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Second malignancies and Richter's syndrome in patients with chronic lymphocytic leukaemia treated with cladribine

T. Robak[a,*], J.Z. Blonski[a], J. Gora-Tybor[a], M. Kasznicki[a], L. Konopka[b], B. Ceglarek[b], M. Komarnicki[c], K. Lewandowski[c], A. Hellmann[d], K. Lewandowski[d], A. Moskwa[e], A. Dmoszyńska[f], B. Sokołowska[f], A. Dwilewicz-Trojaczek[g], A. Tomaszewska[g], K. Sułek[h], M. Całbecka[i]

[a]*Department of Haematology, Medical University of Łódź and Copernicus Memorial Hospital, Pabianicka 62, 93-513 Łódź, ul. Pabianicka 62, Poland*
[b]*Institute of Haematology and Transfusiology, Department of Internal Medicine, Warsaw, Poland*
[c]*Department of Haematology, Medical University of Poznań, Poznań, Poland*
[d]*Department of Haematology, University Medical School of Gdańsk, Gdańsk, Poland*
[e]*Department of Haematology, Gorzów Regional Hospital, Gorzów, Poland*
[f]*Department of Haematology, Medical University of Lublin, Lublin, Poland*
[g]*Department of Haematology, Medical Academy, Warsaw, Poland*
[h]*Haematology Department, Institute of Military Medicine, Warsaw Poland*
[i]*Department of Haematology, Toruń Regional Hospital, Toruń, Poland*

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Abstract

The increased frequency of second malignancies in chronic lymphocytic leukaemia (CLL) is well known. Moreover, antineoplastic therapy additionally increases the risk of secondary cancers. In this study, we analysed whether treatment with cladribine (2-chlorodeoxyadenosine, 2-CdA) during the course of CLL had an impact on the subsequent occurrence of either secondary solid tumours or Richter's syndrome. There were 1487 eligible patients, 251 treated with 2-CdA alone, 913 treated with alkylating agents (AA)-based regimens alone and 323 treated with both 2-CdA and AA. Median time from the start of CLL treatment to the diagnosis of secondary malignancy was 1.9 years (0.5–5.1 years) for the 2-CdA group, 1.8 years (0.3–7.9 years) for the AA group and 3.9 years (0.3–8.4 years) for the 2CdA + AA group. A total of 68 malignancies were reported in 65 patients. Ten events were non-melanotic skin cancers and were excluded from the analysis, leaving 58 events in 58 patients. In the group of patients treated with 2-CdA alone, there were 15 (6.0%) cases, in the group of patients treated with AA alone there were 26 (2.8%) cases, and in the group treated with 2CdA + AA there were 17 (5.3%) cases of secondary malignancies. The differences between the frequency of secondary malignancies in the 2-CdA and 2-CdA + AA versus AA alone groups were not significant ($P = 0.05$ and $P = 0.06$, respectively). Only lung cancers occurred significantly more frequently in the 2-CdA (2.8%) and 2-CdA + AA (2.2%) treated groups compared with the AA patients (0.3%) ($P < 0.001$ and $P < 0.01$, respectively). In conclusion, 2-CdA in CLL patients does not seem to increase the risk of secondary malignancies except for lung cancers. However, further studies are necessary to establish the real risk of lung cancer in CLL patients treated with 2-CdA.

Keywords: Secondary malignancies; Richter's syndrome; Lung cancer; CLL; Cladribine; 2-CdA; Nucleoside analogues; Late complications

1. Introduction

Second malignancies are frequent complications in patients with chronic lymphocytic leukaemia (CLL) [1,2]. Moreover, chemotherapeutic agents may also contribute to subsequent malignancies among CLL survivors [3–5]. The development of a large B-cell lymphoma (LBCL) in a patient with CLL was first described in 1928 by Maurice Richter and the complication is known as Richter's syndrome [6]. This term was initially restricted in its application to a large-cell lymphoma [7]. However, recently the spectrum of secondary lymphoid

* Corresponding author. Tel.: +48-42-689-5191; fax: +48-42-689-5192.
E-mail address: robaktad@csk.am.lodz.pl (T. Robak).

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malignancies complicating CLL includes also prolymphocytic leukaemia, Hodgkin's disease, acute lymphoblastic leukaemia and multiple myeloma. The secondary development of myeloproliferative disorders or myelodysplastic syndrome as well as other neoplastic diseases has also been rarely documented in CLL patients, especially those treated with alkylating agents [7,8].

The nucleoside analogues, fludarabine (FA), cladribine (2-chlorodeoxyadenosine, 2-CdA) and 2-deoxycoformycin (DCF), comprise a novel group of agents with high activity in low-grade lymphoid malignancies [9]. Multiple studies have demonstrated that FA and 2-CdA have major activity in the treatment of advanced CLL [9–12] and low-grade non-Hodgkin's lymphoma (LG-NHL) [9,13–15]. These two agents share similar structures and mechanism of actions, such as the induction of apoptosis [9,16,17]. They also exhibit significant differences, especially in their interactions with enzymes involved in adenosine and deoxyadenosine metabolism activity.

The toxicity profile of purine analogues is similar for all three drugs and consists mainly of dose-limiting myelotoxicity and prolonged immunosuppression [12,13]. Treatment with these agents leads to a decrease in the CD4+/CD8+ ratio for an extensive period of time exceeding 12 months, even up to 3.5 years [18,19]. Prolonged immunosuppression related to nucleoside analogues therapy and the incorporation of these agents or their metabolites into DNA with potentially mutagenic action led to speculation that their therapeutic use might be responsible for an increased incidence of second cancer, especially in patients with diseases such as CLL, in which an already greater risk for this complication has been observed [20–26].

In this study, we analysed the occurrence of second malignancies including Richter's syndrome in the group of CLL patients treated with 2-CdA alone, alkylating agents-based regimens (AA) and both 2-CdA and AA during the last 10 years. To our knowledge, this is the first study to evaluate second malignancies in CLL patients treated with 2-CdA.

2. Patients and methods

The retrospective analysis of patients with B-CLL treated with 2-CdA-based regimens and/or AA-based regimens in eight haematological departments in Poland was performed. The patients were treated between January 1992 and January 2000. All the patients fulfilled the National Cancer Institute sponsored Working Group diagnostic criteria for CLL [27,28]. Lymphocytosis with an absolute lymphocyte count greater than 10×10^9/l and more than 30% lymphocytes in the normal or hypercellular bone marrow had been documented. Most patients had a surface marker analysis providing evidence of monoclonal B-cell CLL proliferation, including CD5, CD19, CD20 and CD23 antigens as well as membrane surface immunoglobulin light chains. The clinical stage of the disease was determined according to the Rai classification at the time of initiation of the first treatment [29]. Patients with stages 0, I and II were eligible if they had evidence of active disease, including progressive lymphocytosis, massive splenomegaly or bulky lymphadenopathy, recurrent disease-related infections, weight loss >10% over a 6-month period or extreme fatigue.

2.1. Treatment

The patients were divided into three groups. Patients in the first group received only 2-CdA. The second group of patients were treated only with alkylating agents (AA): chlorambucil (Chl) as first-line treatment and COP or CHOP as second-line treatment (see below). The third group consisted of patients treated with both 2-CdA and AA (chlorambucil, COP, CHOP).

The doses and schedule of the treatment agents were based on our previous studies [30–32]. 2-CdA was administered at a dose of 0.12 mg/kg/day by 2-h intravenous (i.v.) infusions for 5 consecutive days. The cycles were usually repeated monthly. Treatment was discontinued if a CR was achieved. If there was a partial, but continuing response (PR) up to three additional courses were given. Patients who had relapsed later than 12 months after completing 2-CdA therapy received the same treatment. The group of patients treated with AA alone received Chl as first-line treatment and COP or CHOP as second-line treatment. Chl was given orally at 12 mg/m^2 per day on 7 consecutive days up to CR or PR or grade 3 toxicity. Patients who were resistant to Chl treatment received second-line therapy according to COP (cyclophosphamide 650 mg/m^2 on day 1, vincristine 1 mg/m^2 on day 1 and prednisone 40 mg/m^2 on days 1–5) or CHOP (COP + doxorubicin 25 mg/m^2 on day 1) regimens. The cycles were repeated every 3–4 weeks. The intervals were longer if haematological complications or infections developed. In responding patients, maintenance therapy with the same cycles was administered every 2–3 months until disease progression or the doxorubicin cumulative dose exceeded 550 mg/m^2. The third group consisted of patients treated with both 2-CdA and AA according to the protocols described above.

2.2. Evaluation of second malignancies or Richter's syndrome

The primary objective of this retrospective analysis was to determine whether the treatment with 2-CdA during the course of CLL had an impact on the subsequent occurrence of either secondary malignancies or Richter's syndrome. Malignancies occurring at least 2

months after the start of treatment with cytotoxic agents were included in the analysis. Non-melanotic skin cancers were excluded from the analysis. According to the Revised European-American Lymphoma (REAL) classification, only patients with a histological diagnosis of diffuse large B-cell lymphoma were classified as Richter's syndrome [33].

Data for 3 patients included into this analysis have been reported earlier in single case reports [34–36].

2.3. Statistical analysis

Median values of the measured variables were calculated and the range was given (min–max). The type of distribution of the studied data was evaluated with the Shapiro–Will test. The Mann–Whitney U test was used to compare differences in continuous variables between groups, and χ^2 test or Fisher's exact test were used to compare percentages. The overall survival (OS) was calculated according to the method of Kaplan and Meier [37] and compared between groups by the log rank test. OS was calculated from the first day of treatment to the last day of follow-up or death. All P values <0.05 were considered to be statistically significant.

3. Results

There were 1487 eligible patients, 251 treated with 2-CdA alone, 913 treated with AA alone and 323 treated with both 2-CdA and AA. A total of 68 malignancies were reported in 65 patients. Ten events were non-melanotic skin cancers and were excluded from the analysis. The characteristics of the patients with second malignancies are presented in Table 1. In all CLL patients, we observed 58 (3.9%) cases of secondary malignancies. In the group of patients treated with 2-CdA alone, there were 15 (6.0%) cases, in the group of patients treated with AA alone, there were 26 (2.8%) cases, and in the group treated with 2CdA + AA, there were 17 (5.3%) cases of secondary malignancies. The differences between the frequency of secondary malignancies in the 2-CdA and 2-CdA + AA versus AA alone groups were not significant ($P=0.05$ and $P=0.06$, respectively) (Fig. 1). The median time from the start of treatment to diagnosis of secondary malignancy was 1.9 years (0.5–5.1 years) for 2-CdA group, 1.8 years (0.3–7.9 years) for AA group and 3.9 years (0.3–8.4 years) for the 2CdA + AA group. The median follow up period was 2.6 years (0.5–10.6 years) for the 2-CdA group, 3.3 years (0.3–8.6 years) for the AA group and 4.4 years (0.7–9.9 years) for the 2-CdA + AA group.

Lung cancers were the most common of the 58 malignancies (17, 29%) and Richter's syndrome (13, 22%) (Table 2). Moreover, lung cancers occurred significantly more frequently in both the 2-CdA (2.8%) and 2-CdA + AA (2.2%) treated groups compared with the AA (0.3%) group ($P<0.001$ and $P<0.01$, respectively) (Fig. 2). 71% of the 2-CdA group were cigarette smokers, compared with the 66.7% in the AA group and 57% in the 2-CdA + AA group.

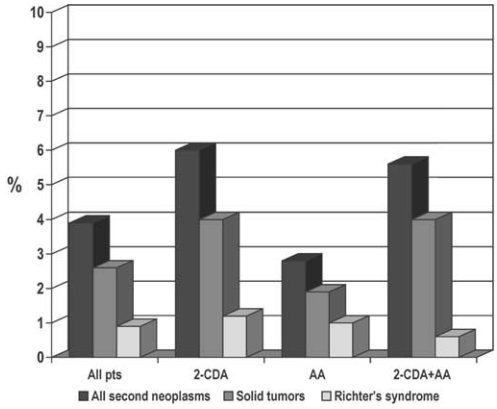

Fig. 1. Second malignancies in 1487 chronic lymphocytic leukaemia patients treated with cladribine (2CdA) alone ($n=251$), alkylating agents-based regimens (AA) alone ($n=913$) or 2CdA + AA ($n=323$). pts, patients.

There were no significant differences between the frequency of Richter's syndrome in 2-CdA and 2-CdA + AA versus AA treated groups ($P=0.3$ and $P=0.8$, respectively) (Fig. 1).

Other secondary cancers, according to the frequency of occurrence, were: colorectal, breast, genitourinary prostate, brain, melanoma, liver, stomach and thyroid (Table 2). We did not observe any significant differences between the frequency of solid tumours in the 2-CdA and 2-CdA + AA versus AA treated groups ($P=0.08$ and $P=0.05$, respectively) (Fig. 1). We also observed 2 patients with Hodgkin's disease and 2 with prolymphocytic leukaemias (Table 2).

There was no significant trend in the frequency of secondary malignancies with an increase in the number of courses of therapy ($P>0.3$, $P>0.6$, $P>0.2$) for 2-CdA, AA and 2-CdA + AA, respectively.

The median survival times of patients with secondary malignancies were 3 years in 2-CdA, 3 years in AA and 5.2 years in the 2-CdA + AA group and did not differ among the groups ($P=0.24$).

4. Discussion

Secondary malignancies occur with an increased frequency in patients with CLL mainly because of the immune defects associated with this disease [1,2,20]. It is of great concern that therapy may further increase the risk of second neoplasm. Dighiero and colleagues have noted the increased frequency of secondary malignancies in patients with CLL treated with AA such as Chl [3]. Others have observed that the cancer risk did

Table 1
Characteristics of patients with secondary malignancies in the group of 1487 chronic lymphocytic leukaemia patients treated with 2-CdA (251), AA (913) or 2-CdA + AA (323)

Characteristics	All pts	Treated with		
		2-CdA	AA	2-CdA + AA
Total	58	15	26	17
Gender				
Male	38 (66%)	10 (67%)	18 (69%)	10 (59%)
Female	20 (34%)	5 (33%)	8 (31%)	7 (41%)
Age in years, median (range)	62 (31–82)	57 (42–66)	65 (32–77)	59 (31–92)
Time from diagnosis to treatment in years, median (range)	0.14 (0.01–9.35)	0.06 (0.02–9.36)	0.81 (0.01–6.24)	0.07 (0.01–2.32)
Time from treatment to second malignancy in years, median (range)	2.29 (0.3–8.4)	1.9 (0.5–5.1)	1.8 (0.3–7.9)	3.9 (0.3–8.4)
Median number of cycles (range)				
2-CdA	2 (1–11)	6 (1–11)		6 (1–9)
AA	6 (1–35)		12 (2–35)	6 (1–18)
Rai's staging				
0	1 (2%)	1 (7%)	0	0
I	11 (19%)	2 (13%)	7 (27%)	2 (12%)
II	27 (47%)	6 (40%)	11 (42%)	10 (59%)
III	10 (17%)	4 (27%)	5 (19%)	1 (6%)
IV	9 (17%)	2 (13%)	3 (12%)	4 (24%)
Disease status at the time of second malignancy diagnosis				
CR	11 (19%)	7 (47%)	2 (8%)	2 (12%)
PR	30 (52%)	7 (47%)	13 (50%)	10 (59%)
NR	17 (29%)	1 (7%)	11 (42%)	5 (29%)
Time from diagnosis to second malignancy (in years)	3.6 (0.1–10.6)	2.6 (0.5–10.6)	3.3 (0.3–8.6)	4.4 (0.7–9.9)
Died	29 (50%)	5 (33%)	17 (65%)	7 (41%)

2-CdA, cladribine; AA, alkylating agents-based regimens; CR, complete remission; PR, partial remission; NR, no remission; pts, patients.

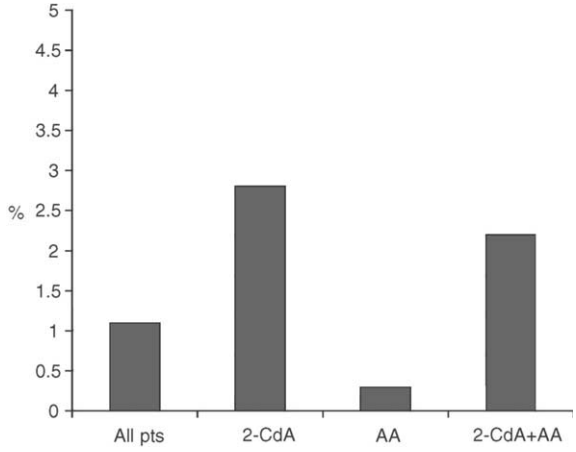

Fig. 2. Lung cancer as second malignance in 1487 chronic lymphocytic leukaemia patients treated with cladribine (2CdA) alone ($n = 251$), alkylating agents based-regimens (AA) alone ($n = 913$) or 2CdA + AA ($n = 323$).

not vary according to the initial AA treatment [5,38]. It still remains unclear whether purine analogues 2-CdA, DCF or FA increase the risk of second malignancies due to profound and prolonged immunosuppression related to therapy with these drugs.

This study was designed to compare the frequency of secondary malignancy in CLL patients who were exposed to 2-CdA, AA and combined 2-CdA and AA treatments. Our data revealed that, except for the lung cancers, there is no difference in the frequency of solid tumours among the groups of patients treated with 2-CdA alone, AA or 2CdA + AA.

Cheson and colleagues studied the frequency of second malignancies in 2014 CLL and hairy cell leukaemia patients treated with 2-CdA, fludarabine or DCF [20]. They concluded that there was no additional risk of secondary malignancies with the use of these purine analogues. However, in their study CLL patients were not treated with 2-CdA. In addition, Flinn and colleagues observed that the frequency of secondary malignancies in the group of 241 hairy cell leukaemia patients treated with DCF was not higher than that expected in the general population [39]. Travis and colleagues analysed data from 9456 CLL patients (5153 untreated, 4303 received radiotherapy or chemotherapy, mainly with AA) [5]. They observed that compared with the general population, CLL patients demonstrated an increased risk of developing second cancers (840 observed, observed to expected ratio O/E = 1.28). The most significant excesses were noted for cancers of the lung, brain, melanoma and Hodgkin's disease. Surprisingly, they observed that the cancer risk did not vary according to the initial treatment

Table 2
Secondary malignancies in the group of 1487 chronic lymphocytic leukaemia patients treated with 2-CdA ($n=251$), AA ($n=913$) and 2-CdA + AA ($n=323$)

Second neoplasm	All pts	Treated with		
		2-CdA	AA	2-CdA + AA
Solid tumour	40	10	17	13
Lung	17	7	3	7
Colon	5	0	2	3
Breast	4	1	3	0
Prostate	3	0	2	1
Thyroid	1	1	0	0
Genitourinary	4	1	2	1
Gastric	1	0	1	0
Brain	2	0	1	1
Melanoma	2	0	2	0
Liver	1	0	1	0
Lymphoma	17	5	9	3
Richter's syndrome (DLBCL)	13	3	8	2
Prolymphocytic	2	1	0	1
Hodgkin's disease	2	1	1	0
Other: PV	1	0	0	1
Total number of second neoplasms	58	15	26	17

2-CdA, cladribine; AA, alkylating agents-based regimens, DLBCL, diffuse large B-cell lymphoma; PV, polycythaemia vera.

and was constant across the time intervals after CLL diagnosis. Lung cancer accounted for almost one-fourth (209/840) of the second tumours, developing during all of the follow-up periods (median occurrence 33 months) and in all of the treatment groups.

In our group of patients we also observed a high frequency of lung cancers. Moreover, patients treated with 2-CdA developed this neoplasm significantly more frequently ($P<0.005$). It is possible that lung cancer development associated with the immunological impairment of CLL patients. An excess of lung cancer has also been reported in patients with non-Hodgkin's lymphoma and Hodgkin's disease—other lymphoid malignancies that are often accompanied by immune defects [5,40]. It should be noted that patients treated with 2-CdA have profound and prolonged immunosuppression. The number of CD4+ cells does not return to normal even after more than 3.5 years following treatment [18,19]. It is also possible that the excess of lung cancer following CLL could be partly due to shared risk factors. Brown and colleagues in their case–control study of CLL reported significant associations with tobacco use [41], although a dose-related relationship was not evident and several earlier studies have not confirmed this link [42,43]. In our group of lung cancer patients, the distribution of smokers and non-smokers was similar in all three groups of patients. So, the increased percentage of lung cancer in the 2-CdA-treated groups is more likely to be explained by profound immunological impairment after drug treatment.

We have not observed any myelodysplastic syndrome or acute myeloid leukaemia. This finding is in keeping with Keating and colleagues who did not observe any second myelodysplastic syndrome/acute myeloid leukaemia in 174 patients treated with FA as their initial therapy [10]. In addition, Cheson and colleagues found only one MDS among 111 second malignancies in 2014 CLL and HCL patients treated with purine analogues [20]. The retrospective analysis of 1374 CLL patients done by Robertson and colleagues did not provide evidence for a heightened risk of AML/MDS in CLL patients, despite treatment with known leukemogenic agents [44]. In contrast, Morrison and colleagues have found MDS/AML in 5 (3.5%) of 142 patients treated with FA plus Chl and in 1 (0.5%) of 188 treated with FA alone [25]. This finding rises the possibility that alkylator-purine analogue combination therapy may increase the risk of therapy-related myeloid malignancies. However, our observations did not confirm this finding in cases treated with 2-CdA.

We have not observed differences in the frequency of RS in all three groups of patients. We observed 1.0% of RS in 2-CdA group, 0.9% in AA and 0.6% in 2-CdA + AA group. This is a lower frequency than in the study of Keating and colleagues who found that 9 (5.2%) among 174 patients with CLL developed large cell lymphoma after FA treatment [10]. In addition, Cheson and colleagues have found 18 (3.0%) patients with non-Hodgkin's lymphoma among 595 patients with CLL treated with FA [20]. In contrast, a significantly higher frequency of Richters's syndrome in CLL patients treated with 2-CdA (12%) or fludarabine (13.8%) was observed by Fridrik and colleagues [45] and Pocock and colleagues [46].

We observed only 2 Hodgkin's disease cases among patients with secondary malignancies, 1 in a patient treated with 2-CdA alone and 1 in a patient treated with AA alone. It does not confirm the significant excess of Hodgkin's disease after fludarabine in CLL patients reported in the preliminary study of Flynn and colleagues [47].

In conclusion, 2-CdA does not seem to increase the risk of secondary malignancies in CLL patients, except for lung cancers. However, further studies are necessary to establish the true incidence of pulmonary cancer in CLL patients treated with 2-CdA.

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References

1. Manusow D, Weinerman BH. Subsequent neoplasia in chronic lymphocytic leukemia. *JAMA* 1975, **232**, 267–269.

2. Greene MH, Hoover RN, Fraumeni Jr JF. Subsequent cancer in patients with chronic lymphocytic leukaemia: a possible immunologic mechanism. *J Natl Cancer Inst* 1978, **61**, 337–340.
3. Dighiero G, Maloum K, Desablens B, *et al*. Chlorambucil in indolent chronic lymphocytic leukemia. *N Engl J Med* 1998, **338**, 1506–1514.
4. Mossafa H, Fourcade C, Pulic M, *et al*. Chronic lymphocytic leukemia associated with myelodysplastic syndrome and/or chronic myeloid leukemia: evidence for independent clonal chromosomal evolution. *Leuk Lymphoma* 2001, **41**, 337–341.
5. Travis LB, Curtis RE, Hankey BF, Fraumeni Jr JF. Second cancers in patients with chronic lymphocytic leukemia. *J Natl Cancer Inst* 1992, **84**, 1422–1427.
6. Richter MN. Generalized reticular cell sarcoma of lymph nodes associated with lymphocytic leukemia. *Am J Pathol* 1928, **4**, 285–291.
7. Seymour J, Campbell J, Richter's syndrome. In: Bruce D, Cheson BD, eds: *Chronic Lymphoid Leukemias*. New York, Basel, Marcel Dekker.
8. Giles FJ, O'Brien SM, Keating MJ. Chronic lymphocytic leukemia in (Richter's) transformation. *Semin Oncol* 1998, **25**, 117–125.
9. Tallman MS, Hakimian D. Purine nucleoside analogs: emerging roles in indolent lymphoproliferative disorders. *Blood* 1995, **86**, 2463–2474.
10. Keating MJ, O'Brien S, Kantarjian H, *et al*. Long term follow-up of patients with chronic lymphocytic leukemia treated with fludarabine as a single agent. *Blood* 1993, **81**, 2878–2884.
11. Piro LD, Carrera CJ, Beutler E, Carson DA. 2-Chlorodeoxyadenosine: an effective new agent for the treatment of chronic lymphocytic leukemia. *Blood* 1988, **72**, 1069–1073.
12. Robak T, Błasińska-Morawiec M, Błoński JZ, *et al*. The effect of 2-h infusion of 2-chlorodeoxyadenosine (cladribine) with prednisone in previously untreated B-cell chronic lymphocytic leukemia. *Eur J Cancer* 1997, **33**, 2347–2351.
13. Adkins JC, Peters DH, Markham A. Fludarabine. An update of its pharmacology and use in the treatment of haematological malignancies. *Drugs* 1997, **53**, 1005–1037.
14. Kay AC, Saven A, Carrera CJ, *et al*. 2-Chlorodeoxyadenosine treatment of low-grade lymphomas. *J Clin Oncol* 1992, **10**, 371–377.
15. 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.
16. Robertson LE, Chubb S, Meyn RE, *et al*. Induction of apoptotic cell death in chronic lymphocytic leukemia by 2-chloro-2′-deoxyadenosine and 9-β-D-arabinosyl-2-fluoroadenine. *Blood* 1993, **81**, 143–150.
17. Robak T, Kasznicki M. Alkylating agents and nucleoside analogues in the treatment of B-cell chronic lymphocytic leukemia. *Leukemia* 2002, **16**, 1015–1027.
18. Seymour JF, Kurzrock R, Freireich EJ, Estey EH. 2-Chlorodeoxyadenosine induces durable remissions and prolonged suppression of CD4+ lymphocyte counts in patients with hairy cell leukemia. *Blood* 1994, **83**, 2906–2911.
19. Cheson BD. Infections and immunosuppressive complications of purine analog therapy. *J Clin Oncol* 1995, **13**, 2431–2448.
20. Cheson BD, Vena DA, Barrett J, Freidlin B. Second malignancies as a consequence of nucleoside analog therapy for chronic lymphoid leukemias. *J Clin Oncol* 1999, **17**, 2454–2460.
21. Saven A, Burian C, Koziol JA, Piro LD. Long term follow-up of patients with hairy cell leukemia after cladribine treatment. *Blood* 1998, **92**, 1918–1926.
22. Kroft SH, Tallman MS, Shaw JM, Thangavelu M, Peterson LC. Myelodysplasia following treatment of chronic lymphocytic leukemia (CLL) with 2-chlorodeoxyadenosine (2-CdA). *Leukemia* 1997, **11**, 170.
23. Van Den Neste E, Louviaux I, Michaux JL, *et al*. Myelodysplastic syndrome with monosomy 5 and/or 7 following therapy with 2-chloro-2′-deoxyadenosine. *Br J Haematol* 1999, **105**, 268–270.
24. Laurencet FM, Zulian BG, Cabrol C, Betticher DC. Myelodysplastic syndrome with biclonal monosomy 7 and trisomy 8 after treatment with cladribine and involved field radiation therapy. *Cancer Genet Cytogenet* 1997, **99**, 85–89.
25. Morrison VA, Raik R, Peterson BL, *et al*. Therapy-related myeloid leukemia after treatment with fludarabine and chlorambucil: results of intergroup study. Cancer and Leukemia Group B 9011. *J Clin Oncol* 2002, **20**, 3878–3884.
26. Van Den Neste E, Delannoy A, Foremans W, Ferrant A, Michaux L. Second primary tumors and immune phenomena after fludarabine or 2-chloro-2′-deoxyadenosine treatment. *Leuk Lymphoma* 2001, **40**, 541–550.
27. Cheson BD, Bennett JM, Rai KR, *et al*. Guidelines for clinical protocols for chronic lymphocytic leukemia: recommendations of the National Cancer Institute-sponsored Working Group. *Am J Hematol* 1988, **29**, 152–163.
28. Cheson BD, Bennett JM, Grever M, *et al*. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood* 1996, **87**, 4990–4997.
29. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. *Blood* 1975, **46**, 219–234.
30. Robak T, Błoński JZ, Kasznicki M, *et al*. Cladribine with or without prednisone in the treatment of previously treated and untreated B-cell chronic lymphocytic leukaemia—updated results of the multicentre study of 378 patients. *Br J Haematol* 2000, **108**, 357–368.
31. Robak T, Błoński JZ, Kasznicki M, *et al*. Cladribine with prednisone versus chlorambucil with prednisone as first line therapy in chronic lymphocytic leukemia—report of prospective randomized multicenter trial. *Blood* 2000, **96**, 2723–2729.
32. Robak T, Błoński JZ, Kasznicki M, *et al*. Cladribine combined with cyclophosphamide and mitoxantrone as front-line therapy in chronic lymphocytic leukemia. *Leukemia* 2001, **15**, 1510–1516.
33. Harris NJ, Jaffe ES, Stein H, *et al*. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994, **84**, 1361–1392.
34. Robak T, Kasznicki M, Bartkowiak J, *et al*. Richter's syndrome following cladribine therapy for chronic lymphocytic leukaemia first manifested as patologic fracture of the femur. *Leuk Lymphoma* 2001, **42**, 789–796.
35. Robak T, Urbanska-Rys H, Strzelecka B, *et al*. Plasmablastic lymphoma in a patient with chronic lymphocytic leukaemia heavily pretreated with cladribine (2-CdA): an unusual variant of Richter's syndrome. *Eur J Haematol* 2001, **67**, 322–327.
36. Robak T, Szmigielska-Kaplon A, Smolewski P, *et al*. Hodgkin's type of Richter's syndrome in familial chronic lymphocytic leukaemia treated with cladribine and cyclophosphamide. *Leuk Lymphoma* 2003, **44**, 859–866.
37. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1993, **53**, 457–481.
38. Mauro FR, Foa R, Giannarelli D, *et al*. Clinical characteristics and outcome of young chronic lymphocytic leukemia patients: a single institution study of 204 cases. *Blood* 1999, **94**, 448–454.
39. Flinn BW, Kopecky KJ, Foucar MK, *et al*. Long-term follow-up of remission duration, mortality, and second malignancies in hairy cell leukemia patients treated with pentostatin. *Blood* 2000, **96**, 2981–2986.
40. Travis LB, Curtis RE, Boice JD Jr, Hankey BF, Fraumeni JF Jr. Second cancers following non-Hodgkin lymphoma. *Cancer* 1991, **67**, 2002–2009.

41. Brown LM, Gibson R, Blair A, *et al*. Smoking and risk of leukemia. *Am J Epidemiol* 1992, **135**, 763–768.
42. Brownson RC. Cigarette smoking and risk of leukemia. *J Clin Epidemiol* 1989, **42**, 1025–1028.
43. Kabat GC, Augustine A, Hebert JR. Smoking and adult leukemia: a case control study. *J Clin Epidemiol* 1988, **41**, 907–914.
44. Robertson LE, Estey E, Kantarjian H, *et al*. Therapy-related leukemia and myelodysplastic syndrome in chronic lymphocytic leukemia. *Leukemia* 1994, **8**, 2047–2051.
45. Fridrik MA, Jager G, Kienzer HR, *et al*. Efficacy and toxicity of 2-chlorodeoxyadenosins (Cladribine)—2 h infusion for 5 days—as first-line treatment for advanced low grade non-Hodgkin's lymphoma. *Eur J Cancer* 1998, **34**, 1560–1564.
46. Pocock C, Matutes E, Wotherspoon A, Cunningham D, Catovsky D. Fludarabine therapy may precipitate large cell transformation in chronic lymphocytic leukemia but not in follicular lymphoma. *Blood* 1998, **92**(Suppl. 1), 429a (abstr 1774).
47. Flynn JM, Howard RS, Byrd JC. Second malignancies in B-cell chronic lymphocytic leukemia (CLL): association with CLL but no additional impact with fludarabine or alkylator treatment. *Blood* 1999, **94**(Suppl. 1), 298b (abstr 4553).