFERENT FORMULATIONS

Clinical pharmacokinetics of 2-CdA have been evaluated in patients with lymphoproliferative diseases and acute leukemia (Table **1**). Initial evaluation of this agent was conducted using a continuous infusion for 5 to 14 days [23]. However, in subsequent studies short intravenous infusions, oral and subcutaneous administrations were used [24-33].

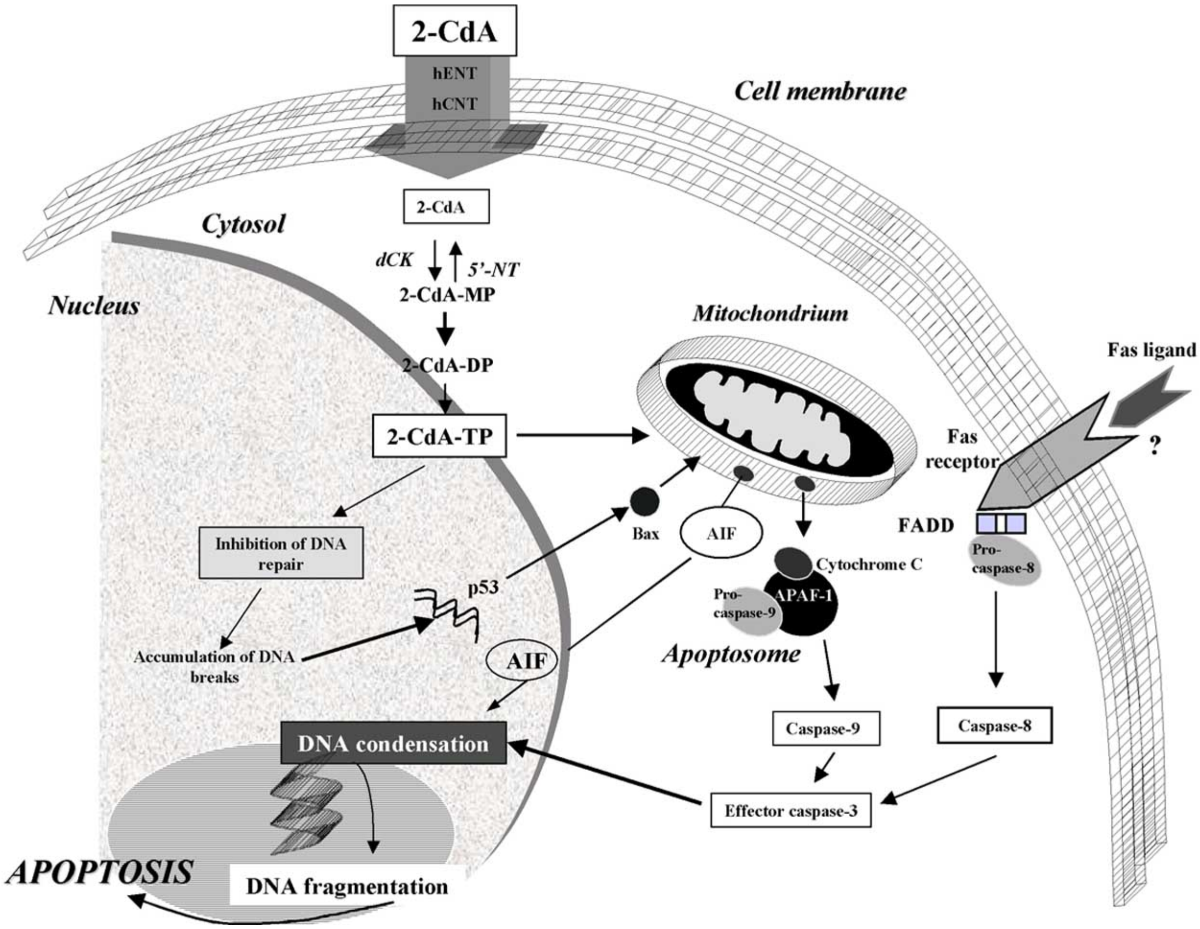


Fig. (**2**)**.** Schematic presentation of 2-CdA pathways.

2-CdA-MP - cladribine monophosphate
2-CdA-DP - cladribine diphosphate
2-CdA-TP - cladribine tiphosphate
dCK - deoxycytidine kinase
5´-NT - 5´-nucleotidase
hENT - human equilibrative nucleoside transporter
hCNT - human concentrative nucleoside transporter
AIF - apoptosis-inducing factor
APAF-1 - apoptotic protease activating factor
FADD - Fas associated death domain

Plasma concentrations of this agent were determined either with high performance liquid chromatography or radioimmunoassay [28,29]. Few different routs of 2-CdA administration have been known: intravenous, subcoutaneous or intramuscular and lastly introduced orall administration [27-32]. Recently, the liguid dosage form contains the most preferably about 10mg/ml of cladribine or its pharmaceutically acceptable salts and includes a cyclodextrin which is generally present in amount necessary to solubilize the cladribine. The preferable concentration of cyclodexrin is from 200 to 300mg/ml [34]. It has been also found that cladribine is more stable against hydrolysis when use in combination with cyclodextrin. This is the particular benefit in the formulation of solid oral dosages forms, when the compounds would be normally undergo hydrolysis in the acid pH of the stomach contents. The addition of cyclodextrin significantly enhances the stability of cladribine at pH 1.4 [34-36]. Dosage forms are optionally formed in a pharmaceutically acceptable vehicle with any of the well-known pharmaceutically acceptable carries, diluents, binders, coloring agents [35].

Intravenous Administration

In the treatment of lymphoid malignancies 2-CdA is usually administered i.v. in a dose of 0.12-0.14 mg/kg in continuous or 2-hour infusion. In the study of Santana *et al*. [30] intracellular nucleoside disappearance rates were high with the values of α and β-half life 1.29 and 2.47 hours respectively, suggesting that continuous intravenous infusion of this drug produces cell death at a greater degree. However, sensitive high performance liquid chromatography (HPLC) assays developed by Lilemark and Juliusson [28,32]

Table 1. Summary of the Pharmacokinetic Properties of Cladribine after Intravenous, Subcutaneous or Oral Administration in Patients with Lymphoproliferative Disorders

Study	Drug regimen		C_{max}	t_{max} (h)	$T_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (h)	CL	V_d	F (%)
	Dose/route	Duration of infusion							
Liliemark & Juliusson 1991 [28]	0.14mg/kg/day i.v. 0.14mg/kg i.v.	2h 24 ci	198nM 21.7nM		35	6.7		17.7 L/kg	
Liliemark *et al*. 1992 [27]	0.14mg/kg/day i.v. 0.14mg/kg/day s.c. 0.14mg/kg/day p.o 0.28mg/kg/day p.o.	2h	169nM 318nM 53nM 196nM	2 0.34 1.17	58	9.9 13.3 10.4	26L/h/kg	54L/m^2 53L/m^2 84 L/m^2	100 48 55
Santana *et al*. 1992 [142]	8.9mg/m^2/day i.v.	ci	54nM	24	3.1	14.2	36.1 L/h/m^2	305 L/m^2	
Kearns *et al*. 1993 [150]	8.9mg/m^2/day i.v.	ci	32.7μM			19.7	39.4 L/h/m^2	356.2 L/m^2	
Albertoni *et al*. 1993 [39]	0.24mg/kg p.o.		83/116nM	0.8					42/46
Albertoni *et al*. 1998 [41]	10mg/m^2/day .po. 5mg/m^2 i.v.	 2h	0.107μM 6μM	1.0 3	1.3	21.1			55 36
Lindemalm *et al*. 2005 [31]	0.9mg/kg/day i.v.	ci				16	39.3L/h	71.7 L/kg	

Abbreviations: i.v.- intravenous; s.c.- subcutaneous; p.o.- oral; C_{max} - peak plasma drug concentration; t_{max} - time to reach C_{max}; $T_{1/2\alpha}$ - initial half-life; $T_{1/2\beta}$ terminal elimination half-life; CL- clearence; V_d - volume of distribution; F- bioavailability.

showed that the terminal half-life of plasma 2-CdA was about 8 hours. The same authors showed the terminal half-life of 6.7 hours after 2-hour infusion of 2-CdA, which permits the drug to be administered as an intermittent infusion without loss of antitumor activity [28]. Following administration of this drug at the dose of 0.14 mg/kg as a 2-hour infusion in 12 patients the mean maximum plasma concentration was 198 nM (range 70-381 nM). The steady -state drug concentration during 24-hour continuous infusion of 2-CdA at a dose of 0.14 mg/kg/day was 21.7 nM. Areas under the plasma concentration curve after 2- and 24-hour infusions were 588 nM and 533 nM, respectively. There is a linear dose relationship between 0.2 and 2.5 mg/m^2/h and elimination follows a 2-or 3-compartment model depending on the sampling procedure [24]. About 30 to 50% of administered 2-CdA is excreted unchanged in the urine within 24 hours [29]. In population pharmacokinetics study performed by Lindelman *et al*. [31] clearance of 2-CdA was 39.3 L/hour with a large interindividual variability. The half-life for the terminal phase was 16 hours.

2-CdA is also an effective drug when administered at a dose of 0.15 mg/kg in 2-hour infusion once a week over 6 courses. In the study by Lauria *et al*. [37] 22/30 (73%) patients with HCL achieved complete responce (CR) and 8 (27%) – partial responce (PR) when 2-CdA was given in this mode. This type of drug administration may be less toxic and reduces the risk of infection complications in comparison with standard 2-CdA daily regimens. In our randomized study we compared weekly administration of 2-CdA (0.12 mg/kg in 2-h i.v. infusion once a week for 6 weeks) with daily administration (0.12 mg/kg in 2-h i.v. infusion for 5 consecutive days) [38]. The updated results of this study indicate that both CR and overall response (OR) rates were similar in compared groups. There was no statistically significant difference in toxicity between groups except for thrombocytopenia. It seems, however, that daily administration of 2-CdA may more frequently induce neutropenia and lead to more frequent infections.

Oral Administration

2-CdA can be also administered orally. However, oral administration of 2-CdA solution induces degradation to 2-chloroadenine, which can be overcome by doubling the dose. The bioavailability after oral administration of 2-CdA is 35-55% [26,27,31]. Maximum oral bioavailability after the dose of 0.28 mg/kg/day dissolved in phosphate buffer solution is 55% [27]. Oral bioavailability of 2-CdA is similar whether taken with or without food (42 vs 46%), or with or without concomitant omeprazole 51% vs 46% [39]. After oral administration 2-CdA is partially deactivated by acid hydrolysis in the stomach and enzyme hydrolysis through intestinal bacteria or as a first - pass effect in the liver [39,40]. Albertoni *et al*. [41] have investigated the pharmacokinetic parameters of 2-CdA in plasma of patients with CLL, treated with 2-CdA orally at a dose 10 mg/m^2 on 3 consecutive days. They correlated plasma 2-CdA levels with intracellular 2-CdA monophosphate (2-CdA-MP) and 2-CdA triphosphate (2-CdA-TP) in circulating leukemia cells after the last dose intake and up to 72h thereafter using specific ion-pair liquid chromatographic method which separates the intracellular 2-CdA nucleotides. The area under the concentration versus time curve (AUC) of 2-CdA-MP in leukemia cells was higher (median 47 μmol/Lxh) than the AUC of 2-CdA-TP (median 21.7 μmol/L/h) and the median ratio between the AUC values of these parameters was 0.66. A significant correlation was found between the maximum plasma 2-CdA and cellular 2-CdA-MP concentrations (r = 0.56, P = 0.02). However, there was no correlation between dCK activity and intracellular pharmacokinetic parameters of 2-CdA-MP and 2-CdA-TP and no relationship between the response to treatment and intracellular concentration of 2-CdA-MP or its active metabolite 2-CdA-TP. However, in some other studies, high dCK activity, as well as low cytoplasmic 5´-NT activity correlated with the response to treatment [42,43]. In contrast, Beghiter *et al.* [44] did not find any correlation between intracellular accumulation of 2-CdA nucleotides and drug sensitivity in CLL leukemia cells.

Subcutaneous Route

The study of Liliemark *et al*. [27,45] has shown that 2-CdA can be also administered in subcutaneous injections. It has been shown that there is 100% 2-CdA bioavailability after subcutaneous administration with high peak concentrations of short duration in the areas under the curve (AUC) very similar to those achieved with 1-hour intravenous infusions. After the subcutaneous dose of 3.4 mg/m^2 body surface area, the median AUC in plasma following first injections was 567.5 μmol/Lxh (range 140 to 356 μmol/Lxh). These values and variability are similar to the corresponding data achieved with intravenous continuous or 2-hour bolus infusions. The median half-life of 2-CdA in plasma was 13.3h (range 3.6-39.8) following subcutaneous injection as compared to 9.9± 4.6 following bolus infusions [27,45]. It should be noted that there were no local side effects when using the subcutaneous route, except for the systemic effects. The subcutaneous route is a substantial improvement in disorders that require repeated courses of 2-CdA, such as CLL.

SIDE EFFECT AND LATE COMPLICATIONS

The tolerability profile of cladribine is distinguished from that of other cytotoxic drugs. Nausea, vomiting, hair loss and abnormal liver or kidney function have been rarely reported in association with its administration at the recommended doses [46]. However, bone marrow suppression with anemia, neutropenia and thrombocytopenia are dose limiting factors. Prolonged thrombocytopenia, neutropenia and anemia are especially pronounced in heavily pretreated patients and after multiple courses of therapy. These observations indicate the necessity of cautious administration of 2-CdA, especially in heavily pretreated patients. In case of severe thrombocytopenia and haemorrhagic diathesis, platelet transfusion may be necessary. Treatment included granulocytopenia constituted a significant clinical problem in over 20% of patients [46]. Our randomized study confirmed strong myelosuppressive effect of 2-CdA that resulted in high incidence of neutropenia (23%) in patients treated with this nucleoside analogue in comparison to Chl with prednisone (11%) (p = 0.02) [47]. Infections or fever of unknown origin were also more frequent after treatment with 2-CdA than with Chl (56% and 40%, respectively, p= 0.02).

Treatment with 2-CdA leads to a decrease in the CD4$^+$/CD8$^+$ ratio for an extensive period of time exceeding 12 to even 24 months. In consequence, infections, including oportunistic ones are frequent events and infections with a fatal outcome have been reported. Previously treated or recently infected patients with CLL were at higher risk for developing infection or fever after 2-CdA, while age, time interval between diagnosis and therapy, neutrophil and lymphocyte count at onset of 2-CdA were not predictive in case of infection after therapy with 2-CdA. Significant decrease of myelotoxicity and risk of infections with similar response and remission duration have been observed in the patients with CLL with 2-CdA dose reduced by 29%.

Reports of neurotoxicity of 2-CdA given at standard schedules of 3-6mg/m^2 for 5 to 7 days are uncommon [46]. However, at higher than the recommended dose life threatening neurotoxicity were seen.

Some reports provide evidence for correlation between 2-CdA therapy and development of autoimmune hemolytic anemia (AIHA) [1,2]. The probability of drug-induced AIHA seems to be higher if hemolysis occurs shortly after drug administration, i.e.1-2 months. If it occurs later it is probably not drug-related and appears by chance alone. Pure red cell aplasia (PRCA) occurs in approximately 5% of CLL patients most often in the course of disease, but also at presentation [1].

Prolonged immunosuppression related to PNA treatment may increase the risk of second malignancies [1]. In our randomized study comparing 2-CdA with Chl in previously untreated patients with CLL we have observed secondary malignancies only in 2 patients treated with 2-CdA (brain glioma and pulmonary cancer) and in one patient treated with Chl (liver cancer) [47]. Recently, we have also analyzed an impact of 2-CdA treatment on the subsequent occurrence of either secondary solid tumors or Richter's syndrome in CLL patients [45]. There were 1487 eligible patients, 251 treated with 2-CdA alone, 931 treated with alkylating agents alone and 323 treated both with 2-CdA and alkylating agents. Our observations indicate that 2-CdA does not significantly increase the risk of secondary malignancies in CLL patients, except for lung cancer. However, longer observations are needed to test the possibility of long-term effect.

INTERACTIONS OF 2-CdA WITH OTHER ANTI-NEOPLASTIC AGENTS

Several *in vitro* and *in vivo* studies have described the interactions between 2-CdA and alkylating agents, anthracyclines and monoclonal antibodies active in CLL [49-52].

Begleiter *et al*. [49] observed a synergistic effect when CLL cells from 12 patients were treated with chlorambucil (Chl) prior to 2-CdA, whereas treatment of human bone marrow with these agents resulted in a low level of synergy for CFU-GM cells and in additive cell killing in erythroid progenitors. Szmigielska *et al*. [50] evaluated the effect of three anthracyclines - doxorubicin, mitoxantrone (MIT) and idarubicin - on murine leukemias L1210 and P388 and showed that all anthracyclines potentiate 2-CdA antileukemic activity both *in vitro* and *in vivo*. These authors have also indicated an additive cytotoxic effect on CLL cells of all anthracyclines applied *in vitro* together with 2-CdA and suggested that all of these combinations induced apoptosis with similar efficacy [51]. In contrast, dexamethasone (DEX) did not enhance antileukemic activity of 2-CdA in mice with both types of murine leukemia [52]. The studies presented by Chow *et al*. [53] suggest that 2-CdA used jointly with cytarabine (Ara-C) acts synergistically on inhibition of proliferation of HL60 and HEL leukemic cell lines as well as on apoptosis of leukemic cells, whereas the combination of Ara-C and FA acts additively or antagonistically. Moreover, 2-CdA combined with Ara-C had higher synergistic activity on AML cells in microculture cytotoxicity assay [54]. Schirmer *et al.* [55] have demonstrated that 2-CdA used jointly with gemcytabine (dFdC) inhibit proliferation of CML cells to a higher degree than that of normal cells. It is worth mentioning that the results of these studies suggest that this combination could be useful in purging before autografting. Maranda *et al*. [56] have revealed that the most effective treatment schedule in both murine leukemias L1210 and P388 was dFdC given before 2-CdA, and the combination of these agents showed an additive effect.

The interactions *in vitro* between PNA and other chemotherapeutic agents such as interferons (IFNα , IFNγ), tumor necrosis factor α (TNF-α) and its muteins and signal transduction inhibitors (imatinib mesylate, tipifarnib) have been also investigated [57-59]. Results of these studies suggest that the combination of 2-CdA with IFN-α in both AML and CML and 2-CdA used jointly with tipifarnib (R115777) in AML patients are promising. 2-CdA used jointly with IFN-α showed a synergistic effect on AML CFU-L and an additive effect on CML CFU-GM cells and enhanced apoptosis of leukemic blasts [59].

Recent data indicate that 2-CdA administered in combination with monoclonal antibodies (MoAbs) such as anti-CD 52 (alemtuzumab) shows proapoptotic activity and significantly increases cytotoxic effect on CLL cells [60].

CLINICAL ACTIVITY OF 2-CdA

2-CdA is the drug of choice in the treatment of hairy cell leukemia (HCL) but is also highly active in low-grade lymphoid malignancies, including chronic lymphocytic leukemia (CLL). Moreover, several investigations have revealed that these agents are also active in other hematologic malignancies including acute myeloid leukemia (AML), Langerhans cell histiocytosis (LCH), and systemic mastocytosis (SM).

Hairy Cell Leukemia

Piro *et al*. [61] were the first to describe sustained CR in 2 HCL patients who had undergone splenectomy and received a single continuous intravenous infusion of 2-CdA for 7 days. Further multiple studies on larger groups of patients demonstrated that 2-CdA induces durable and unmaintained CR in about 80% of patients after a single course of therapy (Table **2**) [62-66]. 2-CdA is administered either as a continuous i.v. infusion at a dose of 0.09 mg/kg over a 5-7 day period or as 2-hour i.v. infusion at a dose of 0.12 mg/kg for 5-7 days [62-65]. However, similar results were achieved when the drug was given in subcutaneous injection [66,67]. Preliminary observations indicated that a CR following 2-CdA administration was durable even without maintenance therapy, so this drug was consistered as potentially curative against HCL [68,69]. However, a long-term clinical follow up of patients who had entered CR also after a single course of 2-CdA revealed that about 20% of them relapsed [70-75]. Patients in apparent clinical and hematological remission following single course of 2-CdA administration may have residual disease detected with the use of flow cytometry or molecular assay [64,74]. These data seem to indicate that a single course of 2-CdA may not be sufficient to eliminate the entire leukemic clone.

2-CdA is an effective drug also in patients with HCL who relapsed or did not enter remission after such therapeutic modalities as splenectomy and/or IFN-α. In our study 20 of 75 patients relapsed and a probability of a 3 year progression free survival (PFS), according to Kaplan-Maier's method, amounted to 75% [63]. The problem of therapy for HCL patients, who relapsed after 2-CdA remains unresolved. In our study 10 of 20 relapsed patients are in good clinical state and their peripheral blood counts are within normal limits, or they have only slight leukopenia and relative lymphocytosis [75]. They are monitored without being treated with 2-CdA. The other 10 relapsed patients became cytopenic or showed clinical progression of disease and were retreated with 2-CdA. Seven of them entered second CR and 3 achieved PR after a 2-CdA course identical to the first one. Similar results have been achieved by Saven *et al*. [76]. This means that 2-CdA does not necessarily produce resistance of the leukemic clone and the drug may be equally effective in the reinduction therapy. The same group reported that 2-CdA may be effective in patients who failed on previous treatment with pentostatin [77].

Chronic Lymphocytic Leukemia

2-CdA has been found to be also quite effective in the treatment of patients with CLL even when they are resistant to other therapies. The therapeutic activity of 2-CdA in patients with CLL resistant to conventional treatment was first reported in 1988 by Piro *et al*. [78]. Out of 18 patients, an overall response was achieved in 10. The same group later reported a response rate of 44% in 90 patients with advanced and previously treated CLL. However, only 4% of the patients achieved CR. Recently we have analyzed the results of treatment with 2-CdA in 378 patients with B-CLL [79]. 2-CdA was given as 2-h infusion for 5 consecutive days. Among 184 pretreated patients CR was obtained in 23 (12.5%) and PR in 66 (35.9%) giving an OR rate of 48.4%. The median duration of OR for these patients was 13.5 (range 3-63) months. The mean survival duration evaluated from the beginning of 2-CdA treatment was 16.3 months.

2-CdA is also more effective in previously untreated patients with CLL than in the pretreated patients. In different studies the OR rate ranged from 56 to 85% and CR from 10 to 47% [80]. In our retrospective analysis of 43 patients treated only with 2-CdA, CR was achived in 16 (37.2%) and OR in 31 patients (85.4% [79]. The median duration of OR was 16.0 (range 3-54) months.

Table 2. Summary of Larger Clinical Studies of 2-CdA in Hairy Cell Leukemia

Study	Treatment	No of pts	CR rate (%)	Duration of CR (median time to relapse)	Rate of relapse at follow-up
Saven *et al*. 1998 [76]	c.i.	349	91	96% survival at 48 months	26% at median 29 months
Goodman *et al*. 2003 [71]	c.i.	207	95	98%	37% at median 44 months
Hoffman *et al*. 1997 [73]	c.i.	49	76	RFS 80% at median 55 months follow-up	NA
Cheson *et al*. 1998 [63]	c.i.	861	50	Median not reached	NA
Robak *et al*. 1996 [65]	2 hour i.v. infusion/5 days or c.i. /7 days	41	76%	NA	NA
Jehn *et al*. 2004 [68]	c.i.	44	98%	DFS 36% at 12 years OS 79% at 12 years	NA
Von Rohr *et al*. 2002 [65]	s.c. bolus injection/5 days	62	76%	NA	NA
Luria *et al*. 1999 [37]	0.15 mg/kg weekly for 6 weeks	25	76%	NA	NA
Robak *et al*. 1999 [75]	2 hour i.v. infusion / 5 days	97	77.3%	37.4 months	26.7%
Chadha *et al*. 2005 [62]	0.1 mg/kg/day x 7,c.i.	86	79%	RFS 54% at 12 years	NA

Abbreviations: CR-complete response, DFS – disease free survival, RFS – relapse free survival, 2-CdA – 2-chlorodeoxyadenosine, NA - not applicable, i.v. –intravenous, s.c.-subcutaneos, c.i. – continuous infusion.

High CR and OR rates in CLL patients treated with 2-CdA as a first-line therapy were also obtained in a multicenter, prospective, randomized trial [47]. In this study we compared the efficacy and toxicity of 2-CdA with prednisone and Chl with prednisone in previously untreated patients with progressive and advanced CLL. 2-CdA was administered at a dose of 0.12 mg/kg daily in 2h infusion for 5 consecutive days and was combined with oral prednisone 30 mg/m^2 daily on days 1 - 5 starting with 2-CdA courses. Chlorambucil was administered at a dose of 12 mg/m^2 per day for 7 consecutive days and prednisone was given at a dose of 30 mg/m^2 per day on days 1 - 7. Both cycles were repeated at monthly, or longer intervals if hematologic complications or severe infections developed. Out of 229 evaluated patients 126 received 2-CdA with prednisone and 103 received Chl with prednisone. The data obtained from this trial indicate that the OR rate after 2-CdA and prednisone therapy was significantly higher than that after Chl and prednisone treatment (87% and 57%, respectively, $p< 0.001$). Moreover, the clinical CR rate after 2-CdA was also significantly higher (47%) than that after Chl treatment (12%) ($p < 0.001$). Likewise, progression free survival (PFS) was significantly longer in the 2-CdA treated group ($p = 0.01$). However, there was no difference in the OS duration between the two groups (Fig. **3**) and no difference in the event-free survival. It should be noted that this trial was designed as a cross-over study and most patients in the Chl group were administered 2-CdA in refractory cases or early relapse. This may have influenced the survival time results.

Recently, an international, randomized study to compare 2-CdA, FA and Chl in previously untreated patients with CLL has been initiated by Karlson *et al*. [81]. Preliminary results of this multicenter study show that both efficacy and toxicity of 2-CdA and FA in first-line treatment are similar. The study comprised 150 patients and 139 were evaluated. Overall response according to NCI criteria and intention to treat were documented in 57% of patients in Chl group, 67% in FA group and 74% in 2-CdA group. Myelotoxicity and infections also did not differ between all three arms. Recently, we have performed an interim analysis of randomized study comparing activity and toxicity of 2-CdA and CY (CC program) versus FA and CY (FC program) in previously untreated progressive or symptomatic CLL [82]. The preliminary results of this study indicate that CC and FC programs used as first-line therapy gave similar CR (36.7% *vs* 56.3%, $p=0.1$) and OR (93.3% *vs* 87.5%, $p=0.37$) and comparable toxicity. The recent data indicate that 2-CdA administered in combination with other chemotherapeutic agents and/or MoAb may produce higher response rates including CR and molecular CR compared with 2-CdA in monotherapy or other treatment regimens.

Among cytotoxic agents alkylating drugs and anthracyclines were the first candidates for combined use with 2-CdA. Montillo *et al*. [83] treated 29 patients with refractory or recurrent CLL or prolymphocytic leukemia (PLL) with 2-CdA (4 mg/m^2 /day) and CY (350 mg/m^2/day) for 3 days every 4 weeks. Eleven patients (38%) had a response with median response duration of 12 months.

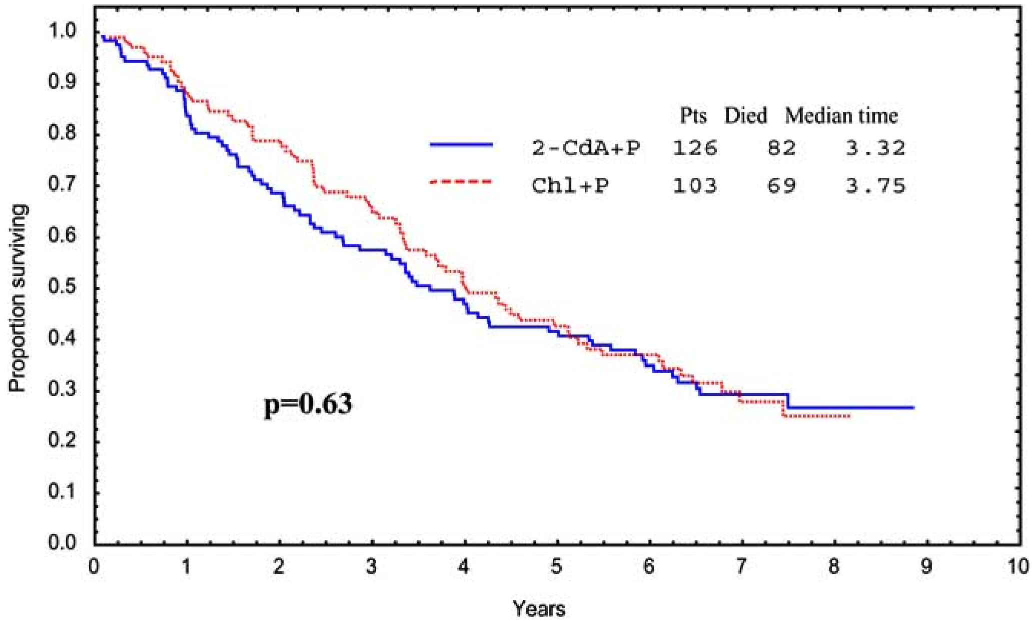


Fig. (**3**)**.** Overal survival time calculated from the first day of 1st line treatment to the last day of follow-up or death for patients treated with 2-CdA+P (continuous line) or Chl+P (dotted line). (Modified and updated from [47]).

Neutropenia was the most common form of hematologic toxicity and was observed after 25% of 84 courses of treatment. We determined the effectiveness and toxicity of 2-CdA in combination with CY in patients with previously untreated CLL [84]. 2-CdA was administered at a dosage of 0.12 mg/kg for 3 consecutive days and CY at a dose of 650 mg/m^2 on day 1. The cycles were repeated at 4-week, or longer intervals if severe myelosuppression occurred. In the analyzed group of 82 patients the OR rate of 87.8% was achieved including CR in 29.3% patients. Minimal residual disease (MRD) was only detected in six out of 24 (25%) patients with CR. Grade 3/4 thrombocytopenia was seen in four patients (5%) and neutropenia in 10 (12%). Severe infections were noted in 21(25%) patients. The efficacy of this regimen seems to be higher than observed earlier after treatment with 2-CdA alone.

In our study we determined the effectivenes and toxicity of combined chemotherapy consisting of 2-CdA, mitoxantrone (MIT) and CY (CMC regimen) in the treatment of refractory or relapsed indolent lymphoproliferative disorders [85,86]. The treatment course consisted of 2-CdA at a dose of 0.12 mg/kg/day in 2-h intravenous infusion for 5 (CMC5) or 3 (CMC3) consecutive days, MIT 10 mg/m^2 i.v. on day 1 and CY 650 mg/m^2 i.v. on day 1. The overall response rate was 48.6%. There was no difference in the frequency of response between the CMC3 and CMC5 treated groups. However, infections and fever of unknown origin complicated the treatment with CMC5 more often than with CMC3. The CMC program is an active combined regimen both in pretreated and therapy naive CLL patients.

Recently, we performed a randomized multicenter study to compare the CMC3 regimen with 2-CdA alone and in combination with CY (CC program) [87]. All regimens were repeated every 28 days or longer intervals if hematologic complications occurred. MRD was evaluated by flow cytometry. The updated results seem to indicate that CMC program used as first-line therapy gives higher CR rate (39.6%) than 2-CdA alone (25.9%) or 2-CdA combined with CY (28.3%) (p=0.03). However, OR rate was similar in all three groups (79.1%, 74.1%, 82.2% respectively, p=0.1). Moreover, 2-CdA and CC programs were less myelotoxic than CMC.

The chimeric anti-CD20 - monoclonal antibody, rituximab has emerged as an effective single agent for patients with CD20 positive B-cell indolent lymphoid malignancies. In *in vitro* studies on fresh CLL cells we showed that the combination of rituximab with 2-CdA induces additive pro-apoptotic effect [88]. Subsequently, we started a clinical study to determine the efficacy and toxicity of combined therapy consisting of rituximab and 2-CdA (RC regimen) in patients with refractory or relapsed indolent lymphoproliferative disorders [89]. RC regimen consisted of rituximab at a dose of 375 mg/m^2 in 6-hour infusion on day 1 and 2-CdA at a dose 0.12 mg/kg in 24-hour infusion, given on days 2-6. The RC courses were repeated monthly. Twenty six CD20 antigen positive patients, 15 with CLL and 11 with low-grade non-Hodgkin's lymphoma, were enrolled in the study. The total number of RC courses given to the entire group amounted to 78. Four patients (16.4%) achieved CR and 14(53.8%) PR. Hypersensitivity to rituximab was the major toxicity of RC regimen and occurred in 9 (34.6%) patients, mostly during the first infusion of MoAb. There was no treatment related mortality. The results of our study indicate that RC regimen has substantial activity both in CLL and LG-NHL patients. However, the results of RC treatment seem to be better than the results observed in a similar group of patients who received 2-CdA at the same dose of monotherapy in our earlier studies.

The effectivenes and toxicity of the oral formulation of 2-CdA in CLL patients has been also investigated [90,91]. Juliusson *et al*. [90] treated 63 patients with symptomatic but previously untreated CLL with 2-CdA solution 10 mg/m^2 /d orally for 5 consecutive days in monthly courses. OR was achieved in 47 (75%) patients including CR in 24 (38%) patients. The median response duration was not reached at 2 years and the overall survival rate at 2 years was 82%. However, grade 3/4 infections toxicity occurred in 22 (35%) patients. More recently, the same group presented the results of a phase II trial of oral 2-CdA with a 3-d, 3-weekly schedule in untreated and pretreated patients [91]. In the group of 63 previously untreated patients OR was achieved in 81% including CR in 20% of the patients. The results of previously untreated patients were retrospectively compared with 63 patients from previous study [90] using a 5-d monthly schedule. The oral dose corresponded to 5 mg/m^2 by the intravenous route. No significant difference regarding the response in these two cohorts of patients was observed. The above study demonstrates that oral 2-CdA has efficacy and safety similar to the intravenous formulation. Unfortunately oral formulation of this agent is not commercially available yet.

The subcutaneous route of 2-CdA administration has been also investigated in refractory or relapsed CLL patients. Betticher *et al.* [92] compared the efficacy and toxicity of this agent administered at a dose 0.7 mg/kg/cycle (20 patients) as continuous i.v. infusion over 7 days and 0.5 mg/kg/cycle given as s.c. bolus injection for 5 days (35 patients). The OR rate for all 55 patients was 38% with 5% CR and 33% PR. Response frequency and duration was similar in both groups of patients. However, reduced dose of subcutaneous 2-CdA was less myelotoxic and reduced risk of infection was observed as compared to intravenous route. Further studies on larger numbers of patients should be undertaken to establish the optimal dose and route of 2-CdA administration in CLL patients.

Waldenström Macroglobulinemia

2-CdA was administered to previously treated or untreated patients with Waldenstrom macroglobulinemia (WM) either at a dose of 0.1 mg/kg/day as a 7-day continuous intravenous infusion or at a dose of 0.12 mg/kg/day in 2-hour intravenous infusion for 5 consecutive days at monthly intervals [93-100]. Objective responses were seen in 64% to 100% of previously untreated patients and in 14% to 78% of refractory or relapsed patients. Most patients received between 2 to 4 courses of treatment (Table **3**). The number of cycles administered in these studies varied considerably but significant tumor reduction was observed as

Table 3. 2-CdA in Waldenstrom's Macroglobulinemia

Study	Regimen	Duration of treatment	Median age (years)	Prior treatment	No. of pts	Overall response (%)	MS or MRD (month)
Dimopoulos *et al*. 1993 [93]	0.1 mg/kg c.i.	2 cycles	65	None I^o refractory Refractory/ relapsed Relapse of treatment	9 9 7 75	100 44 14	
Dimopoulos *et al*. 1994 [94]	0.1 mg/kg c.i. for 7 days	2 cycles	65	None	26	85	MRD 13
Dimopoulos *et al*. 1995 [95]	0.1 mg/kg c.i. for 7 days	2 cycles	60	I^o refractory Refractory/ relapsed Relapse of treatment	20 17 9	45 17 78	MS 28 MRD 12
Delannoy *et al*. 1999 [96]	4 mg/m^2/day c.i. for 7 days (5pts) or 2h infusion for 5 days(13 pts)	Median of 2 cycles	69	None or refractory/relapse	11 16	73 50	
Betticher *et al*. 1995 [97]	0.5 mg/kg s.c. for 5 days	Median of 3 cycles	65	Refractory/ relapsed Relapse of treatment	18 6	39 50	MRD 8
Liu *et al* 1998 [98]	0.12 mg/kg i.v. for 5 days	3 cycles, then 1 cycle if CR or continue until max response if PR	66	None	7	57	86% survival at 48 months MRD 28
Hellman *et al*. [99]	0.14 mg/kg i.v. for 5 days	4 cycles	62	Yes None Relapse	13 9 15	53 44 38	MRD
Veber *et al*. 2004 [102]	0.1 mg/kg i.v.		67	None	16	94	MRD 23

Abbreviations: c.i.-continuous infusion, sc-subcutaneously; i.v.-intravenously; MRD - median response duration; MS - median survival.

early as after two courses of 2-CdA. The median time to response ranged from 1.2 months to 5.8 months in various studies. The median time to response to 2-CdA in previously untreated patients varied between 13 and 28 months [101,102]. Longer responses were found in patients receiving more cycles of therapy. 2-CdA was also administered to patients in whom primary treatment with alkylating agents had failed. The rate of objective responses ranged from 14% to 53% in different studies (Table **3**) [93,95,97,99]. The response rate was higher and the duration of response was longer when 2-CdA was given to patients with primary refractory disease or to patients relapsing of therapy rather than to patients with disease in resistant relapse [101].

In the majority of studies 2-CdA was administered in continuous intravenous infusion at a dose 0.1 mg/kg/day for 7 days [93-95], or 0.12-0.14 mg/kg/day in 2- hour infusion for 5 days [98,99]. However in one study the drug was given as subcutaneous bolus injections at a dose of 0.1 mg/kg/day over 5 days [97]. Twenty five previously treated refractory or relapsed patients received therapy every 4 weeks or at longer intervals for a maximum of 6 cycles. Overall response rate was 68% and median remission duration was 8 months. The authors suggest that such schedule of 2-CdA administration should be considered in second or third line therapy of WM.

Low Grade Non-Hodgin's Lymphoma

2-CdA showed remarkable activity in both previously treated and untreated low grade non Hodgkin's lymphoma (LG-NHL). In relapse/refractory LG-NHL patients 2-CdA induced durable response, with OR rates ranging from 36% to 56% and CR rates between 10% and 20% [103-116]. The most significant toxicity of 2-CdA was myelosuppression with neutropenia and thrombocytopenia which occured at an acceptable incidence rate.

2-CdA was effective in combination with alkylating agents and mitoxantrone in the treatment of refractory or relapsed advanced stage LG-NHL [117-120]. The combination of 2-CdA with MIT and cyclophosphamide (CY) (CMC regimen) proved to be very active in heavily pretreated LG-NHL patients; however, due to its significant toxicity the period of 2-CdA administration was recommended to be reduced from 5 to 3 days [87]. Recently, we reported high efficacy and good tolerability of 2-CdA in combination with rituximab (RC regimen) in heavily pretreated patients with indolent NHL [89]. The demonstrated activity of 2-CdA in pretreated LG-NHL led to its application in untreated patients. The data reported so far indicate a significant activity with response rates ranging from 64 to 100% and CR rates of 7 to 32% [107-111].

Recently, lower doses of 2-CdA (5 mg/m^2/week) have been investigated both in monotherapy [121] and in combination with MIT [122]. The results demonstrate that these reduced doses of 2-CdA are highly active and possibly better tolerated than standard doses in the treatment of indolent lymphoid malignancies.

2-CdA shows some activity in advanced cutaneous T-cell lymphoma (CTCL) patients, including Sezary syndrome (SS) and mycosis fungoides. This drug produced 24% OR in CTCL with high incidence of septic complications and significant treatment related mortality [123,124]. However, in a phase II study on relapsed or refractory adult T-cell lymphoma conducted by Tobinai *et al* [125], 2-CdA showed no effect and patient enrollment was discontinued.

2-CdA has been reported to produce 57% OR with 28.5% sustained CR in patients with angioimmunoblastic lymphadenopathy with dysproteinemia (AILD). These results in the absence of effective standard treatment for AILD suggest that further studies using 2-CdA for the treatment of patients with AILD are recomended.

Langerhans Cell Histiocytosis

2-CdA has major clinical activity in both pediatric and adult patients with Largenhans cell histocytosis (LCH) [125-132]. Weitman *et al*. [129] analysed 15 patients with LCH including 12 children and found OR rate 60% including 40% CR. The dosage of 2-CdA varied from 0.1 mg/kg/day to 13 mg/m^2 per day by continuous infusion for 5 days and the number of cycles varied from 2 to 6. Similar results have been recently presented by Stine *et al*. [131]. Ten children with multiple reactivations or high risk disease were treated with continous infusion of 2-CdA with starting dose 5 mg/m^2/day for 3 days and escalated to 6.5 mg/m^2/day for 3 days if tolerated. The maximum number of courses of 2-CdA per patient was limited to 6.0. All 10 patients had clinical response. Seven patients required no additional therapy and remained disease free a median of 50 months from completing therapy.

Seven and Burian [128] observed 13 adults with LCH who were treated with a median of 3 courses of 2-CdA at a dosage of 0.14 mg/kg per day by 2-hour infusion for 5 consecutive days. The OR rate was 75% including 58% CR and 17% PR. The median response follow up duration was 33 months. More recently, Pardanani *et al.* [130] reported 5 adult patients with LCH treated with 2-CdA. They received 2-CdA 0.7 mg/kg over 5 to 7 days. The median number of courses was 4. All patients responded including 3 (60%) who achieved CR. In the majority of studies treatment was well tolerated, with myelosuppression being main toxic effect.

Combination of 2-CdA with CY may be even more effective in the treatment of LCH. We observed a patient with systemic lupus erythematosus, who developed LCH [131]. The disease was refractory to treatment with high doses of prednisone and vincristine but CR was achieved after treatment with 2-CdA combined with CY. Treatment course consisted of 2-CdA at a dose of 0.12 mg/kg/daily in a 2-hour infusion for 3 consecutive days and CY 650 mg/m^2 i.v. on day 1. The cycles were repeated every 28 days to complete 5 courses of treatment and there was no relapse of LCH in the following 4 years of observation. In conclusion, 2-CdA displays major activity in adults and children with LCH and warrants further investigation as a single agent and in combination with other drugs.

Acute Myeloid Leukemia

2-CdA as a single agent has been also investigated in patients with acute myeloid leukemia (AML), both in children and in adults (Table **4**) [33,133-139]. A better outcome was achieved in pediatric patients rather than in adults. Santana *et al*. [30] conducted a phase I study in 31

Table 4. Larger Studies Evaluating the Efficacy of 2-CdA in AML

Study	2-CdA doses	Patients characteristics	No. of patients	Age (years)	Complete remission	Median duration of CR
Santana *et al*. 1994 [134]	8.9 mg/m^2/d for 5d (CI)	Untreated	22	7 (0.6-18.9)	6 (27%)	NR
Krance *et al*. 2001 [137]	8.9 mg/m^2/d for 5d (CI))	Untreated primary AML	73	4.9 (0-18.8)	37(51%)	NR
Vahdat *et al*. 1994 [138]	5-21 mg/m^2/d for 5d (CI)	Relapsed/refractory	36	47 (14-84)	3 (8%)	3 m
Gordon *et al*. [135]	17 mg/m^2/d for 5d (CI)	Relapsed/refractory	15	60 (29-75)	0	0
Van den Neste *et al*. 1998 [134]	2-CdA 0.1 mg/kg/d x7+DNR 50 mg/kg/d x 3	Relapsed/refractory	14	57 (19-66)	0	0
Kornblau *et al*. 1996 [135]	2-CdA 12 mg/m^2/d x5+Ara-C 1 g/m^2/d over 24x 5d	Relapsed/refractory	17	61 (27-73)	2(12%)	14 wks
Wrzesien-Kus *et al*. 2003 [149]	2-CdA 5 mg/m^2/d x5+Ara-C 2 g/m^2 x5d+G-CSF 300 ug/d sc x 6	Relapsed/refractory	58	45(18-67)	29(50%)	17wks
Juliusson *et al*. 2001 [143]	2-CdA 5mg/m^2/dx 4+Ara-C 61 g/m^2/dx 4+IDA (10mg/m^2/dx2	Primary treatment	34	71(60.5-84.5)	24 (71%)	NR
Holowiecki *et al*. 2004 [144]	200mg/m^2/d x5+ DNR 60 mg/m^2/d x 3	Untreated	200	45 (16-60)	144(72%)	
Wrzesien-Kus *et al*. 2005 [141]	2-CdA 5mg/m^2 dx5+Ara-C 2g/m^2/d x5d+MIT 10mg/m^2 d1+G-CSF 300 μg/d sc x 6	Relapsed/refractory	43		21 (49%)	NR

Abbreviations:AML- acute myleoid leukemia; 2-CdA- 2-chlorodeoxyadenosine; Ara-C -cytarabine; IDA - idarubicin; DNR- daunorubicin; MIT - mitoxantrone; G-CSF - granulocyte-colony stimulating factor; CI - continuous infusion; d -day; wk-week; NR - not reported.

pediatric patients, 18 with AML and 13 with acute lymphoblastic leukemia. 2-CdA was given as a continuous 5-day infusion at doses of 3-10.7 mg/m^2/day. At dose levels above 6.2 mg/m^2/day significant oncolytic responses occurred in all patients. The only dose-limiting toxicity was myelosuppression. The maximal tolerated dose was established at the level of 8.9 mg/m^2/day for 5 days. Recently, the same group published updated results of this study [137]. Between 1991 and 1996 73 children with newly diagnosed primary AML and 20 children with secondary AML or myelodysplastic syndrome (MDS) underwent treatment. They received one or two 5-day courses of 2-CdA (8.9 mg/m^2 /day) given by continuous infusion. In patients with primary AML assessed for response CR rate was 24% after one course and 40% after two courses of 2-CdA. The agent was well tolerated and its toxicity was acceptable.

Unfortunately, the encouraging results with 2-CdA in childhood AML have not been confirmed in adults [136-138]. Vahdat *et al*. [138] reported their preliminary results of the treatment with 2-CdA in 30 adult patients with relapsed AML. 2-CdA was administered at a dose of 5-21 mg/m^2 /day in continuous infusion for 5 days. CR was achieved only in one patient who received a dose of 15 mg/m^2 /day. The most significant adverse event was the development of a progressive sensorimotor peripheral neuropathy.

In the study performed by Van den Neste *et al*. [134] 2-CdA was given at a dose of 0.1 mg/kg/day for 7 days with (10 patients) or without (5 patients) daunorubicin in relapsed or refractory adult AML patients. No CR was achieved and only one patient had a PR. All the patients experienced peripheral blood cytopenia and the median time to reach the leukocyte nadir was 12 days. These unsatisfactory results can be explained at least in part by the study population. The relapsed patients had very short first CR duration, more than 36% had refractory leukemia and all the patients were previously treated with anthracyclines. Moreover, the patients were older (median age 57 years), and only 5% had good prognosis karyotypic abnormalities.

Another phase II study of 2-CdA in adult patients with relapsed or refractory AML (median 60 years) performed by ECOG has been recently published [136]. 2-CdA was administered in 15 patients at a dose of 17 mg/m^2 /day for 5 days. Second course of therapy was given to patients who had not achieved aplasia by day 21. There were no CR, though in eight patients bone marrow aplasia was observed.

The results presented above indicate that 2-CdA used as a single agent is an active drug in children with AML, even with relapsed or refractory disease. However, this agent has little activity in adult patients with relapsed or refractory disease. *In vitro* and *in vivo* pharmacological studies performed to determine the effect of pretreatment with 2-CdA on Ara-CTP accumulation in leukemic blasts demonstrated a 50-65% increase in the rate of Ara-CTP accumulation [135,139]. Basing on these observations, Kornblau *et al*. [135] treated 17 relapsed patients (15 with AML and 2 with MDS) with 2-CdA at a dose of 12 mg/m^2/day and Ara-C at a dose of 1g/m^2 over 2-hour infusion/day, for 5 consecutive days. Two patients with AML achieved CR lasting 10 and 17 weeks, respectively.

It is also thought that the addition of G-CSF may further improve the effects of 2-CdA in combination with Ara-C. In our preliminary study we evaluated both the efficacy and toxicity of the combination of 2-CdA with high dose Ara-C and G-CSF (CLAG regimen) in 58 patients with refractory or relapsed AML [140]. The protocol consisted of an infusion of 5 mg/m^2 of 2-CdA over 2 hours daily for 5 consecutive days. A 4-hour infusion of Ara-C (2/g/m^2) was started 2 hours after each infusion of 2-CdA. G-CSF at a dose of 300 μg s.c. was given 2-hours before the first dose of 2-CdA for 6 days. The rate of CR was 50%. Disease free survival after 1 year was 29%. Subsequently, we evaluated the efficacy and toxicity of induction treatment consisting of 2-CdA (5 mg/m^2), Ara-C (2g/m^2), mitoxantrone (10 mg/m^2) and G-CSF (CLAG-M) in 42 refractory AML patients (141). CR was achieved in 21 (49%) patients. Hematologic toxicity was the most prominent toxicity of this regimen. The overall survival after 1 year for all 42 patients was 43%, whereas OS for 20 patients in CR was 73%. Disease free survival (1 year) was 68.6%. We concluded that CLAG-M efficacy in refractory AML seems to be better than the efficacy of many other regimens.

Juliusson *et al*. [142] studied 2-CdA with high dose Ara-C (HDAC) or HDAC and IDA. In the group treated with IDA six out of eight patients achieved CR without excessive toxicity. However such promising results with a triple drug regimen were obtained in a relatively young and good-prognosis population of adult AML patients. In the study of Van den Neste *et al*. [134] out of 14 cases of relapsed or refractory AML no patient reached CR after treatment with 2-CdA, Ara-C and daunorubicin (DNR) combination regimen. Encouraging results with combination regimen of 2-CdA, Ara-C and IDA have been also obtained in elderly patients. Juliusson *et al*. [143] performed a randomized phase II study in previously untreated AML patients over 60 years of age. All patients received Ara-C 1g/m^2/day bid for 4 days and IDA 10 mg/m^2 /day for 2 days. Two thirds were randomized to receive additional 2-CdA 5 mg/m^2 given before Ara-C bid for 4 days. CR rate was 71% in the group treated without 2-CdA. Moreover, 2-CdA did not increase early death, or time of granulocyte and platelet regenerations, or median time with fever >38^{o}C, or intravenous antibiotics.

Efficacy and safety of 2-CdA combined with Ara-C and DNR (DAC-7) has been recently compared with the standard 3+7 (DA-7) chemotherapy in previously untreated AML patients in randomized multicenter study in Poland [144]. DAC-7 protocol consists of DNR 60 mg/m^2 /day on days 1-3, Ara-C 200 mg/m^2 /day on days 1-7 and 2-CdA 5 mg/m^2 in 2-hour infusion on days 1-5. In DA-7 therapy the patients receive the same doses of Ara-C and DNR but without 2-CdA. A total of 200 patients were randomized to the DAC-7 and another 200 patients were assigned to the DA-7. The overall CR rate equaled 72% for DAC-7 and 69% for DA-7 arm (p= NS). After a single course of DAC-7 induction, the CR rate equalled 64% and was significantly higher compared to 47% in the DA-7 arm (p=0.0009). Median hospitalization time during the induction was 7 days shorter for DAC-7 compared to the DA-7 group (33 vs 40 days, p=0.002). Toxicity was comparable in both groups. The probability of 3-year leukemia –free survival (LFS) for DAC-7 and DA-7 group equalled 43 and 34%, respectively (p=NS) . There was a trend toward a higher LFS rate for patients aged >40 years receiving DAC-7 compared with DA-7 regimen (44 vs 28%, P=0.05). This study proves that addition of 2-CdA increases antileukemic potency of DNR+Ara-C regimen, thus resulting in a higher CR rate after one induction cycle when compared to DA-7 without additional toxicity. It shortens hospitalization time and may improve long-term survival in patients aged >40 years.

Systemic Mastocytosis

Systemic mastocytosis (SM) is a rapidly progressing disease with multiorgan involvement, resistant to conventionally used cytotoxic drugs, with fatal prognosis. Recent *in vivo* study showed that 2-CdA exerts cytotoxic, anti - apoptotic effect on mast cell leukemia derived cell line HMC-1 cells [145]. Some recent reports have demonstrated that 2-CdA plays a role in the treatment of agressive SM [146-148] and described the efficacy of 2-CdA in patients with interferon-α (IFNα) resistant SM. Six cycles of 5 days of 2-CdA (0.3 mg/kg in 2-hour infusion) induced an almost complete disappearance of the mast cell infiltration in the skin and bone marrow. Pardanami *et al*. [147] used 3 to 6 courses of 2-CdA given over a period of 3-36 months to treat 4 patients with SM that was resistant to IFN-α. Treatment with this agent resulted in a major response in 2 patients and a good partial response in another patient. Treatment was well tolerated and duration of remission ranged from 2 months to 4 years. These good results have been recently confirmed by Kluin-Nelemans *et al*. [148]. They treated 10 patients with different subtypes of SM. 2-CdA was administered at a dose 0.10-0.13 mg/kg in a 2-hour infusion for 5 consecutive days. The courses were repeated every 4-8 weeks to complete 6 cycles. All responded with respect to signs, symptoms and mast cell parameters although none achieved CR. Side effects were mainly related to bone marrow suppression.

These reports demonstrate that 2-CdA has a role in the treatment of symptomatic mastocytosis which is unresponsive to conventional therapy with IFN-α. However, the optimal method of 2-CdA administration remains to be determined on a larger number of patients.

CURRENT & FUTURE DEVELOPMENTS

The purine nucleoside analogue, cladribine (2-chlorodeoxyadenosine, 2-CdA) represents a novel cytotoxic agent with high efficacy in indolent lymphoid malignancies. This agent exhibits also significant activity in Langerhans cell histiocytosis, acute myeloid leukemia, systemic mastocytosis and other hematological malignancies. 2-CdA is the drug of choice in the treatment of hairy cell leukemia.

However, in some European countries it has been also registered for the second line treatment of chronic lymphocytic leukemia. The drug was originally administered for 7 consecutive days by continuous intravenous infusion and this mode of administration is now recommended. However, optimal doses and the best method for 2-CdA administration remain to be determined.

Pharmacokinetics data suggest that 2-CdA may be also given over a 2-hour infusion period and that the treatment course may be shortened to 5 days. This approach has shown the same high efficacy, especially in patients with indolent lymphoid malignancies such as hairy cell leukemia and chronic lymphocytic leukemia. Recently, subcutaneous and oral formulations have been developed. However, after oral administration 2-CdA is partially deactivated by acid hydrolysis in the stomach and doses double to intravenous or subcutaneous formulation should be given.

Recent preclinical and clinical studies indicate that other cytotoxic agents and some monoclonal antibodies act synergistically with 2-CdA. Combined treatment with 2-CdA and alkylating agents, anthracyclines and/or rituximab (anti-CD20 monoclonal antibody) may have important clinical value especially in indolent lymphoid malignancies. Further studies evaluating the optimal doses, methods of administration and combinations with other agents are justified and indeed necessary in the years to come.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Ministry of Science, Warsaw, Poland (No 2P05B01828). We thank Ms Grazyna Laskowska for correction of the English version of the manuscript and Ms Krystyna Marszalek for typing the paper. The authors have no conflicts of interest that are directly relevant to the contents of this manuscript.

REFERENCES

[1] Robak T, Korycka A, Kasznicki M, Wrzesien-Kus A, Smolewski P. Purine nucleoside analogues for the treatment of hematological malignancies: Pharmacology and clinical applications. Curr Cancer Drug Targets 2005; 5:421-44

[2] Bryson HM, Sorkin EM. Cladribine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in haematological malignancies. Drugs 1993; 76: 872-94.

[3] Carson DA, Wasson DB, Kaye J, *et al*. Deoxycytidine kinase mediated toxicity of deoxyadenosine analogs toward malignant human lymphoblasts in vitro and toward murine L1210 leukemia *in vivo*. Proc Natl Acad Sci USA 1980; 77: 6865-69.

[4] Kazimierczuk Z, Cottam HB, Revankar GR, Robins RK. Synthesis of 2-deoxynucleosides via a novel direct sterospecific sodium salt glycocylation procedure. J Am Chem Soc. 1984; 106: 6379-82.

[5] Carson DA, Kay J, Seegmiller JE. Lymphospecific toxicity in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency: possible role of nucleoside kinase. Proc Natl Acad Sci USA 1977; 74: 5677-81.

[6] Beutler E. Cladribine (2-chlorodeoxyadenosine). Lancet 1992; 340: 952-6.

[7] Pastor-Anglada M, Molina-Arcas M, Casado FJ, Bellosillo B, Colomer D, Gil J. Nucleoside transporters in chronic lymphocytic leukemia. Leukemia 2004; 18: 385-93.

[8] Molina-Arcas M, Marce S, Villamor N. Equilibrqtive nucleoside transporter 2- (hENT2) protein expression correlates with *ex vivo* sensitivity to fludarabine in chronic lymphocytic leukemia (CLL) cells. Leukemia 2005; 19: 64-8.

[9] Carson DA, Wasson DB, Taetle R, Yu A. Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. Blood 1983; 62: 737-43.

[10] Avery TL, Regh JE, Lumm WC, Harwood FC, Santana VN, Blakley RL. Biochemical pharmacology of 2-chlorodeoxyadenosine in malignant human hematopoietic cell lines and therapeutic effects of 2-bromodeoxyadenosine in drug combinations in mice. Cancer Res 1989; 49: 4972-8.

[11] Seto S, Carrera CJ, Kubota M, Wasson DB, Carson DA. Mechanism of deoxyadenosine and 2-chlorodeoxyadenosine toxicity to nondividing human lymphocytes. J Clin Invest 1985; 75: 377-83.

[12] Griffig J, Koob R, Blakley RL. Mechanism of inhibition of DNA synthesis by 2-chlorodeoxyadenosine in human lymphoblastic cells. Cancer Res 1989; 49: 6923-28.

[13] Warzocha K, Fabianowska-Majewska K, Blonski J, Krykowski E, Robak T. 2-chlorodeoxyadenosine inhibits activity of adenosine deaminase and S-adenosylhomocysteine hydrolase in patients with chronic lymphocytic leukemia. Eur J Cancer 1997; 33: 170-3.

[14] Robertson LE, Chubb S, Meyn RE, *et al*. W. Induction of apoptotic cell death in chronic lymphocytic leukemia 2-chloro-2´-deoxy-adenosine and 9-β-D-arabinosyl-2-fluoroadenine. Blood 1993; 81: 143-50.

[15] Castejon R, Vargas JA, Briz M, *et al*. Induction of apoptosis by 2-chlorodeoxyadenosine in B-cell chronic lymphocytic leukemia. Leukemia 1997; 11: 1253-1257.

[16] Pettit AR. Mechanism of action of purine analogues in chronic lymphocytic leukemia. Br J Haematol 2003; 121: 692-702.

[17] Kawasaki H, Carrera CJ, Piro LD, Saven A, Kipps TJ, Carson DA**.** Relationship of deoxycytydine kinase and cytoplasmic 5´nucloetidase to the chemothrapeutic efficacy of 2-chloro-deoxyadenosine. Blood 1993; 81: 597-601.

[18] Carson DA, Carrera CJ, Wasson DB, Yamanaka H. Proogrammed cell death and adenine deoxynucleotide metabolism in humans lymphocytes. Adv Enzyme Regul 1988; 27, 395-404.

[19] Genini D, Adachi S, Chao O, *et al*. Deoxyadenosine analogues induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. Blood 2000: 96, 3537-43.

[20] Marzo I, Perez-Gala P, Rubio-Felix D, Anel A, Naval J. Cladribine induces apoptosis in human leukemia cells by caspase-dependent and -independent pathways acting on mitochondria. Biochemistry 2001, 359: 537-46.

[21] Nomura Y, Inanami O, Takahashi K, Matsuda A, Kuwabara M. 2-Chloro-2´-deoxyadenosine induces apoptosis through the Fas/Fas ligand pathway in human leukemia cell line MOLT-4. Leukemia 2000; 14: 299-306.

[22] Klopfer A, Hasenjager A, Belka C, Schulze-Osthoff K, Dorken B, Daniel, P. Adenine deoxynucleotides fludarabine and cladribine induces apoptosis in a CD95/Fas receptor, FADD and caspase-8-independent manner by activation of the mitochondrial cell death pathway. Oncogene 2004; 16: 9408-18.

[23] Carson DA, Wasson DB, Beutler E. Antileukemic and immunosuppressive activity of 2-chlorodeoxyadenosine. Proc Natl Acad Sci USA 1984; 81: 2232-36.

[24] Johnson SA. Clinical pharmacokinetics of nucleoside analogues. Focus on haematological malignancies. Clin Pharmacokinet 2000; 39: 5-26.

[25] Beutler E, Piro LD, Saven A, *et al*. 2-Chlorodeoxyadenosine (2-CdA). A potent chemotherapeutic and immunosuppressive nucleoside. Leuk Lymphoma 1991; 5: 1-8.

[26] Saven A, Cheung WK, Smih I, *et al*. Pharmacokinetics study of oral and bolus intravenous 2-chlorodeoxyadenosine in patients with malignancy. J Clin Oncol 1996; 14: 978-83.

[27] Liliemark J, Albertioni F, Hassan M, *et al*. One the bioavailability of oral and subcutaneous 2-chloro-2´-deoxyadenosine in humans: alternative routes of administration. J Clin Oncol. 1992; 10: 1514-8.

[28] Liliemark J. and Juliusson G. On the pharmacokinetics of 2-chloro-2´-deoxyadenosine in humans. Cancer Res 1991; 51: 5570-2.

[29] Albertioni F, Petterson B, Reichalova V, *et al*. Analysis of 2-chloro-2´-deoxyadenosine in human blood plasma and urine by high-performance liquid chromatography. Ther Drug Monit 1995; 16: 413-8.

[30] Santana VN, Mirro J Jr, Harwood FC, *et al*. A phase I clinical trial of 2-chlorodeoxyadenosine in pediatric patients with acute leukemia. J Clin Oncol 1991; 9: 416-22.

[31] Lindemalm S, Savic RM, Karlsson MO, Liliemark J, Juliusson G, Albertioni F. Application of population pharmacokinetics to cladribine. BMC Pharmacology 2005; 5: 4.

[32] Liliemark J, Pettersson B, Juliusson G. Determination of 2-chloro-2´-deoxyadenosine in human plasma. Biomed Chromatogr 1991; 5: 262-4.

[33] Santana VM, Mirro Jr. J, Kearns C, Schell MJ, Crom W. Blakley R.L 2-Chlorodeoxyadenosine produces a high rate of complete hematologic remission in relapsed acute myeloid leukemia. J Clin Oncol 1992; 10: 364-70.

*[34] Schultz, T.W., Naeff, R.: US6194395 (**2001**).

*[35] Bodor, N.S., Dandiker, Y.: WO04087101A3 (**2004**).

*[36] Wrenn, Jr.S.M.: US6174873 (**2001**).

[37] Lauria F, Boachia M, Marrotta G, Respadori D, Zinzani PL, Rondelli D. Weekly administration of 2-chlorodeoxyadenosine in patients with hairy cell leukemia is effective and reduces infectious complications. Haematologica 1999; 84: 22-5.

[38] Robak T, Kasznicki M, Góra-Tybor J, *et al*. Randomized comparison of weekly administration and daily courses of cladribine in patients with hairy cell leukemia. Updated results. Blood 2004; 104 (suppl. 1): 948a (abstract 3478).

[39] Albertioni F, Juliusson G, Liliemark J. On the bioavailability of 2-chloro-2´-deoxyadenosine (CdA). The influence of food and omeprazole. Eur J Clin Pharmacol 1993; 44: 579-82.

[40] Albertioni F, Juliusson G, Liliemark J. On the pharmacokinetics of 2-chloro-deoxyadenosine (CdA) and renal excretion after intravenous and oral administration. Proc. Am Soc Clin Oncol Ann Meet 1994; 13, A421 (abstract).

[41] Albertioni F, Lindemalm S, Reichelova V, *et al*. Pharmacokinetics of cladribine in plasma and its 5´-monophosphate and 5'-triphosphate in leukemic cells of patients with chronic lymphocytic leukemia. Clin Cancer Res 1998; 4: 653-58.

[42] Arner ES, Spasokoukotskaja T, Juliusson G, Liliemark J, Eriksson S. Phosphorylation of 2-chlorodeoxyadenosine (CdA) in extracts of peripheral blood mononuclear cells of leukaemic patients. Br J Haematol 1994; 87: 715-8.

[43] Kawasaki H, Carrera CJ, Piro LD, Saven A, Kipps TJ, Carson DA. Relationship of deoxycytidine kinase and cytoplasmic 5´-nucleotidase to the chemotherapeutic efficacy of 2-chlorodeoxyadenosine. Blood 1993; 81: 597-601.

[44] Begleiter A, Verburg L, Ashique A, *et al*. Comparison of antitumor activities of 2-chlorodeoxyadenosine and 9-β-arabinosyl-2-fluoroadenine in chronic lymphocytoc leukemia and marrow cells *in vitro*. Leukemia 1995; 9: 1875-81.

[45] Juliusson G, Heldal D, Hippe E, *et al*. Subcutaneous injections of 2-chlorodeoxyadenosine for symptomatic hairy cell leukemia. J Clin Oncol 1995; 13: 989-95.

[46] Robak T. Cladribine in the treatment of chronic lymphocytic leukemia. Leuk Lymphoma 2001; 40; 551-64

[47] Robak T, Blonski JZ, Kasznicki M, *et al*. Cladribine with prednisone versus chlorambucil with prednisone as first-line therapy in chronic lymphocytic leukemia: report of a prospective, randomized, multicenter trial. Blood 2000; 96: 2723-9.

[48] Robak T, Blonski JZ, Gora-Tybor J, *et al*. Second malignancies and Richter's syndrome in patients with chronic lymphocytic leukemia treated with cladribine Eur J Cancer 2004; 40: 383-9.

[49] Begleiter A, Wang H, Verburg L, *et al*. *In vitro* cytotoxicity of 2-chlorodeoxyadenosine and chlorambucil in chronic lymphocytic leukemia. Leukemia 1996; 10: 1959-65.

[50] Szmigielska-Kaplon A, Ciesielska E, Szmigiero L, Robak T. Anthracyclines potentiate activity against murine leukemias L1210 and P388 *in vivo* and *in vitro*. Eur J Haematol 2002; 68: 370-5.

[51] Szmigielska-Kaplon A, Smolewski P, Najder M, Robak T. Evaluation of apoptosis induced *in vitro* by cladribine (2-CDA) combined with anthracyclines in lymphocytes from patients with B-cell chronic lymphocytic leukemia. Ann Hematol 2002; 81: 508-13.

[52] Robak T, Szmigielska A. Dexamethasone does not enhance antileukemic activity of cladribine in mice with leukemia L1210 and P388. Neoplasma 2000; 47: 168-71.

[53] Chow KU, Boehrer S, Napieralski S, *et al*. In AML cell lines Ara-C combined with purine nucleoside analogues is able to exert synergisitic as well antaginistic effects on proliferation and disruption of mitochondrial membrane potential. Leuk. Lymphoma 2003; 44: 165-173.

[54] Kristensen J, Nygren P, Liliemark J, *et al*. Interactions between cladribine (2-chlorodeoxyadenosine) and standard antileukemic drugs in primary cultures of human tumor cells from patients with acute myelocytic leukemia. Leukemia 1992; 8: 1712-7.

[55] Schirmer M, Geisen F, Tiefenthalter M, Konwalinka G. Differential effects of cladribine and gemcitabine on erythroid and granulocytic progenitors from patients with chronic myeloid leukemia. Leuk Res 1999; 23: 1121-6.

[56] Maranda E, Szmigielska A, Robak T. Additive action of gemcitabine (2´,2´-difluorodeoxycytidine) and 2-chlorodeoxyadenosine on murine leukemias L1210 and P388. Cancer Invest 1999; 17: 95-101.

[57] Robak T, Korycka A. The comparison of 2-chlorodeoxyadenosine (2-CdA) in combination with i interferon α (IFNα) and interferon γ (IFNγ) on granulocyte-macrophage progenitor cells (CFU-GM) and clonogenic blasts (CFU-L) in cultures *in vitro*. Leuk Lymphoma 1996; 21: 161-8.

[58] Korycka A, Robak T. The influence of imatinib mesylate (STI571) used alone or in combination with purine analogues on the normal and chronic myelogenous leukemia progenitor cells *in vitro*. Leuk Lymphoma 2003, 44, 1549-55.

[59] Korycka A, Smolewski P, Robak T. The influence of farnesyl protein transferase inhibitor R115777 (Zarnestra) alone and in combination with purine nucleoside analogues on acute myeloid leukemia progenitors *in vitro*. Eur J Haematol 2004; 73: 418-26.

[60] Smolewski P, Szmigielska-Kaplon A, Cebula B, *et al*. Proapoptotic activity of alemtuzumab alone and in combination with rituximab or purine nucleoside analogues in chronic lymphocytic leukemia cells. Leuk. Lymphoma 2005; 46: 87-100.

[61] Piro LD, Carrera CJ, Carson DA, Beutler E. Lasting remissions in hairy cell leukemia induced by a single infusion of 2-chlorodeoxyadensine. N. Engl J Med 1990; 322:1117-21

[62] Chadha P, Rademaker AW, Mendirata P, *et al*. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine (2-CdA): long-term follow-up of the Northwestern University experience. Blood 2005; 106: 241-6

[63] Cheson BD, Sorensen JM, Vena DA. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine via the Group Protocol mechanism of the National Cancer Institute: a report of 979 patients. J Clin Oncol 1998; 16: 3007-15.

[64] Juliusson G, Lenkei R, Liliemark J. Flow cytometry of blood and bone marrow cells from patients with hairy cell leukemia phenotype of hairy cell and lymphocyte subsets after treatment with 2-chlorodeoxyadenosine. Blood 1994; 83: 3672-81.

[65] Robak T, Blasinska-Morawiec M, Krykowski E, *et al.* 2-chlorodeoxyadenosine (2-CdA) in 2-hour versus 24-hour intravenous infusion in the treatment of patients with hairy cell leukemia. Leuk Lymphoma 1996; 22: 107-11.

[66] von Rohr A, Schmitz SF, Tichelli A, *et al*. Treatment of hairy cell leukemia with cladribine (2-chlorodeoxyadenosine) by subcutaneous bolus injection: a phase II study. Ann Oncol 2002; 13: 1641-9.

[67] Tallman MS, Zakarija A. Hairy cell leukemia: survival and relapse. Long-term follow-up of purine analog-based therapy and approach for relapsed disease. Transfus Apheresis Sci 2005; 32: 99-103.

[68] Jehn U, Bartl R, Dietzfelbinger H, Haferlach T, Heinemann V. An update: 12-year follow-up of patients with hairy cell leukemia following treatment with 2-chlorodeoxyadenosine. Leukemia 2004; 18: 1476-81.

[69] Bastie JN, Cazals-Hatem D, Daniel MT, *et al*. Five years follow-up after 2-chlorodeoxyadenosine treatment in thirty patients with hairy cell leukemia: evaluation of minimal residual disease and $CD4^+$ lymphocytopenia after treatment. Leuk Lymphoma 1999; 35: 555-65.

[70] Zinzani PL, Magagnoli M, Bandandi M, *et al*. Long-term follow-up of hairy cell leukemia patients treated with 2-chlorodoexy-adenosine. Haematologica 2000; 85: 922-5.

[71] Goodman GR, Burian C, Koziol JA, Saven A. Extended follow-up of patients with hairy cell leukemia after treatment with cladribine. J Clin Oncol 2003; 21: 891-6.

[72] Tallman MS, Hakkimian D, Variakojis D, *et al*. A single cycle of 2-chlorodeoxyadenosine results in complete remission in the

majority of patients with hairy cell leukemia. Blood 1992; 80: 2203-9.

[73] Hoffman MA, Janson D, Rose E, Rai KR. Treatment of hairy cell leukemia with cladribine: Response, toxicity and long-term folow-up. J Clin Oncol 1997; 15: 1138-42.

[74] Filleul B, Delannoy A, Ferrant A, *et al*. A single course of 2-chlorodeoxyadenosine does not eradicate leukemic cells in hairy cell leukemia patients in complete remission. Leukemia 1994; 8: 1153-16.

[75] Robak T, Blasinska-Morawiec M, Blonski J, *et al*. 2-chloro-deoxyadenosine (cladribine) in the treatment of hairy cell leukemia and hairy cell leukemia variant 7-year experience in Poland. Eur J Haematol 1999; 62: 49-56.

[76] Saven A, Burian C, Koziol JA, Piro LD. Long term follow-up of patients with hairy cell leukemia after cladribine treatment. Blood 1998; 92: 1918-26.

[77] Saven A, Piro LD. Complete remissions in hairy cell leukemia with 2-chlorodeoxyadenosine after failure with 2´-deoxycoformycin. Ann Intern Med 1993; 119: 278-3.

[78] Piro LD, Carera CJ, Beutler E, Carson DA. 2-Chloro-deoxyadenosine: an effective new agent for the treatment of chronic lymphocytic leukemia. Blood 1988; 71: 1069-73.

[79] Robak T, Kasznicki M. Alkylating agents and nucleoside analogues in the treatment of B-cell chronic lymphocytic leukemia. Leukemia 2002; 16: 1015-27.

[80] Robak T, Blonski JZ, Kasznicki M, *et al*. Cladribine with or without prednisone in the treatment of previously treated and untreated B-cell chronic lymphocytic leukemia: updated results of the multicenter study of 378 patients. Br Haematol 2000; 108: 357-68

[81] Karlson K, Stromberg N, Jonsson V. Cladribine (CdA), fludarabine (F) or high-dose intermitten chlorambucil (Chl) as first-line treatment of symptomatic chronic lymphocytic leukemia. First interim analysis of data from the international randomized phase III trial. Blood 2004; 104 (suppl 1): 945 (abstract 3470).

[82] Robak T, Blonski JZ, Góra-Tybor J, *et al*. Cladribine with cyclophosphamide v.s. fludarabine with cyclophosphamide as first line treatment in chronic lymphocytic leukemia an early report of prospective randomized study (PALG CLL3). Hematologica Hematology J 2005; 90; 143 (abstract 364)

[83] Montilo M, Tedeschi A, P'Brien S, *et al.* Phase II study of cladribine and cyclophosphamide in patients with chronic lymphocytic leukemia and prolymphocytic leukemia. Cancer 2003; 97: 114-20.

[84] Robak T, Blonski JZ, Kasznicki M *et al*. Cladribine combined with cyclophosphamide is highly effective in the treatment of chronic lymphocytic leukemia. Hematol J 2002; 3: 244-50.

[85] Robak T, Góra-Tybor J, Lech-Maranda E, Blonski JZ, Kasznicki M. Cladribine in combination with mitoxantrone and cyclophosphamide (CMC) in the treatment of heavily pre-treated patients with advanced indolent lymphoid malignancies. Eur J Hematol 2001; 66: 188-94.

[86] Robak T, Blonski JZ, Kasznicki M, *et al*. Cladribine combined with cyclophosphamide and mitoxantrone as front line therapy in chronic lymphocytic leukemia. Leukemia 2001; 15: 1510-6.

[87] Robak T, Blonski JZ, Góra-Tybor J, *et al*. Cladribine alone or in combination with cyclophosphamide or cyclophosphamide and mitoxantrone as first line treatment in chronic lymphocytic leukemia: an early report of prospective randomized study. Blood 2004; 104 (suppl 1): 100a (abstract 337).

[88] Smolewski P, Szmigielska A, Cebula B, Sobczak A, Darzynkiewicz Z, Robak T. Pro-apoptotic effect of rituximab alone and in combination with purine nucleoside analogues in B-CLL chronic lymphocytic leukemia cells measured by fluorochrome labeled inhibitors of caspases (FLICA) assay. Blood 2002; 100: (suppl 1). 387a (abstract 1503).

[89] Robak T, Smolewski P, Urbanska-Rys H, Góra-Tybor J, Blonski JZ, Kasznicki M. Rituximab followed by cladribine in the treatment of heavily pretreated patients with indolent lymphoid malignancies. Leuk Lymphoma 2004; 45: 937-44.

[90] Juliusson G, Christiansen I, Hansen MM, *et al*. Oral cladribine as primary therapy for patients with B-cell chronic lymphocytic leukemia. J Clin Oncol 1996; 14: 2160-6.

[91] Karlson K, Stromberg N, Liliemark J, *et al*. Oral cladribine for B-cell chronic lymphocytic leukemia: report of a phase II trial with a 3-4, 3 weekly schedule in untreated and pretreated patients and a long-term follow up of 126 previously treated patients. Br J Hematol 2002; 116: 538-48.

[92] Betticher DC, Ratschiller D, Hsu SF, *et al*. Reduced dose of subcutaneous cladribine induces identical response rates but decreased toxicity in pretreated chronic lymphocytic leukemia. Ann Oncol 1998; 9: 721-6.

[93] Dimopoulos MA, Kantarjian H, Estey E, *et al*. Treatment of Waldenström macroglobulinemia with 2-chlorodeoxyadenosine. Ann Intern Med 1993; 118: 195-8.

[94] Dimopoulos MA, Kantarjian H, Wela D, *et al*. Primary therapy of Waldenstrom's macroglobulinemia resistant to standard therapy with 2-chlorodeoxyadenosine. J Clin Oncol 1994; 12: 2694-8.

[95] Dimopoulos MA, Weber DN, Delasalle KB, Keating M, Alexanian R. Treatment of Waldenström's macroglobulinemia resistant to standard therapy with 2-chlorodeoxyadenosine: identification of prognostic factors. Ann Oncol 1995; 6: 49-52.

[96] Delannoy A, Van den Nesste E, Michaux JL, Bosly A, Ferrant A. Cladribine for Waldenstrom's macroglobulinemia. Br J Haematol 1999; 104: 928-36.

[97] Betticher DC, Hsu Schmitz SF, Ratschiller D, *et al*. Cladribine (2-CdA) given as subcutaneous bolus injections is active in pre-treated Waldenstrom's macroglobulinemia. Br J Haematol 1997; 99: 358-363.

[98] Liu ES, Burian C, Miller HE, *et al*. Bolus administration of cladribine in the treatment of Waldenström macroglobulinemia. Br J Haematol 1998; 103: 690-5.

[99] Hellmann A, Lewandowski K, Zaucha JM, Bieniaszewska M, Halaburda K, Robak T. Effect of a 2-hour infusion of 2-chlorodeoxyadenosine in the treatment of refractory or previously untreated Waldenström's macroglobulinemia. Eur J Haematol 1999; 63: 35-41.

[100] Weber DM, Dimopoulos MA, Delasalle K, Rankin K, Gavino M, Alexanian R. 2-Chlorodeoxyadenosine alone and in combination for previously untreated Waldenström's macroglobulinemia. Semin Oncol 2003; 30:243-7

[101] Dimopoulos M, Mertim G, Leblond A, Anagnostopoulos A, Alexanian R. How we treat Waldenström's macroglobulinemia. Haematologica Hematology J 2005; 90: 117-25.

[102] Johnson SA, Oscier DG, Leblond V. Waldenström's macroglobuli-nemia. Blood Rev 2002; 16: 175-84.

[103] Kay AC, Saven A, Carrera CJ, *et al*. 2-Chlorodeoxyadenosine treatment of low-grade non-Hodgkin's lymphomas. J Clin Oncol 1992; 10: 371-7.

[104] Betticher DC, Fey MF, von Rohr A, *et al*. High incidence of infections after 2-chlorodeoxyadenosine (2-CdA) therapy in patients with malignant lymphoma and chronic and acute leukemias. Ann Oncol 1994; 5: 57-64.

[105] Liliemark J, Porwit A, Juliusson G. Intermittent infusion of cladribine (CdA) in previously treated patients with low-grade non-Hodgkin's lymphoma. Leuk Lymphoma 1997; 25: 313-8.

[106] Armitage JO, Tobinai K, Hoelzer D, Rummel J. Treatment of indolent non-Hodgin's lymphoma with cladribine as single agent therapy and in combination with mitoxantrone. Int J Hematol 2004; 79: 311-21.

[107] Hoffman M, Tallman MS, Hakimian D, *et al*. 2-chlorodeoxy-adenosine is an active salvage therapy in advanced indolent non-Hodgkin's lymphoma. J Clin Oncol 1994; 12: 788-92.

[108] Robak T, Góra-Tybor J, Krykowski E, *et al.* Activity of 2-chlorodeoxyadenosine (cladribine) in 2-hour intravenous infusion in 94 previously treated patients with low-grade non-Hodgkin's lymphoma. Leuk Lymphoma 1997; 26: 99-105.

[109] Ogura M, Morishina Y, Kobayashi Y, *et al*. Durable response but prolonged cytopenia after cladribine treatment in relapsed patients with indolent non-Hodgkin's lymphomas: results of a Japanese phase II study. Int J Hematol 2004; 80: 267-77.

[110] Zinzani PL. Non-Hodgkin's lymphoma: the evolving role of purine analogues. Best Pract Res Clin Haematol 2002; 15: 505-16.

[111] Pott-Hoeck C, Hiddemann W. Purine analogs in the treatment of low-grade lymphomas and chronic lymphocytic leukemias. Ann Oncol 1995; 6:421-33.

[112] Saven A, Emanuele S, Kosty M, *et al*. 2-Chlorodeoxyadenosine activity in patients with untreated indolent non-Hodgkin's lymphoma. Blood 1995; 86: 1710-6.

[113] Rummel MJ, Chow KU, Jager E, *et al.* Intermittent 2-hour-infusion of cladribine as first-line therapy or in first relapse of

progressive advanced low-grade and mantle cell lymphomas. Leuk Lymphoma 1999; 35:129-38.

[114] Canfield VA, Vose J, Guiney P, *et al*. 2-Chlorodeoxyadenosine as initial therapy for advanced low-grade non-Hodgkin's lymphomas. Leuk Lymphoma 1997; 24: 335-9.

[115] Fridrik M, Linkesch W, Jager G, *et al*. Efficacy and toxicity of 2-chlorodeoxyadenosine (Cladribine) - 2-h infusion for 5 days - as first - line treatment for advanced low-grade non-Hodgkin's lymphoma. Eur J Cancer 1998; 34: 1560-64.

[116] Liliemark J, Martinsson U, Cavalln-Stahl E, *et al*. Cladribine for untreated or early low-grade non-Hodgkin's lymphoma. Leuk Lymphoma 1998; 30: 573-81.

[117] Laurencet FM, Zulian GB, Guetty-Alberto, M, *et al*. Cladribine with cyclophosphamide and prednisone in the management of low-grade lymphoproliferative nalignancies. Br J Cancer 1999; 79: 1215-9.

[118] Robak T, Góra-Tybor J, Urbanska-Rys H, *et al*. Combination regimen of 2-chlorodeoxyadenosine (cladribine), mitoxantrone and dexamethasone (CMD) in the treatment of refractory and recurrent low-grade non-Hodkin's lymphoma. Leuk Lymphoma 1999**;** 32: 359-368.

[119] Van den Neste E, Louviaux I, Michaux JL, *et al*. Phase I/II study of 2-chloro-2-deoxyadenosine with cyclophosphamide in patients with pretreated B cell chronic lymphocytic leukemia and indolent non-Hodgkin's lymphoma. Leukemia 2000; 14: 1136-42.

[120] Robak T, Szmigielska-Kaplon A, Blonski JZ, Kasznicki M, Chojnowski K. Activity of combined cladribine with etoposide in heavily pretreated patients with indolent lymphoid malignancies. Chemotherapy 2005; 51: 247-51.

[121] Riccioni R, Caracciolo F, Galimberti S, Cecconi N, Petrini M. Low dose 2-CdA schedule activity in splenic marginal zone lymphoma. Hematol Oncol 2002; 21: 163-8

[122] Rummel MJ, Chow KU, Karahas T, *et al*. Reduced -dose cladribine (2-CdA) plus mitoxantrone is effective in the treatment of mantle-cell and low-grade non-Hodgkin's lymphoma. Eur J Cancer 2002; 38: 1739-46

[123] Kong LR, Samuelson E, Rosen ST. 2-Chlorodeoxyadenosine in cutaneous T-cell lymphoproliferative disorders. Leuk Lymphoma 1997; 26: 89-97.

[124] Bouwhuis SA, el-Azhary RA, McEvoy MT, *et al.* Treatment of late-stage Sezary syndrome with 2-chlorodeoxyadenosine. Int J Dermatol 2002; 41: 352-6.

[125] Tobinai K, Uike N, Saburi Y, Chou T, *et al.* Cladribine/ATL Study Group, Japan. Phase II study of cladribine (2-chloro-deoxyadenosine) in relapsed or refractory adult T-cell leukemia-lymphoma. Int J Hematol 2003; 77: 512-7.

[126] Saven A, Figueroa ML, Piro LD, Rosenblatt JD. 2-Chlorodeoxyadenosine to treat refractory histiocytosis X. N Engl J Med 1993; 329: 734-5.

[127] Saven A, Foon KA, Piro D. 2-Chlorodeoxyadenosine-induced complete remissions in Langerhans cell histiocytosis. Ann Intern Med 1994; 121: 430-2.

[128] Saven A, Burian C. Cladribine activity in adult Langerhans-cell histiocytosis. Blood 1999; 93: 4125-30.

[129] Weitman S, Wayne A, Arceci R, Lipton JM, Whitlock JA. Nucleoside analogues in the therapy of Langerhans cell histiocytosis: a survey of members of the histiocyte society and review of the literature. Med Pediatr Oncol 1999; 33: 476-81.

[130] Pardanani A, Phyliky RL, Li CY, Tefferi A. 2-chlorodeoxyadenosine therapy for disseminated Langerhans cell histiocytosis. Mayo Clin Proc 2003; 78: 301-6.

[131] Stine KC, Saylors RL, Saccente S, McClain KL, Becton DL. Efficacy of continuous infusion 2-CdA (cladribine) in pediatric patients with Langerhans cell histiocytosis. Pediatr Blood Cancer 2004; 43: 81-4.

[132] Robak T, Kordek P, Robak E, *et al*. Langerhans cell histiocytosis in a patient with systemic lupus erythematosus a clonal disease responding to treatment with cladribine and cyclophosphamide. Leuk Lymphoma 2002; 43: 2041-46.

[133] Santana VM, Hurwitz CA, Blakley RL, *et al.* Complete hematologic remissions induced by 2-chlorodeoxyadenosine in children with newly diagnosed acute myeloid leukemia. Blood 1994; 84: 1237-42.

[134] Van den Neste E, Martiat P, Mineur P, *et al.* 2-Chlorodeoxyadenosine with or without daunorubicin in relapsed or refractory acute myeloid leukemia. Ann Hematol 1998; 76: 19-23.

[135] Kornblau SM, Gandhi V, Andreeff HM, *et al.* Clinical and laboratory studies of 2-chlorodeoxyadenosine + cytosine arabinoside for relapsed or refractory acute myelogenous leukemia in adults. Leukemia 1996; 10: 1563-1569.

[136] Gordon MS, Young ML, Tallman MD, *et al*. Phase II trial of 2-chlorodeoxyadenosine in patients with relapsed/refractory acute myeloid leukemia: a study of the Eastern Cooperative Oncology Group (ECOG), E5995. Leuk Res 2000; 24: 871-5.

[137] Krance RA, Hurwitz CA, Head DR, *et al.* Experience with 2-chlorodeoxyadenosine in previously untreated children with newly diagnosed acute myeloid leukemia and myelodysplastic disease. J Clin Oncol 2001; 19: 2804-11.

[138] Vahdat L, Wong E, Wile M, Rosenblum M, Foley KM, Warell Jr. RP. Therapeutic and neurotoxic effects of 2-chlorodeoxyadenosine in adults with acute myeleoid leukemia. Blood 1994; 84: 3429-34.

[139] Gandhi V, Estey E, Keating MJ, Chucrallah A, Plunkett W. Chlorodeoxyadenosine and arabinosylcytosine in patients with acute myelogenous leukemia: pharmacokinetic, pharmacodynamic and molecular interactions. Blood 1996; 87: 256-64.

[140] Robak T, Wrzesien-Kus A, Lech-Maranda E, Kowal M, Dmoszynska A. Combination regimen of cladribine (2-chlorodeoxyadenosine), cytarabine and G-CSF (CLAG) as induction therapy for patients with relapsed or refractory acute myeloid leukemia. Leuk Lymphoma 2000; 39: 121-9.

[141] Wrzesien-Kus A, Robak T, Wierzbowska A, *et al*. A multicenter open, noncomparative, phase II study of the combination of cladribine (2-chlorodeoxyadenosine), cytarabine, granulocyte colony-stimulating factor and mitoxantrone as induction therapy in refractory acute myeloid leukemia: a report of the Polish Adult Leukemia Group. Ann Hematol 2005; 84: 557-64

[142] Juliusson G, Liliemark J. 2-Chlorodeoxyadenosine (CDA) with and without cytosine arabinoside (Ara-C) and idarubicin for acute myeloid and lymphoid leukemia - clinical and pharmacokinetic studies. Br J Haematol 1994; 87: 48a (Abstract),

[143] Juliusson G, Lofgren C, Mollgard L, *et al*. No additional toxicity from cladribine (CdA) as primary treatment of acute myeloid leukemia in elderly patients: results from a randomized phase II study from the Leukemia Group of Middle Sweden (LGMS) Blood 2001; 98 (suppl 1): 123a (abstract 518).

[144] Holowiecki J, Grosicki S, Robak T, *et al*. Addition of cladribine to daunorubicin and cytarabine increases complete remission rate after a single course of induction treatment in acute myeloid leukemia. Multicenter phase III study. Leukemia 2004; 18: 989-97.

[145] Samorapoompichit P, Steiner M, Lucas T, *et al.* Induction of apoptosis in the human mast cell leukemia cell line HMC-1 by various antineoplastic drugs. Leuk Lymphoma 2003*;* 44*:* 509-15.

[146] Tefferi A, Li CY, Butterfield JH, Hoagland HC. Treatment of systemic mast cell disease with cladribine. N Engl J Med 2001; 344: 307-9.

[147] Pardanami A, Hoffbrand AV, Butterfield JH, Tefferi A. Treatment of systemic mast cell disease with 2-chlorodeoxyadenosine. Leuk Res 2004; 28: 127-31.

[148] Kluin-Nelemans HC, Oldhoff JM, van Doormoal JJ, *et al*. Cladribine therapy for systemic mastocytosis. Blood 2003; 102: 4270-6.

[149] Wrzesien-Kus A, Robak T, Lech-Maranda E, *et al*. Polish Adult Leukemia Group. A multicenter, open, non-comparative, phase II study of the combination of cladribine (2-chlorodeoxyadenosine), cytarabine and G-CSF as induction therapy in refractory acute myeloid leukemia – a report of the Polish Adult Leukemia Group (PALG) Eur J Haematol 2003; 71: 155-62.

[150] Kearns CM, Blakley RL, Santana VM, Crom W. Pharmacokinetics of cladribine (2-chlorodeoxyadenine) in children with acute leukemia. Cancer Res 1994; 54: 1235-9.