

Treatment of hairy cell leukemia with cladribine (2-chlorodeoxyadenosine) by subcutaneous bolus injection: a phase II study

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Background: To assess the activity and toxicity of 2-chlorodeoxyadenosine (cladribine, CDA) given by subcutaneous bolus injections to patients with hairy cell leukemia (HCL).

Patients and methods: Sixty-two eligible patients with classic or prolymphocytic HCL (33 non-pretreated patients, 15 patients with relapse after previous treatment, and 14 patients with progressive disease during a treatment other than CDA) were treated with CDA 0.14 mg/kg/day by subcutaneous bolus injections for five consecutive days. Response status was repeatedly assessed according to the Consensus Resolution criteria.

Results: Complete and partial remissions were seen in 47 (76%) and 13 (21%) patients, respectively, for a response rate of 97%. All responses were achieved with a single treatment course. Most responses occurred early (i.e. within 10 weeks) after start of CDA therapy, but response quality improved during weeks and even months after treatment completion. The median time to treatment failure for all patients was 38 months. Leukopenia was the main toxicity. Granulocyte nadir (median $0.2 \times 10^9/l$) was strongly associated with the incidence of infections ($P = 0.0013$). Non-specific lymphopenia occurred early after CDA treatment, and normal lymphocytes recovered slowly over several months. No significant associations were found between infections and nadir count of lymphocytes or any lymphocyte sub-population. No opportunistic infections were observed.

Conclusions: One course of CDA given by subcutaneous bolus injections is very effective in HCL. The subcutaneous administration is more convenient for patients and care providers, and has a similar toxicity profile to continuous intravenous infusion. The subcutaneous administration of CDA is a substantial improvement and should be offered to every patient with HCL requiring treatment with CDA.

Key words: 2-chlorodeoxyadenosine, cladribine, hairy cell leukemia

Introduction

Hairy cell leukemia (HCL) is a chronic lymphoproliferative disease of B-cell lineage with an indolent, but progressive course [1, 2]. The disease is characterized by the presence of abnormal mononuclear cells with irregular cytoplasmic pro-

jections. These 'hairy cells' contain cytoplasmic vesicles with positive cytochemical staining for tartrate-resistant acid phosphatase (TRAP) in more than 95% of all cases [3], and they typically express a panel of mature B-cell surface markers [4] including CD19, CD20 and CD22, as well as lineage-non-specific markers such as CD11c, CD25 and CD103. In spite of the characteristics observed in classic HCL, the disease may present with a wide range of features which overlap with other hematological malignancies [5, 6]. Combination of morphological and clinical criteria, cytochemistry and immunophenotype analysis is therefore required for accurate diagnosis of

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HCL [7]. A prolymphocytic variant of HCL has been described which may be a disease intermediate between HCL and B-cell prolymphocytic leukemia [8–11].

HCL is characterized by infiltration of bone marrow and spleen, but other organs may be affected as well. Common presenting symptoms are splenomegaly, peripheral cytopenia, a variable proportion of circulating tumor cells, frequent infections due to impaired immunity, bleeding tendency, and fatigue. Splenectomy, aimed at improving cytopenias secondary to hypersplenism, was the treatment of choice before interferon- α (IFN- α) and deoxycoformycin (DCF) became available in the 1980s. Treatment with IFN- α results in hematological responses in 70–90% of patients; however, complete remissions (CRs) are unusual [12–14]. DCF has an overall response rate of 79–100% in HCL patients, and CRs in the bone marrow were seen in 56–89% of patients [15–18]. The reluctance of many investigators to recommend DCF as first-line treatment for HCL is primarily based on concerns over the toxicity of the drug [19].

More recently, 2-chlorodeoxyadenosine (cladribine, CDA) has been introduced as yet another treatment for HCL [20] resulting in response rates of 90–100%, with 70–90% of patients achieving a CR as defined by complete disappearance of hairy cells in the bone marrow [21–24]. The remissions are usually long-lasting, and the outcome after CDA treatment seems not to be affected by previous therapies [25, 26]. Notably, CDA therapy is associated with minimal acute and sub-acute side-effects. Moderate bone marrow depression is the main toxicity. The granulocyte nadir is usually seen during the second week after initiation of a 7-day treatment course. Febrile temperatures occurring early after treatment usually result from non-infectious reasons such as lysis of hairy cells with release of pyrogens. Infections during the granulocytopenic period are less common and may be promoted by concomitant treatment with immunosuppressive agents such as steroids [23]. As concerns late toxicities, there is evidence from a number of studies that CDA treatment induces a marked and long-lasting, yet transient reduction in the number of normal peripheral blood lymphocytes including T cells, natural killer (NK) cells and B cells [22, 23]. The clinical significance of this immunosuppression with regard to the occurrence of opportunistic infections, the incidence of secondary neoplasms, and the subsequent course of HCL is still controversial.

The standard schedule for CDA in most studies was an intravenous infusion of 0.1 mg/kg/day for 7 days, and remissions were achieved after one single treatment course in the majority of patients. However, this schedule requires either an infusion pump or hospitalization of the patients. In addition, a substantial rate of phlebitis (up to 19%) has been observed in some series after continuous intravenous CDA infusion for 7 days [27]. CDA has also been administered as a 2-h infusion for five consecutive days [28]. Still, a more convenient and cost-saving route of administration is desirable for HCL patients. We [29] and others [30–32] have shown that subcuta-

neous bolus administration of CDA is tolerated without complications and with a 100% bioavailability. We therefore wished to evaluate the clinical activity and toxicity of CDA given by subcutaneous bolus injections to HCL patients.

Patients and methods

Study design and treatment

In this multicenter phase II study, all patients received a first treatment cycle with CDA at a dose of 0.14 mg/kg/day by subcutaneous bolus injection for five consecutive days (total dose 0.7 mg/kg). Patients not responding to this first cycle subsequently received a maximum of two additional cycles with CDA given at the same total dose but as a continuous intravenous infusion over seven consecutive days. CDA was produced by Lipomed AG, Basel, Switzerland, and provided in vials containing 10 mg of the drug in NaCl 0.9% at a concentration of 2 mg/ml.

Patients were advised to maintain a good hydration state during administration of CDA. Allopurinol was recommended at a dose of 100 mg/day (300 mg/day in case of elevated serum uric acid) orally for a period of 2 weeks, starting on day 1 of each CDA cycle.

An acute infection had to be controlled by antibiotic treatment before CDA therapy was started. Patients who developed neutropenia with fever $>38^{\circ}\text{C}$ following CDA therapy were initially given intravenous antibiotics after taking at least three blood cultures. However, the majority of these patients had sterile blood cultures and no evidence of infection. Therefore, the approach to neutropenic fever changed during the course of the study, and febrile patients with sterile blood cultures were given oral antibiotics for a period depending on the patient's condition and the level of suspicion for infection. An infection was assumed if fever $>37^{\circ}\text{C}$ was accompanied by organ-related symptoms (such as cough or dysuria) or in the case of documented bacteremia. Administration of hematopoietic growth factors was allowed in cases of severe documented infection in neutropenic patients. Other ancillary treatments were given as indicated clinically. Prophylactic antiemetics were not given routinely. Packed red blood cells were administered for symptomatic anemia. Prophylactic platelet transfusions were given if the platelet count was $<5 \times 10^9/l$ in afebrile patients or $<10 \times 10^9/l$ in febrile patients.

Eligibility and monitoring of patients

Patients of any age with newly diagnosed HCL or progressive disease (PD) after previous treatment not including CDA (splenectomy, IFN- α , DCF) were eligible for this study. Eligibility criteria included WHO performance score ≤ 2 , expected survival time >3 months, serum creatinine $<200 \mu\text{mol/l}$ and bilirubin $<35 \mu\text{mol/l}$ (unless due to HCL), and negative HIV serology. Patients were not eligible in the case of pregnancy, any ongoing treatment for HCL (cytotoxic drugs, IFN- α), prior therapy within 4 weeks before start of study treatment, unresolved toxicity from previous treatment, previous or concurrent additional malignancy other than *in situ* carcinoma of the cervix and basal or squamous cell carcinoma of the skin. All patients gave written informed consent to participate in the study, which was approved by the local ethical committee of each participating institution.

In all patients, the diagnosis of classic or variant HCL was established by morphology including peripheral blood smear and bone marrow examination (trephine biopsy and, if possible, aspirate). In the majority of patients, bone marrow slides were also stained for TRAP and reticulin fibers. Pretreatment evaluation of patients included clinical examination, complete blood count (including reticulocytes), immunophenotyping of peripheral blood lymphocytes by flow cytometry (including T cells, T

helper cells, T suppressor cells, NK cells, normal B cells and hairy cells), biochemical parameters [including calcium, albumin, creatinine, aspartate aminotransferase, alkaline phosphatase, bilirubin, uric acid, lactate dehydrogenase (LDH), cholesterol, serum protein electrophoresis], a chest X-ray and an abdominal computed tomography scan or sonography.

After start of the study treatment, peripheral blood counts, biochemical parameters, and peripheral blood lymphocyte immunophenotyping (flow cytometry) were repeated weekly during the first month, at 6 weeks and at 10 weeks after each treatment cycle, then every 3 months for 2 years, and every 6 months thereafter.

Response and toxicity criteria

Response status (including bone marrow examination and imaging of other tumor manifestations) was assessed at 4 weeks and at 10 weeks after treatment start, and every 6 months thereafter, according to the Consensus Resolution [33] using the following criteria.

A CR was defined as the complete disappearance of all evidence of disease and required all of the following: normal peripheral blood counts (hemoglobin >120 g/l, neutrophils $>1.5 \times 10^9/l$, platelets $>100 \times 10^9/l$); absence of hairy cells (by morphological examination) in the peripheral blood and in the bone marrow, with reversion of TRAP stain to negative (if positive initially); regression to normal of disease-related organomegaly.

A partial remission (PR) required all of the following: normal peripheral blood counts (as in CR); circulating hairy cells $\leq 5\%$ of lymphocytes; $>50\%$ reduction of bone marrow infiltration by hairy cells; $>50\%$ reduction of palpable disease-related organomegaly.

A minor response (MR) required all of the following: $>50\%$ reduction of circulating hairy cells; improvement of one or more of the peripheral blood counts (hemoglobin, neutrophils, platelets).

Non-responders were patients not meeting the criteria for CR, PR or MR.

Relapse was defined as the reappearance of hairy cells in the bone marrow or as any other new disease manifestations in patients with previously documented CR.

PD was defined as the occurrence of new disease manifestations, as $>50\%$ increase in the percentage of residual tumor cells, or as $>50\%$ increase of residual disease-related organomegaly in patients with previously documented PR.

Time to treatment failure (TTF) was defined as the time between treatment start and progression, relapse, second tumor, or death, whichever occurred first.

Overall survival (OS) was measured from the commencement of treatment until death from any cause.

Early death was defined as death within 4 weeks after administration of the last CDA dose.

Toxic death was defined as any death to which CDA toxicity may have contributed.

Hematological and non-hematological side-effects were graded according to the expanded National Cancer Institute toxicity criteria.

Statistical considerations

The endpoints of the study were: (i) to define the rates of CR and PR, time to achieve a remission, TTF, and OS in HCL patients treated with CDA by subcutaneous bolus injections; (ii) to determine the incidence of side-effects of treatment with CDA; and (iii) to assess by serial immunophenotyping the proportion of peripheral blood lymphocyte subpopulations (T cells, T-cell subsets, NK cells, normal and neoplastic B cells) during and after CDA treatment, and to investigate the possible clinical significance of lymphocyte subpopulation shifts with special regard to the subsequent course of disease and rate of infections.

The sample size of this phase II study was determined as suggested by Simon [34]. A two-stage design was used, setting the significance level at 5% and the power of the study at 90%. The minimal response rate of interest was defined as 70%. Fifteen patients were necessary in the first stage, and, if 11 or less were responding, the trial was to be closed. Otherwise, an additional 21 patients were planned in order to complete the study, for a total number of 36. The planned sample for the second stage was reached in August 1994. At that time, considering the high response rate and the lack of an immediately available better therapeutic option for this group of patients, it was decided to continue the study. The study was closed in May 1995, soon before activation of the succeeding study. The increased sample allows a more precise estimation of the response rate.

Associations were tested with a chi-square or Fisher's exact test as appropriate. The correlation between pretreatment values of laboratory parameters and qualitative parameters was determined using the Wilcoxon two-sample or the Kruskal-Wallis test as appropriate. Correlation was determined with Spearman's rank correlation. Laboratory parameters were analyzed as both continuous and categorical variables.

Predictive factors for 'early CR' (within 10 weeks) and 'overall CR' were analyzed with contingency tables and multivariate logistic regression [35]. Odds ratios [ORs, with 95% confidence intervals (CI) and *P* values] were estimated with respect to the reference category for each covariate using binary variables.

TTF and OS were estimated according to Kaplan and Meier [36] and compared using the log-rank test [37]. Observations were censored for patients last known to be without events for TTF or alive for OS. Standard errors were estimated using Greenwood's formula. Univariate analysis was used to assess individual correlations with prognosis (TTF). Multivariate analysis using the Cox proportional hazard model was performed [38]. A likelihood ratio test was used as an indicator of the overall significance of a new variable in addition to the model including only clinical variables [39]. All *P* values are two-sided. No correction has been performed for multiple evaluations.

Results

Accrual and patients' characteristics

A total of 63 consecutive patients were registered within 21 months. One registered patient was ineligible because of a concomitant renal cell cancer. All reported results refer to the 62 eligible patients. The characteristics of these patients are shown in Table 1. The male:female ratio was 47:15. In two cases, a prolymphocytic variant was diagnosed, whereas 60 patients had classic HCL. Thirty-three patients had newly diagnosed HCL, 15 patients had a relapse after previous treatment, and 14 patients had PD during a previous medical treatment other than CDA. No patient had been treated with DCF, and no patient had been treated with CDA given intravenously before entering the study. Most patients without previous splenectomy had an enlarged spleen, and a minority of patients had hepatomegaly or lymphadenopathy. The median time from diagnosis was 13 months (range 0–227 months).

Responses

The responses induced by CDA treatment are shown in Table 2. Sixty patients achieved a CR or PR, for an overall remission

Table 1. Characteristics of eligible patients ($n = 62$)

	No.	Percent
Gender		
Male	47	76
Female	15	24
Performance status		
0	46	74
1	13	21
2	3	5
Hairy cell leukemia subtype		
Classic	60	97
Prolymphocytic	2	3
Status at study entry		
Non-pretreated	33	52
First/second/third relapse	15	24
Progressive disease during treatment with		
Interferon- α	10	16
Interferon- α and chlorambucil	3	5
Chlorambucil and prednisone	1	2
Previous therapies ^a		
Splenectomy	10	16
Radiotherapy	0	–
Chemotherapy alone ^b	3	5
Interferon- α \pm chemotherapy	24	39
Organ involvement ^a		
Splenomegaly	38	61
Hepatomegaly	10	16
Lymphadenopathy	15	24
Other (multiple lung nodules)	1	2

^aPatients may have had more than one previous therapy or organs involved.

^bWith alkylating agents.

rate of 97%. Forty-seven patients achieved a CR (76%; 95% CI 63% to 86%), and 13 patients (21%) achieved a PR. A single patient who received one CDA cycle only did not achieve a remission. No additional CDA was given to this patient because of medical problems unrelated to HCL and not due to toxicity. This patient died 13 months after CDA therapy from cardiac failure. One patient was not evaluable for response because treatment with IFN- α was initiated 2 weeks after CDA therapy.

Two patients were given more than one treatment cycle. One patient who was in PR after the first course received a second cycle and achieved a CR; the second patient who was in PR after the first course received two additional cycles but remained in PR.

A summary of the responses at serial evaluations is given in Table 2. The percentages were calculated including all eligible patients and therefore slightly underestimate the response rate in evaluable patients. Most responses occurred early (i.e. within 10 weeks) after the start of CDA therapy. However, the response quality kept improving during weeks and even months after treatment completion, with most complete responses occurring later than 10 weeks after start of the first CDA cycle.

The patients' characteristics listed in Table 1 as well as baseline hematological parameters (hemoglobin, leukocytes, granulocytes, lymphocytes, platelets) and biochemical parameters (LDH, albumin, cholesterol) were examined for possible associations with achievement of either early CR (within 10 weeks) or overall CR. Univariate analysis revealed that early CR was more frequent in patients with normal baseline hemoglobin ($P = 0.05$), but there was no other significant association with any of the factors examined. Multivariate analysis confirmed that patients with a normal baseline hemoglobin had a higher probability to achieve an early CR (OR 3.6, 95% CI 1.02–12.9, $P = 0.047$), and among pretreated patients, females may have a lower probability to achieve overall CR (OR 9.5, $P = 0.06$).

Table 2. Responses

	Best response at any time		Four weeks		Ten weeks		Any follow-up (>10 weeks)	
	<i>n</i>	%	<i>n</i>	% ^a	<i>n</i>	% ^a	<i>n</i>	% ^a
Complete response	47	76	7	11	11	18	46 ^b	74
Partial response	13	21	41	66	38	61	7	11
Minor response/no response	1	2	8	13	2	3	1	2
PD after previous response	n.a.	n.a.	0	0	1	2	0	0
Not assessed/not evaluable	1	2	6	10	10	16	8	13
Total	62	100	62	100	62	100	62	100

^aAll eligible patients included in the percentage calculations.

^bOne patient with complete remission at 10 weeks not evaluable at follow-up due to early death.
n.a., not applicable; PD, progressive disease.

Time to treatment failure and overall survival

At a median follow-up of 3.8 years, the following events indicating treatment failure were observed: progression after PR in seven patients (one of whom died); relapse after CR in eight patients; second tumors in three patients (two of whom died); and death from other causes in two patients.

The second malignancies were a brain tumor (glioblastoma multiforme) detected 2 months after CDA treatment (HCL in CR) and an adenocarcinoma of the pancreas detected 28 months after CDA treatment (HCL in CR); these two patients died from their second tumors. An additional patient developed a renal cell cancer 5 months after CDA therapy; this patient is alive and in CR from both HCL and renal cell cancer following surgical resection of the second tumor.

One patient with progressive HCL (after PR) died from a neutropenic infection 22 months after CDA treatment. Another patient, who had no response to CDA therapy, died from cardiac failure 13 months after CDA.

The median TTF for all patients was 38 months (Figure 1). The failure-free proportion is 0.90 [standard error (SE): 0.04] at 1 year and 0.76 (SE: 0.05) at 2 years, respectively. Using univariate analysis, TTF was shorter for patients without systemic pretreatment as compared with patients in relapse or with progression after systemic pretreatment (failure-free proportion at 2 years 0.68 versus 0.83, $P = 0.04$). Patients with hepatomegaly had a significantly shorter TTF as compared with patients with normal liver size (failure-free proportion at

2 years 0.60 versus 0.79, $P = 0.03$). Shorter TTF was also observed in patients with decreased baseline hemoglobin (failure-free proportion at 2 years 0.69 versus 0.87, $P = 0.06$) and platelets (failure-free proportion at 2 years 0.70 versus 0.93, $P = 0.04$).

By multivariate analysis, the OR for failure was 2.46 (95% CI 0.88–6.84, $P = 0.09$) for patients without systemic pretreatment in a model adjusted for hepatic involvement and baseline platelet count. Pretreatment was considered for subgroup analysis. In non-pretreated patients, the estimated OR for hepatic involvement was 3.1 (95% CI 0.97–9.8, $P = 0.055$) in a model adjusted for baseline hemoglobin. Interpretation of these results should be cautious since the subgroups contain few patients, and few failures were observed.

The median OS has not been reached so far. The proportion alive is 0.97 (SE: 0.02) at 1 year and 0.93 (SE: 0.03) at 2 years.

Toxicities

All but one patient were evaluable for hematological toxicity. Pretreatment and nadir values of hematological parameters are given in Table 3. CDA treatment induced considerably low leukocyte nadirs. Granulocyte nadir was less than 50% of the pretreatment value in 73% of patients, and lymphocyte nadir was less than 50% of the pretreatment value in 88% of patients. Serial assessment of peripheral blood lymphocytes and lymphocyte subpopulations including T cells, T-cell subsets, non-malignant B cells and NK cells revealed a marked

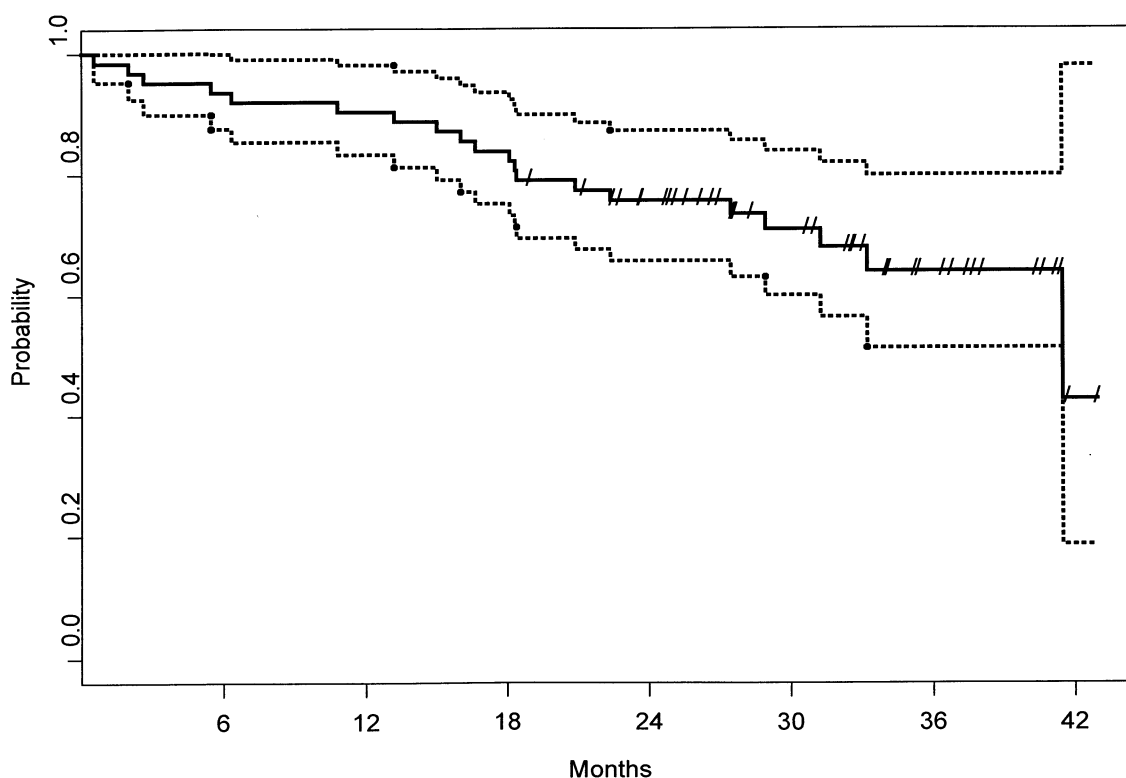


Figure 1. Time to treatment failure (with 95% confidence intervals).

Table 3. Baseline and nadir values of hematological parameters

	Baseline values		Nadir values	
	Median	Range	Median	Range
Hemoglobin (g/dl)	10.6	4.5–16.3	8.1	4.1–14
Leukocytes ($\times 10^9/l$)	2.4	0.2–41.1	0.5	0.02–2.1
Granulocytes ($\times 10^9/l$)	0.7	0.02–2.3	0.2	0–1.6
Lymphocytes ($\times 10^9/l$)	1.3	0.1–13.9	0.2	0–1.2
Platelets ($\times 10^9/l$)	64	43–318	52	17–236

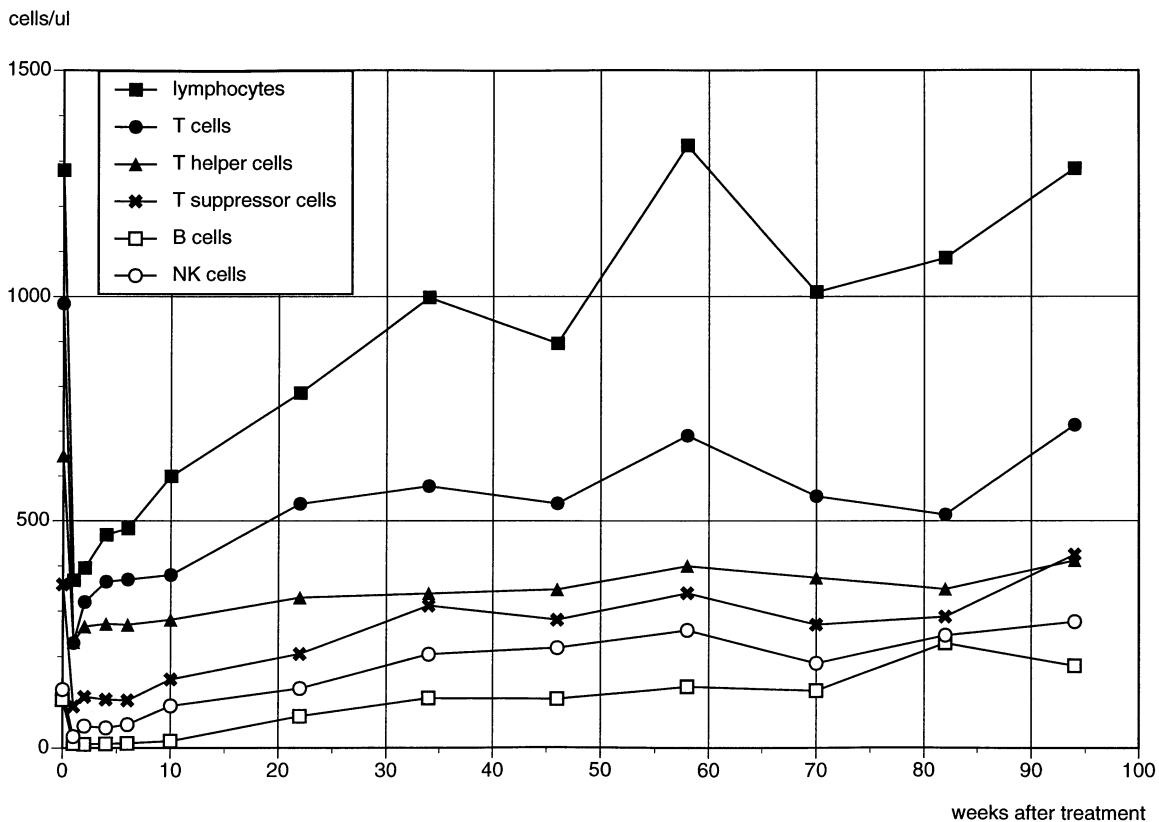
but non-specific lymphopenia with rapid onset after treatment with CDA (see Figure 2). Nadir values were recorded 1 week after treatment start for all subpopulations except for B cells (nadir after 2 weeks). All subpopulations subsequently recovered, but recovery was generally slow and required several months. In one patient with excessive (>95%) bone marrow infiltration by hairy cells and agranulocytosis at study entry, granulocyte recovery required 9 months, whereas normal granulocyte counts were reached within some weeks in all other patients.

In contrast to white blood cells, toxicity was less severe for hemoglobin and platelets. In 67% of patients, hemoglobin nadirs were higher than 75% of pretreatment values, and, of

particular interest, no decrease in platelet counts was observed in 36% of patients.

A total of 50 infectious episodes were seen in 35 patients at any time after CDA. Early infections (≤ 10 weeks after CDA treatment) were observed in 36 cases (26 infections within 4 weeks and 10 infections between 4 and 10 weeks after CDA treatment), and late infections (>10 weeks after CDA treatment) in 14 instances. Most infections were mild, but nine were graded as moderate (grade II) and 12 as severe (grade \geq III, requiring oral or intravenous antibiotics). While the infectious agent was not identified in most cases, herpes simplex virus was found in three patients, *E. coli* in one patient, and *S. pneumoniae* in one patient. No opportunistic germs were identified. One patient with progressive HCL (after previous PR) and neutropenia died from pneumonia 22 months after CDA treatment. All other infections resolved after appropriate treatment. No association was found between infections and nadir counts of lymphocytes or lymphocyte subpopulations. However, infections were strongly associated with nadir granulocyte count ($P = 0.0013$).

Other severe (grade \geq III) clinical toxicities included a gastrointestinal hemorrhage (in a patient with preexisting peptic ulcer), a cardiac failure (in a patient with preexisting coronary heart disease) and a mental depression in one patient each, and CDA treatment was probably not a major underlying factor in these events. As mentioned earlier, second tumors were

**Figure 2.** Medians of lymphocytes and lymphocyte subpopulations before and after cladribine therapy. NK, natural killer cells.

diagnosed in three patients. Mild (grade I or II) side-effects included hemorrhages, high temperature without evidence of infection, cutaneous rashes, diarrhea, symptoms of anemia, nausea, edema, bone pain and headache. No patient had mucositis, vomiting, neurological symptoms or alopecia.

Discussion

Our data confirm the excellent activity of CDA in HCL. In our series, the overall remission rate was 97%, and 76% of the patients achieved a CR, defined according to the very stringent Consensus Resolution criteria. Juliusson et al. [40] reported a similar CR rate of 75% in their study with subcutaneous CDA for HCL. Remarkably, our results were obtained with a single treatment cycle in 60 patients, and only two patients required more than one cycle. The response quality improved over weeks and even months after application of a single treatment cycle without further therapy, as suggested before by limited data from other investigators [23, 25]. Clearance of hairy cells from the peripheral blood was seen a few days after treatment start, whereas clearance from bone marrow was slower, and normalization of organomegaly (spleen, lymphadenopathy) usually required 3–6 months. Therefore, observation is justified in most patients after a single treatment course, unless a rapid treatment effect is required by clinical features such as massive organomegaly or severe cytopenia.

We found that rapid achievement of a CR (i.e. within 10 weeks after treatment) was favored by a normal pretreatment hemoglobin. In agreement with other reports, no other clinical features predicted the quality of response. The present study demonstrates that CDA given by subcutaneous bolus injections may have a clinical efficacy equivalent to the continuous intravenous application of the same dose. In trials using the intravenous schedule, CR rates ranged from 50% to 91%, depending on the methods used to assess the remission status [22, 41–44].

The median TTF of our cohort was 38 months, with 90% of patients failure-free at 12 months and 76% at 24 months. Seventy-five percent of treatment failures were due to relapses after CR or tumor progression after PR. Few data on TTF only are available for comparison from other studies in which regular follow-up bone marrow samples were obtained. Juliusson et al. [40] reported a progression-free proportion of 68% at 18 months after subcutaneous CDA, but no statements were made concerning treatment failures other than HCL progression. In a series using intravenous therapy, failure-free survival was 93% at 24 months and 81% at 48 months [43]. In other studies with intravenous therapy, progression-free survival was 77% at 36 months [45], 72% at 48 months [41], and 80% at 55 months [42]. The differences may be explained by variable follow-up schedules and methods, since in contrast to our study no regular bone marrow samples were taken during follow-up, and less strict response criteria have been applied in most series.

We observed that TTF was adversely correlated with hepatic involvement ($P = 0.03$), while a trend to longer TTF was observed in patients with normal pretreatment hemoglobin, normal platelet count and pretreated patients. The latter group may represent a population with less aggressive disease and therefore better prognosis. However, these results require cautious interpretation because subgroups contained few patients only. In the large Scripps Clinic series, an increased pretreatment peripheral leukocyte count ($>15 \times 10^9/l$) and the presence of a splenomegaly were significant predictors of TTF [43]. In contrast to a previous report with a small patient number [46], abdominal lymphadenopathy was not associated with a shorter TTF in our series.

A pronounced depression of peripheral leukocytes was the main toxicity of CDA. We found a median granulocyte nadir of $0.2 \times 10^9/l$, and the granulocyte nadir was $<25\%$ of the pretreatment count in 45% of patients. CDA is also known to induce profound lymphopenia, and we could demonstrate that this lymphopenia is non-specific, with all subpopulations being affected. The median lymphocyte nadir of $0.2 \times 10^9/l$ occurred 1 week after the start of CDA treatment. Suppression of lymphocyte subpopulations was long-lasting, and recovery took months or even years. In keeping with other studies [47, 48], NK cells were the fastest to recover, whereas recovery of non-malignant B cells was slower. T-cell recovery was very prolonged, and all T-cell subpopulations did not reach normal levels within the observation period of nearly 2 years.

Infections occurred in a significant proportion of patients predominantly during the first weeks after CDA therapy. The incidence of infections was strongly associated with granulocyte nadir ($P = 0.0013$), but no correlations were found between infections and nadir counts of lymphocytes or any lymphocyte subpopulation. Moreover, we did not observe any opportunistic infections. It appears therefore that the long-lasting immunosuppression resulting from CDA-induced lymphopenia has no clinical consequences. This is in accord with other reasonably large studies in which the rate of opportunistic infections was also low despite a prolonged suppression of CD4⁺ T cells [22, 41, 42, 45]. We conclude that neutropenia but not lymphopenia is predictive for infections in HCL patients receiving one CDA treatment course. This may be different in patients with other lymphoproliferative diseases who are sometimes heavily pretreated or receive several treatment courses with CDA.

Second malignancies were detected in three patients. Two of these malignancies seem not to be related to CDA therapy since they were detected at 2 and 5 months after CDA treatment. In addition, a pancreatic adenocarcinoma was detected in one patient 28 months after CDA therapy. An increased incidence of second malignancies, particularly lymphoid neoplasms, in HCL patients is well known [49, 50] and seems to be due to an intrinsic susceptibility of HCL patients to second tumors rather than to CDA treatment or CDA-induced immunosuppression [42, 45, 49–51]. A particularly high incidence of

second neoplasms has been reported after treatment with IFN- α [52].

Data from numerous studies suggest that CDA is the treatment of choice for HCL. In most studies, the drug has been given intravenously. Oral administration is also possible, but the bioavailability of oral CDA may considerably vary between individual patients depending on food intake and stomach acid [30, 53], and this administration route has not been pursued by clinical investigators during recent years. The present study shows that subcutaneous bolus administration of CDA is very effective. Most patients may find the subcutaneous administration more convenient than intravenous infusions, since it allows self-administration of the drug and infusion devices can be avoided. The subcutaneous administration should be offered to every patient with HCL (or any other lymphoproliferative disorder) requiring treatment with CDA. At present we are performing a subsequent study in which CDA is given to HCL patients by weekly subcutaneous bolus injections.

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