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# IN VITRO SENSITIVITY OF CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS TO FLUDARABINE, 2-CHLORODEOXYADENOSINE AND CHLORAMBUCIL: CORRELATION WITH CLINICO-HEMATOLOGICAL AND IMMUNOPHENOTYPIC FEATURES

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**ABSTRACT**

***Background.*** **Chlorambucil (CLB), 2-chlorodeoxyadenosine (2-CDA) and fludarabine (FAMP) are among the most widely used drugs in chronic lymphocytic leukemia (CLL). Therefore we evaluated *in vitro* sensitivity to these drugs and cross-resistance of purine analogs. In addition, we correlated the *in vitro* data with the main clinico-hematological variables and surface markers.**

***Patients and Methods.*** **Eighty CLL samples obtained from 63 untreated and 17 treated CLL patients were tested *in vitro* with the MTT assay. Lethal dose (LD)50 values were calculated to determine sensitivity to CLB, 2-CDA and FAMP.**

***Results.*** **Samples were clustered either for a one-log increase of LD50 values or for LD50 threshold values of 3 μM for FAMP, 0.3 μM for 2-CDA and 7 μM for CLB, which correspond to the therapeutically achievable plasmatic levels of these drugs. A higher number of samples sensitive to 2-CDA were identified by the first approach; with the second method the relative order of sensitivity was FAMP>2-CDA>CLB. Concerning 2-CDA and FAMP cross-resistance, out of 61 samples resistant to 2-CDA, 29.5% were sensitive to FAMP. Conversely, 13.9% out of 43 samples resistant to FAMP were sensitive to 2-CDA. No correlation was found between the main clinico-hematological features and the LD50 values of each drug either considering the whole series or only the untreated cases. *In vitro* drug sensitivity was also evaluated during the steady-state of the disease and at disease progression in six untreated cases. We observed a mean increase in the LD50 values of about 13, 38 and 22 times for CLB, FAMP and 2-CDA, respectively. Among the treated cases, the LD50 values of both purine analogs and CLB correlated with bone marrow histology. CLL cells expressing CD14, CD11c, CD11b, and FMC7 were more resistant *in vitro* to purine analogs but not to CLB.**

***Conclusions.*** **This study suggests that i) the purine analogs exert a greater cytotoxic effect on CLL cells; ii) 2-CDA and FAMP are not cross-resistant *in vitro* in a percentage of CLL samples, iii) a possible change in LD50 values may be related to modification of the disease status, and iv) the expression of certain surface markers, which are CLL-unrestricted, identifies samples with higher *in vitro* resistance to purine analogs.**

*Key words: CLL, 2-chlorodeoxyadenosine, fludarabine, chlorambucil, chemosensitivity*

Chronic lymphocytic leukemia (CLL) is a disorder characterized by a B-lymphocyte expansion.[1-3] Conventional treatment with alkylating agents is largely palliative, while higher doses of chlorambucil (CLB) and the use of anthracycline-containing regimens have provided substantial improvement in the response rate.[1-5] The cytotoxic potential of the purine analogs fludarabine (FAMP) and 2-chlorodeoxyadenosine (2-CDA) has also led to complete remissions with minimal or moderate residual disease.[1-3, 5-9]

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Response to therapy depends upon several factors, both clinico-hematological and biological. In addition to the role of disease stage, whatever the method used, and to bone marrow histology, a clinical impact has been suggested for the expression of some surface markers in CLL. It is worth noting that certain markers, which are CLL-unrestricted, seem to identify more aggressive clinical forms of the disease.[10-12] In addition, CD14 expression has been shown to be associated with the production of IL-1,[13] a factor which notably influences the autocrine loop of neoplastic B-cell proliferation in CLL.

In this paper we present data on the *in vitro* chemosensitivity of peripheral cells to CLB, FAMP and 2-CDA in 80 CLL cases using a method based on the ability of living cells to reduce a yellow tetrazolium salt (MTT) to a purple formazan product.[14] This method has been successfully employed as a chemosensitivity test for G0 slow-dividing B-lymphocytes of CLL.[15-19] The main aims of this study were to determine the cytotoxic effect induced by CLB, FAMP and 2-CDA and to assess the *in vitro* cross-resistance of the two purine analogs, although both drugs have similar mechanisms of action. In addition, we correlated the MTT assay results with both clinico-hematological data and the expression of CLL-unrestricted surface antigens that recognize more aggressive clinical forms of CLL,[10-12] in order to detect a possible difference in drug activity in specific CLL subsets.

## *Materials and Methods*

### *Patient population and cell separation*

Eighty CLL samples were studied after obtaining informed consent from the respective patients. Sixty-three were untreated and 17 were treated. The diagnosis was based on clinical, morphological and phenotypic findings.[20] Fifty-four were assigned to Binet stage A, 7 to B and 14 to C. Thirty-three cases were Rai stage 0, 7 were in stage I, 24 in II, 5 in III and 11 in IV. Forty-nine patients showed long doubling time (DT>12 months).[21] Bone marrow histology presented a non-diffuse pattern in 47 patients and diffuse bone marrow involvement in 14 cases. Bone marrow biopsy was not performed in 19 patients. At the time of the study the mean total tumor mass (TTM) score[20] for both untreated and treated patients was 7.2 (median TTM 6.0, range 2.3-28.4). The mean lymphocyte count was $47\times10^9$/L (median $24\times10^9$/L). Among the treated patients, therapy consisted of CLB in 5 cases, CLB plus α-interferon in 3, Binet's modified CHOP in 3, and different drug combinations without anthracyclines in the 6 remaining cases. Median time from the start of treatment to the *in vitro* study was 17 months (range 1-71 months), while median follow-up was 28 months (range 1-202 months). At the time of the study, among the treated cases, 10 patients were in relapse and 7 were resistant.

Mononuclear cells were separated from fresh venous blood samples by Ficoll-Hypaque gradient centrifugation (J-Bio, Les Ulis Cedex, France). After isolation, cells were washed twice and resuspended in RPMI 1640 medium (Gibco, BRL, Life Technologies LTD, Paisley, Scotland, UK) supplemented with 15% heat-inactivated FCS (Gibco), 100 IU/mL penicillin, 100 μg/mL streptomycin (Imperial, UK), 0.125 μg/mL fungizone (Squibb Inc., New York, NY) and 2 mmol/L l-glutamine (J-Bio).

### *Immunological analysis*

A total of $0.5\times10^6$ PBMC were labeled with either FITC-conjugated CD5, CD14, CD19, CD25 (Ylem, Italy) monoclonal antibodies (MoAb), or with purified CD11b (Ylem), CD23 and CD11c (Becton Dickinson, Mountain View, CA, USA), FMC7 (Techno Genetics, Italy) Moab and subsequently with a FITC-labeled secondary reagent, F(ab')2 fragments of a rabbit anti-mouse Ig (Ylem). From 5,000 to 10,000 cells were examined for fluorescence with an EPICS Profile II flow cytometer (Coulter Electronics, Inc., Hialeah, FL, USA). The lymphocytic population was gated on a two-parameter forward angle versus 90° light scatter histogram. The percent of cells expressing the surface antigen of interest was determined by establishing a cursor position channel, using as negative control an irrelevant FITC-conjugated mouse IgG subclass (Becton Dickinson) or a FITC-labeled secondary reagent. All samples

studied were CD5-, CD19- and CD23-positive. Leukemic cell percentage ranged from 80% to 95%, as assessed by the anti-CD19 MoAb.

*Drug preparation*

CLB (Sigma, St. Louis, MO) was dissolved in absolute ethanol, which did not affect cell viability at the final concentration (data not shown). 2-CDA was kindly provided by Dr. D. Lutz on behalf of the International Society for Chemo and Immunotherapy (IGCI, Austria). Commercially available FAMP (Fludara, Berlex, Alameda, CA, USA) was diluted in medium. Stock solutions of drugs were diluted in RPMI 1640 and aliquoted at 100%, the maximal final concentration used, and stored at –80°C.

*Cytotoxicity assay*

To estimate cell viability, the MTT assay was performed as previously described.[16,18] Briefly, 100 µL of $10\times10^6$/mL CLL cell suspensions were plated in 96 round-bottom wells (Nunc, Roskilde, Denmark) with several concentrations of CLB, FAMP or 2-CDA diluted in a volume of 25 µL. Four replicate wells were used as control and for each drug dose. The plates were then incubated in a humidified atmosphere for 4 days at 37°C in a 5% $CO_2$ incubator. Fifty µL of 5 mg/mL MTT (Sigma) in PBS were added to every well and the plates were re-incubated for an additional 4 hr. Plates were then centrifuged at 2000 rpm for 10' and 150 µL of the supernatant were carefully discarded from every well by vacuum aspiration with a hand-made device. Fifty µL of 100% dimethylsulfoxid (DMSO) (Calbiochem, La Jolla, CA, USA) were added to each well to solubilize the MTT formazan. The plates were kept in the incubator for 1 h, then spectrophotometric adsorbance at 540 nm was determined using a multiwell scanning spectrophotometer (EL 310, Biotek Instruments). The dose-dependent cytotoxic effect of the drugs was studied by culturing CLL cells with 0.00437-437 µM 2-CDA, 0.027-270 µM FAMP and 0.0328-328 µM CLB for 4 days. Drug dose-response curves were drawn and lethal dose (LD)50 values (i.e. the drug dose able to kill 50% of cells) were determined for each drug with previously employed software.[19]

*Statistical analysis*

Descriptive statistics, the Pearson correlation coefficient, the Wilcoxon rank-sum test and Fisher's exact test (2 tails) were calculated with the SAS/STAT software package, release 6.06 of SAS Institute Inc., 1993. The clinico-hematological variables considered for the statistical analysis were Rai and Binet stage systems, TTM score, DT, bone marrow histology, IgG and LDH serum levels at the time of the study; among the surface antigens, the percentage and the absolute number of CD14, CD11c, CD11b and FMC7 entered the study.

## *Results*

*MTT assay results and definition of drug sensitivity*

Drug dose-response curves were drawn and lethal dose 50 values were calculated for each drug. Table 1 shows mean LD50 values for 2-CDA, FAMP and CLB in the 80 CLL samples. Based on the LD50 median values expressed in µM, 2-CDA was 2.9 and 34.2 times more potent than FAMP and CLB, respectively. 2-CDA and CLB LD50 mean values were similar in the untreated and the treated group, while a trend toward greater *in vitro* resistance to FAMP was detected in treated cases.

Since a wide range of individual dose-response curves was seen when testing all three drugs, samples were ranked on the basis of a one-log increase in LD50 values. Thus six levels of drug sensitivity were generated and the

Table 1. LD50 values resulting from the drug dose-response curves drawn for 2-CDA, FAMP and CLB in 80 samples, 63 from untreated and 17 from treated CLL cases. The data are expressed as mean value ± s.e. (median).

| | LD50 (µM) | | |
|---|---|---|---|
| | | samples obtained from cases | |
| *Drugs* | *all samples* | *untreated* | *treated* |
| 2-CDA | 39.9±12.1 (1.58) | 32.9±12.2 | 35.2±34.8 |
| FAMP | 54.6±12.5 (4.59) | 40.1±11.3 | 106.8±38.9 |
| CLB | 104.2±12.7 (54.1) | 105.1±13.8 | 101.2±31.2 |

*In some cases the LD50 values were higher than the highest concentration of the drug tested or lower than the lowest concentration tested. In calculating the mean, the highest and lowest concentrations are taken as the LD50 values for these samples.*

Table 2. Six levels of sensitivity to 2-CDA, FAMP and CLB generated in 80 CLL samples obtained on the basis of a one-log increase in LD50 values. LD50 values are expressed as μM.

| | 2-CDA | | FAMP | | CLB | |
|---|---|---|---|---|---|---|
| Levels of sensitivity | No. of samples | LD50 mean±sd(median) | No. of samples | LD50 mean±sd(median) | No. of samples | LD50 mean±sd(median) |
| 1 | 9 | 0.004±0.0005 (0.004) | 1 | 0.001 | – | – |
| 2 | 5 | 0.0414±0.0224 (0.038) | 7 | 0.038±0.006 (0.035) | 1 | 0.033 |
| 3 | 19 | 0.552±0.247 (0.476) | 7 | 0.436±0.080 (0.519) | 2 | 0.367 |
| 4 | 20 | 3.315±2.867 (2.074) | 34 | 3.501±0.290 (3.569) | 12 | 5.753±0.860 (7.331) |
| 5 | 17 | 27.31±5.861 (19.74) | 16 | 30.29±6.839 (17.74) | 36 | 40.06±5.100 (25.53) |
| 6 | 8 | 321.9±52.17 (410.2) | 13 | 281.2±27.90 (350.6) | 29 | 235.5±15.16 (209.3) |

results are reported according to increasing sensitivity in Table 2. Considering the samples falling into the first four levels (beyond which resistant samples were likely to be found), 53, 49 and 15 samples were defined on a molar basis as being sensitive to 2-CDA, FAMP and CLB, respectively. We also approached the definition of sensitivity in another way; we considered the results obtained at the drug concentration corresponding to the therapeutically achievable plasma level, namely 0.3, 3 and 7 μM for 2-CDA,[22] FAMP[23] and CLB,[24] respectively (Table 3). Sensitivity to 2-CDA was demonstrated in 14/61 (22.9%) samples from the untreated cases and in 3/17 (17.6%) from the treated ones. FAMP showed the most marked cytotoxic effect in samples obtained from both untreated (40.9%) and treated (23.5%) cases. Finally, 6/63 and 3/17 samples from the untreated and treated cases, respectively, were sensitive to CLB.

Table 3. Number of sensitive CLL samples detected on the basis of LD50 threshold values of 0.3, 3 and 7 μM, which correspond to the plasma levels of the drugs achievable after standard therapy with 2-CDA, FAMP and CLB, respectively.

| | | samples obtained from cases | |
|---|---|---|---|
| *Drugs* | *all samples* | *untreated* | *treated* |
| | LD50 (μM) cutoff values | sensitive/total (%) | sensitive/total (%) |
| 2-CDA | 0.3 | 14/61 (22.9) | 3/17 (17.6) |
| FAMP | 3.0 | 25/61 (40.9) | 4/17 (23.5) |
| CLB | 7.0 | 6/63 (9.5) | 3/17 (17.6) |

Furthermore, we studied the LD50 values in the steady-state of the disease and at disease progression in some untreated cases in order to evaluate whether changes in the LD50 value were related to the need for treatment. Since the median follow-up from the time of the first MTT assay in the untreated cases was too short for an extended longitudinal evaluation, we could investigate only six cases. We observed a mean increase in LD50 values of 12.7 (range 0.95-48), 37.29 (range 1.05-98) and 22.54 (range 1.85-76) times for CLB, FAMP and 2-CDA, respectively (data not shown).

*Cross-resistance between 2-CDA and FAMP*

Most of the CLL samples showing sensitivity to FAMP were generally sensitive to 2-CDA (r=0.30068, P=0.0075 by Pearson analysis; data not shown). However, detailed analysis of the data showed that 18/61 (29.5%) samples resistant to 2-CDA were sensitive to FAMP (Table 4); conversely, only 6 out of the 43 (13.9%) samples resistant to FAMP responded to 2-CDA.

| | | FAMP | | |
|---|---|---|---|---|
| | No. of samples | No. of samples | | Fisher's exact test |
| | | Resistant | Sensitive | (2-Tail) *P*= |
| 2-CDA | Resistant | | | |
| | 61 | 43 | 18 | 0.011 |
| | Sensitive | | | |
| | 17 | 6 | 11 | |

Table 4. Cross-table between 2-CDA and FAMP. Sensitivity was defined on the basis of LD50 cutoff values corresponding to 0.3 and 3 μM for 2-CDA and FAMP, respectively.

*Correlation of MTT assay data with clinico-hematological and immunophenotypic variables*

No correlation was found between the LD50 values of each drug and the Rai and Binet staging systems, or the DT and TTM scores, either when the whole series was analyzed or when samples from the untreated and treated cases were considered separately. In addition, serum LDH levels correlated positively with 2-CDA (r=0.33107, p=0.0136) and CLB (r=0.27666, p=0.0409) for the whole series, but only 2-CDA maintained a statistically significant correlation in the treated cases (r=0.8644, p=0.0001). The pattern of bone marrow histology, diffuse or non-diffuse, seen at diagnosis was significantly correlated with the higher and the lower LD50 values, respectively, for all drugs exclusively in the treated group (data not shown).

The correlation between LD50 values and the expression of some CLL-unrestricted surface markers, namely CD11b, CD11c, CD14, and FMC7, is shown in Table 5. 2-CDA LD50 values were significantly correlated with the percentage and absolute count of CD14 in both the whole series and the treated cases; there was also a correlation with the absolute count of FMC7 and CD11b in the whole series, and with CD11c in the untreated group. With regard to FAMP LD50 values, we found a correlation with the percentage and absolute count of CD14 in the whole series, and only with the percentage of CD14 in the treated patients; in the untreated group, the LD50 values were significantly related to CD11c percentage. Finally, CLB LD50 values did not correlate with any of these markers except for CD14 expressed as percentage in the treated cases.

The distribution of marker expression was investigated by the Wilcoxon rank-sum test in both drug-sensitive and resistant samples (data not shown). We found a statistically significant difference between 2-CDA-sensitive and resistant samples with respect to FMC7 (p=0.05) and CD11c (p=0.0430) percentage, and between FAMP subgroups with respect to FMC7 absolute count (p=0.0289).

## *Discussion*

Attention in the field of hematological malignancies has focused on the availability of an *in vitro* test for accurate, rapid and economical measurement of resistance and sensitivity to chemotherapeutic agents.[14] The MTT assay was used in this study to evaluate the *in vitro* cytotoxic effect of 2-CDA, FAMP and CLB. Examined on a molar basis, our overall MTT assay results indicate that a greater number of samples exhibit sensitivity to 2-CDA than to FAMP, while very low *in vitro* sensitivity to CLB was found. In addition, we utilized a second approach to determine drug sensitivity; we considered the therapeutically achievable plasma level of each drug, which corresponds to 0.3, 3 and 7 μM for 2-CDA,[22] FAMP,[23] and CLB,[24] respectively. With this method FAMP exhibited the highest activity. We recently performed an *in vitro*/*in vivo* study in a relatively small cohort of CLL patients and demonstrated that when *in vitro* drug sensitivity is evaluated using these cutoffs it is possible to predict complete clinical response.[19] Another more extended, prospective study focusing on the possible *in vivo* correspondence of *in vitro* MTT results in CLL is in

Table 5. Correlation between CLL-unrestricted surface markers and LD50 values for 2-CDA, FAMP and CLB. Statistical analysis was carried out using Pearson's coefficient.

| | Whole series | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CD14 (%) | CD14 (AbC) | FMC7 (%) | FMC7 (AbC) | CD11c (%) | CD11c (AbC) | CD11b (%) | CD11b (AbC) |
| 2-CDA | *r*= 0.40014 *P*= 0.0036 n= 51 | *r*= 0.60830 *P*= 0.0001 n= 51 | NS | *r*= 0.32950 *P*= 0.0405 n= 39 | NS | NS | NS | *r*= 0.32101 *P*= 0.0316 n= 45 |
| FAMP | *r*= 0.38781 *P*= 0.0049 n= 51 | *r*= 0.42690 *P*= 0.0020 n= 51 | NS | NS | NS | NS | NS | NS |
| CLB | NS | NS | NS | NS | NS | NS | NS | NS |
| | Samples from untreated cases | | | | | | | |
| 2-CDA | NS | NS | NS | NS | *r*= 0.55508 *P*= 0.0008 n= 33 | NS | NS | NS |
| FAMP | NS | NS | NS | NS | *r*= 0.48126 *P*= 0.0046 n= 33 | NS | NS | NS |
| CLB | NS | NS | NS | NS | NS | NS | NS | NS |
| | Samples from treated cases | | | | | | | |
| 2-CDA | *r*= 0.82138 *P*= 0.0011 n= 12 | *r*= 0.7112 *P*= 0.0036 n= 12 | NS | NS | NS | NS | NS | NS |
| FAMP | *r*= 0.79553 *P*= 0.0020 n= 12 | NS | NS | NS | NS | NS | NS | NS |
| CLB | *r*= 0.67201 *P*= 0.0167 n= 12 | NS | NS | NS | NS | NS | NS | NS |

*NS: not significant; AbC: absolute count.*



progress in our institution. However, the reported efficacy of FAMP has been demonstrated in previously treated CLL patients by complete clinical response rates up to 29%.[1-3] Previously untreated cases achieved 33% complete responses and 39% complete responses with persistent lymphoid nodules in the bone marrow.[1-3] These results mirror, at least in part, our *in vitro* data. Moreover, 2-CDA is very effective in CLL and although initial reports showed a complete response rate of 4%,[8] possibly due to the high incidence of Binet's stage C patients, more recently Juliusson reported 39% complete responses in previously treated CLL patients,[9] while among untreated patients a 25% complete remission rate was reported by Saven.[25] Again, these results are in line with our *in vitro* data that predict 22.9% and 17.6% complete responses for samples obtained from untreated and treated patients, respectively. Finally, although the present *in vitro* results confirm the reportedly low complete remission rate after CLB therapy,[1-3,5] we should take into consideration that this finding is probably due to the low dose commonly employed. In fact, significantly higher doses of CLB may induce better clinical responses.[4,26] It would be of interest therefore to re-evaluate the MTT assay results using as threshold value the plasma concentration of the drug determined by the new pharmacokinetic study with high-dose CLB.

Although 2-CDA and FAMP are similar in structure and mechanism of action, it is still unclear whether there is cross-resistance between the two purine analogs, leaving the question of whether they can be used interchangeably unanswered. In the present study, samples sensitive to FAMP generally responded *in vitro* to 2-CDA. However, a preferential sensitivity to FAMP and 2-CDA was demonstrated in 29.5% and 6% of samples, respectively. In a recent report, 10% of samples showed markedly

different *in vitro* sensitivity to 2-CDA and FAMP.[27] On the other hand, whether 2-CDA may be clinically useful in patients resistant to FAMP is a matter of controversy. Juliusson reported that four consecutive CLL patients who were refractory to FAMP responded to 2-CDA.[28] However, O'Brien observed that only 7% of patients (2/28) refractory to FAMP showed a partial response to 2-CDA, and no patient achieved a complete remission.[29] Nevertheless, the possible lack of cross-resistance between the two purine analogs *in vitro* and *in vivo* needs to be investigated further in CLL.

The LD50 values were also assessed in the steady-state of the disease and at disease progression in six untreated cases. We observed a mean increase in the LD50 values of all 3 drugs, thus suggesting a possible change in the chemosensitivity pattern related to the expansion of the disease. However, given the small number of cases investigated, this conclusion requires confirmation in a larger series.

In the second part of this study, a correlation analysis was employed to detect possible associations between *in vitro* drug sensitivity and main hematological parameters. We found no significant correlation between LD50 values and the Rai and Binet staging systems, or the DT and TTM scores, while among treated patients a significant association was documented between the diffuse pattern of bone marrow infiltration and elevated LD50 values. These findings are not surprising considering that disease status is a function of spreading and tumor mass, which are also variables for the achievement of complete remission, in addition to the intrinsic drug resistance of CLL cells.

Finally, the finding that FMC7$^+$ and myelomonocytic antigen-positive CLL likely represent distinct, more aggressive clinical forms of the disease[10-12] and that CD14 expression is associated with CLL B-cells production of IL-1,[13] a factor which influences the self-maintaining loop of CLL B-cells, prompted us to correlate the expression of CLL-unrestricted surface markers CD14, FMC7, CD11b and CD11c with the *in vitro* MTT assay results in order to identify a possibly different pattern of *in vitro* sensitivity of unusual CLL forms. Our findings seem to be in agreement with the above mentioned clinical and biological features. In fact, the results obtained in the whole series suggest that CLL lymphocytes highly expressing CD14, CD11b and FMC7 are more resistant to purine analogs *in vitro*, while no correlation was found with CLB.

In conclusion, we demonstrated that the reportedly high activity of purine analogs in the majority of CLL patients is supported by the *in vitro* chemosensitivity of CLL cells to these drugs, while further data are needed concerning CLB at higher doses than those commonly used.

The ongoing prospective *in vitro*/*in vivo* study on the correspondence between MTT results and response rate to these drugs will determine the predictive value of this assay.



## *References*


1. O'Brien S, Del Giglio A, Keating M. Advances in the biology and treatment of B-cell chronic lymphocytic leukemia. Blood 1995; 85:307-18.
2. Faguet G. Chronic lymphocytic leukemia: an updated review. J Clin Oncol 1994; 12:1974-90.
3. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N Engl J Med 1995; 19:1052-7.
4. Jaksic B, Brugiatelli M. High dose continuous chlorambucil vs. intermittent chlorambucil plus prednisone for treatment of B-CLL - IGCI CLL-01 trial. Nouv Rev Fr Hematol 1988; 30: 437-42.
5. Montserrat E, Rozman C. Chronic lymphocytic leukemia treatment. Blood Rev 1993; 7:164-75.
6. Keating MJ, Kantarjian HM, Talpaz M, et al. Fludarabine a new agent with major activity against chronic lymphocytic leukemia. Blood 1989; 74:19.
7. O'Brien S, Kantarjan H, Beran M, et al. Results of fludarabine and prednisone therapy in 264 patients with chronic lymphocytic leukemia with multivariate analysis-derived prognostic model for response to treatment. Blood 1993; 82:1695-704.
8. Piro LD, Carrera CJ, Beutler E, Carson DA. 2-chlorodeoxyadenosine: an effective new agent for the treatment of chronic lymphocytic leukemia. Blood 1988; 72:1069-73.
9. Juliusson G, Liliemark J. High complete remission rate from 2-chloro-2'deoxyadenosine in previously treated patients with B-cell chronic lymphocytic leukemia: response predicted by rapid decrease of blood lymphocyte count. J Clin Oncol 1993; 11: 679-89.
10. Catovsky D, Cherchi M, Brooks D, Bredley J, Zola H. Heterogeneity of B cell leukemia demonstrated by the monoclonal antibody FMC7. Blood 1981; 58:406-8.
11. Pinto A, Zagonel V, Carbone A, et al. Expression of myelomonocytic antigens on chronic lymphocytic leukemia B cells identifies a subset of patients with a "variant" CLL phenotype and different biological and clinical features. (abstr). Proceedings of American Society of Clinical Oncology 1989; pp. 204 .
12. Wormsley SB, Baird SM, Gadol N, Rai KR, Sobol RE. Characteristics of CD11c$^+$ CD5$^+$ chronic B-cell leukemias and the

identification of novel peripheral blood B-cell subsets with chronic lymphoid leukemia immunophenotypes. Blood 1990; 76:123-30.
13. Morabito F, Prasthofer EF, Dunlap NE, Grossi CE, Tilden AB. Expression of myelomonocytic antigens on chronic lymphocytic leukemia B cells correlates with their ability to produce interleukin 1. Blood 1987; 70:1750-7.
14. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J Immunol Meth 1983; 65:55-63.
15. Hanson JA, Bentley DP, Bean EA, Sarah R, Nute SR, Moore JL. In vitro chemosensitivity testing in chronic lymphocytic leukaemia patients. Leuk Res 1991; 15:565-9.
16. Morabito F, Messina G, Oliva B, et al. In vitro chemosensitivity of chronic lymphocytic leukemia B-cells to multidrug regimen (CEOP) compounds using the MTT colorimetric assay. Haematologica 1993; 78:213-8.
17. Zinzani PL, Tosi P, Visani G, et al. Apoptosis induction with three nucleoside analogs on freshly isolated B-chronic lymphocytic leukemia cells. Am J Hematol 1994; 47:301-6.
18. Silber R, Degar B, Costin D, et al. Chemosensitivity of lymphocytes from patients with B-cell chronic lymphocytic leukemia to chlorambucil, fludarabine, and camptothecin analogs. Blood 1994; 84: 3440-60.
19. Morabito F, Callea I, Rodinò A, et al. Modulation of purine analogs- and chlorambucil-induced cytotoxicity by alpha-interferon and interleukin-2 in chronic lymphocytic leukemia. Leukemia 1995; 9:1425-33.
20. International Workshop on CLL. Chronic lymphocytic leukemia: recommendation for diagnosis, staging, and response evaluation. Ann Intern Med 1989; 110:236-8.
21. Jaksic B, Vitale B. Total Tumor Mass score (TTM): a new parameter in chronic lymphocytic leukaemia. Br J Haematol 1981; 49:401-13.
22. Liliemark J, Pettersson B, Juliusson G. The relationship between plasma 2-chlorodeoxyadenosine (CdA) and cellular CdA nucleotides (CdAN) after intermittent and continuous I.V. in patients with chronic lymphocytic leukemia. (abstr.) Blood 1991; 78 (suppl 1):33a.
23. Danhauser L, Plunkett W, Keating M, Cabanillas F. 9-beta-D-arabinofuranosyl-2-fluoroadenine 5'-monophosphate pharmacokinetics in plasma and tumor cells with relapsed leukemia and lymphoma. Cancer Chemother Pharmacol 1986; 18:142-5.
24. Adair CG, Bridges JM, Desai ZR. Can food affect the biovailability of chlorambucil in patients with haematological malignancies? Cancer Chemother Pharmacol 1986; 17:99-102.
25. Saven A, Lemon RH, Kosky M, Beutler E, Piro LD. 2-chlorodeoxyadenosine (2-CDA) activity in patients with untreated chronic lymphocytic leukemia. J Clin Oncol 1995; 13:570-4.
26. Brugiatelli M, Jaksic B, Planinc-Peraica A, et al. Treatment of chronic lymphocytic leukemia in early and stable phase of the disease: long-term results of a randomized trial. Eur J Haematol 1995; 55:158-63.
27. Begleiter A, Verburg L, Ashique A, et al. Comparison of antitumor activities of 2-chlorodeoxyadenosine and 9-β-arabinosyl-2-fluoroadenine in chronic lymphocytic leukemia and marrow cells in vitro. Leukemia 1995; 9:1875-81.
28. Juliusson G, Elmhorn-Rosemberg A, Liliemark J. Response to 2-chlorodeoxyadenosine in patients with B-cell chronic lymphocytic leukemia resistant to fludarabine. N Engl J Med 1992; 327:1056-61.
29. O'Brian S, Kantaryian H, Estey E, et al. Lack of effect of 2-chlorodeoxyadenosine therapy in patients with chronic lymphocytic leukemia refractory to fludarabine therapy. N Engl J Med 1994; 330:319-22.