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THE IN VITRO CYTOTOXIC EFFECT OF MITOXANTRONE IN COMBINATION WITH FLUDARABINE OR PENTOSTATIN IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

FORTUNATO MORABITO,* IDA CALLEA,* GIUSEPPE CONSOLE,* CATERINA STELITANO,° GIUSEPPINA SCULLI,# MONICA FILANGERI,* BIANCA OLIVA,* CATERINA MUSOLINO,# PASQUALE IACOPINO,* MAURA BRUGIATELLI@

**Centro Trapianti di Midollo Osseo e Terapia Sovramassimale Emato-Oncologica A. Neri, °Divisione di Ematologia, @Centro per le Microcitemie e Biologia Molecolare, Dipartimento di Emato-Oncologia, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria; #Istituto di Medicina Interna, Università di Messina; Italy*

ABSTRACT

Background and Objective. Clinical studies indicate that combination chemotherapy with mitoxantrone (Mitox) and a purine analog can improve the response rate in indolent lymphoproliferative disorders. We explored the *in vitro* Mitox- fludarabine (FAMP)- and pentostatin (Pento)-induced cytotoxicity and their interactions in CLL.

Methods. The peripheral lymphocytes of 24 CLL patients were tested at different drug concentrations, with Mitox, FAMP or their combinations in 22 cases, and with Mitox, Pento or their combinations in 20cases, 18 of which were the same from the FAMP group. The MTT assay was chosen for the drug-induced cell cytotoxicity and flow cytometry analysis of the DNA hypodiploid peak for the study of the apoptotic process. Drug interactions were calculated in the MTT assay according to both *multiplicative* and *maximum* models.

Results. According to the lethal dose (LD) 50 values, when the three drugs were tested alone, 11 out of 22 and 8 out of 20 samples were sensitive to Mitox in the FAMP and Pento groups, respectively; on the other hand, 2 out of 22 and 0 out of 20 samples appeared sensitive to FAMP or Pento alone, respectively. Analyzing the MTT assay data with the *multiplicative* and *maximum* model, the combinations of Mitox+FAMP and Mitox+Pento at different drug concentrations were synergistic in 28.2% and 39.3%, respectively. At leukemic cell survival ≤ 50%, 11.7% and 11.1% of all combinations were synergistic in the Pento and FAMP group, respectively. The number of synergistic interactions at a therapeutically achievable plasma-drug concentration was an inverse function of the Mitox concentration. In the FAMP group, a direct correlation was found between the LD50 values of both FAMP and Mitox and the number of synergistic interactions, while the Pearson correlation coefficient was not significant in the Pento group. Finally, as measured by the DNA hypodiploid peak, Mitox (0.25 µg/mL) plus Pento (0.16 µg/mL) showed a significantly enhanced apoptosis in comparison to each single drug, while Mitox failed to demonstrate an additive effect with FAMP (1 µg/ml).

Interpretation and Conclusions. This experience demonstrates the extent of the *in vitro* synergism of Mitox with FAMP and Pento in inducing cell cytotoxicity; it also shows an adjunctive apoptotic effect for the Mitox-Pento association only.

Key words: chronic lymphocytic leukemia, mitoxantrone, fludarabine, pentostatin, MTT assay, apoptosis

Mitoxantrone (Mitox) is an anthracenedione which exerts its anti-tumor activity by interacting with DNA and causing DNA strand breakage.[1] Mitox is active apart from cell-cycle, inhibiting both resting and dividing neoplastic cells and thus resulting effective in both acute leukemia[2] and lymphoma.[3] In particular, a high response rate was reported in low-grade lymphoma either as a single agent[3] or in combination with purine analogs.[4,5]

B-cell chronic lymphocytic leukemia (CLL) is a clonal proliferation of malignant B-lymphocytes that is considered incurable with conventional chemotherapy and is generally palliated with chlorambucil.[6,7] Other conventional therapeutic options consist of anthracycline-containing regimens and, more recently, purine analogs and high-dose chlorambucil which have resulted in a higher remission rate.[7-10]

Apoptosis has increasing significance considering that it represents an important and ubiquitous mode of action by anti-tumor drugs.[11] CLL cells constitute resistance genes[12] and show a poor propensity to apoptosis. A combined use of Mitox

Correspondence: F. Morabito, MD, Centro Trapianti di Midollo Osseo e Terapia Sovramassimale Emato-Oncologica, Dipartimento di Emato-Oncologia, Azienda Ospedaliera Bianchi-Melacrino-Morelli, 89100 Reggio Calabria, Italy. Tel. international +39.965.27191. Fax: international +39.965.25082. E-mail: sp00404@relay.it.net
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with either fludarabine (FAMP) or pentostatin (Pento) might be effective in CLL patients. This prompted us to examine CLL cell chemosensitivity to *in vitro* exposure to Mitox either alone or in combination with FAMP or Pento. The non-clonogenic 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay, repeatedly used for the *in vitro* study of a single-drug or drug-combination chemosensitivity of CLL cells,[13-17] was employed. We also examined whether the anthracenedione-induced apoptotic profile, evaluated by flow-cytometry analysis, might be modified by the addition of either FAMP or Pento. Our results indicate that Mitox may synergize *in vitro* with FAMP and Pento, although in a low percentage of cases, while additive or antagonistic effects have been reported more frequently.

Materials and Methods

Patients and cell separation

Samples were taken from 24 previously untreated (14 cases) and previously treated CLL patients who had not undergone therapy from at least one month. Three patients were in Rai stage 0, 7 were in stage I, 8 were in stage II, 2 were in stage III and 4 were in stage IV. The diagnosis was based on the clinical picture and on the typical morphological and immunological findings.[18] Peripheral blood mononuclear cells were separated by centrifugation onto a Ficoll-Hypaque gradient (Lymphoprep, Nycommed AS, Oslo, Norway). After isolation, cells were washed twice and re-suspended in RPMI 1640 medium (Flow Laboratories, Opera, Italy) supplemented with 15% heat-inactivated FCS, 100 IU/mL penicillin, 100 µg/mL streptomycin, 0.125 µg/mL fungizone, 2 mmol/L l-glutamine (Flow). Moreover, 5 µg/mL of insulin and transferrin and 5 ng/mL of sodium selenite (Sigma) were added to the medium in order to improve CLL cell survival. All samples contained 80-90% leukemic cells.

Drug preparation and MTT assay

Commercially available Mitox (Novantrone, Wyeth-Lederle, Milan, Italy), FAMP (Fludara, Schering AR, Germany) and Pento (Nipent, Wyeth-Lederle, Milan, Italy), were diluted in medium. MTT assay for cell viability was used to estimate drug chemosensitivity as previously described.[12,13,15-17] In brief, 1×10^6 CLL cells were incubated in 96 round-bottom wells (Nunc, Roskilde, Denmark) with different concentrations of Mitox, FAMP and Pento in quadruplicate in a humidified atmosphere for four days at 37°C in 5% CO_2. Fifty µL of 5 mg/mL MTT (Sigma) in PBS were added to every well and the plates were re-incubated for an additional four hours. Fifty µL of 100% dimethylsulfoxide (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 hour and then their spectrophotometric adsorbance at 540 nm was determined using a multi-well scanning spectrophotometer (EL 310, Biotek Instruments, Burligton, VT, USA). Leukemic cell survival (LCS) was calculated by the formula: (mean OD treated wells/mean OD control wells) ×100%. The higher the cytotoxicity induced by a drug or by a drug combination, the lower the LCS. The dose-dependent cytotoxic effects of the drugs were studied in each sample by culturing CLL cells with 2.5-0.0025 µg/mL Mitox, 100-0.01 µg/mL FAMP and 16-0.0016 µg/mL Pento. Drug dose-response curves were drawn and their lethal dose (LD)50 values (i.e. the drug dose capable of killing 50% of the cells) were determined by using our own customized computer software.

Assessment of in vitro drug interactions

For the calculations of drug interactions, the *multiplicative* model and the *maximum* model were followed as previously described.[17,19,20] The *multiplicative* model predicts the effect of a drug combination as the product of the effect of each single drug, as is expected LCS (drug A + drug B)=LCS (A) × LCS (B). The *maximum* model predicts that the effect of a drug combination is similar to that of the most active single drug (*D*max). Consequently, we defined the drug combination as *synergistic* when the observed LCS for a drug combination was lower than the product of the effect of each single drug: observed LCS (A+B) < expected LCS (A) × LCS (B). For the definition of *additivity* the following formula was applied: expected LCS (A) × LCS (B) < observed LCS (A+B) < LCS (*D*max), where *D*max is the lowest LCS value between A and B. Likewise, the definition of *antagonism* was satisfied when expected LCS (A) × LCS (B) < observed LCS (A + B) > LCS (Dmax). Sensitivity, additivity and antagonism calculation were performed by using our own software.

For the study of the interactions between Mitox with either FAMP or Pento, four Mitox (2.5, 0.25, 0.025 and 0.0025 µg/mL), five FAMP (100, 10, 1, 0.1 and 0.01 µg/mL) and five Pento (16, 1.6, 0.16, 0.016 and 0.0016 µg/mL) concentrations were tested for a total of 20 drug combinations.

Flow cytometry analysis of apoptotic nuclei

Freshly isolated CLL cells that had been incubated for 4 days with either no drug, Mitox 0.025 µg/mL, FAMP 1 µg/mL or Pento 0.16 µg/mL, or combinations thereof, were used for the flow cytometry determination of the apoptotic nuclei. CLL cells were then re-suspended in a 1 µL hypotonic solution of propidium iodide (Sigma) (PI 50 µg/mL in 0.1% sodium citrate plus 0.1% Triton X-100, Sigma) and the tubes were left at 4°C overnight in the dark.[21] The PI-fluorescence of the nuclei was measured by an EPICS Profile II flow

cytometer (Coulter Electronics, Hialeah, FL, USA). The flow cytometer was aligned using the highest quality 10 µm fluorospheres (Coulter EPICS, DNA-check beads) that had a population % HPCV of less than 2% for FL2 and FS. The nuclei traversed the light beam at 488 nm laser. A 600 nm dichroic mirror and a 635 band pass filter (bandwidth 20 nm) were used for collecting the PI red fluorescence, and data of the hypodiploid DNA peak was acquired on a logarithmic scale.

Statistical analysis

Descriptive statistics, Pearson correlation coefficient and Wilcoxon matched-pairs signed-ranks test were performed using the SAS/STAT software package, release 6.06 of SAS Institute Inc., 1993.

Results

Mitox effect on apoptotic cell death induced by FAMP and Pento

The flow cytometry analysis results of apoptotic nuclei after a 4-day culture with either medium, each single drug or drug combination is depicted in Figure 1. In the first set of experiments carried out in 22 samples by using a single drug concentration, Mitox and FAMP significantly enhanced the percentage of spontaneous apoptosis (Mitox versus medium, p=0.04; FAMP versus medium, p=0.0042), while the addition of the anthracenedione to FAMP did not further improve the apoptosis induced by the purine analog (Figure 1A). Similar results were obtained in a second set of experiments using Mitox and Pento (Mitox versus medium, p=0.01372; Pento versus medium, p=0.038), but in this case Mitox-Pento combination significantly enhanced the apoptotic effect of each single drug (Mitox+Pento versus Mitox, p = 0.0007; Mitox+Pento versus Pento, p=0.0008) (Figure 1B).

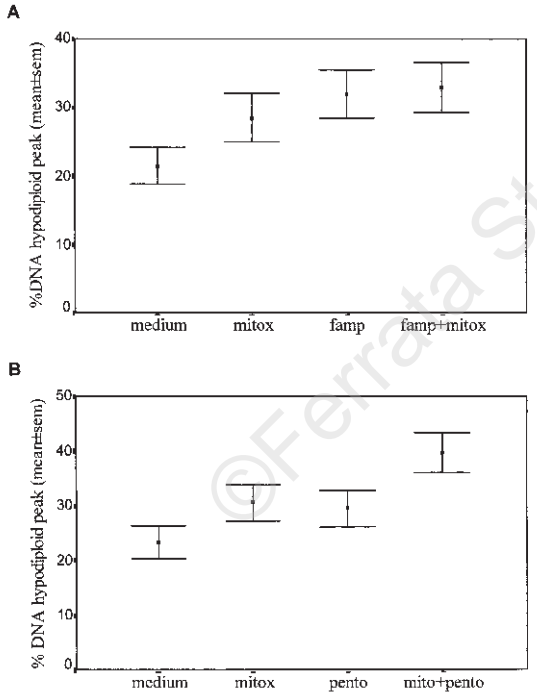

Figure 1. Flow cytometry propidium iodide/DNA analysis corresponding to apoptotic nuclei (hypodiploid peak) determined after 4-day incubation in medium, Mitox 0.025 µg/mL FAMP 1 µg/mL and Mitox+FAMP (A) in 22 CLL samples, or in medium, Mitox, Pento 0.16 µg/mL and Mitox+Pento (B) in 20 samples. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test: A) medium versus Mitox p=0.04, medium versus FAMP p=0.0042, FAMP+Mitox versus either FAMP or Mitox p=not significant; B) medium versus Mitox p=0.01372, medium versus Pento p=0.0038, Pento+Mitox versus Mitox p=0.0007, Pento+Mitox versus Pento p=0.0008.

Mitox-, FAMP- and Pento-induced cytotoxicity

The drug effect on cell survival was also analyzed by the MTT colorimetric assay. CLL cell samples were cultured with several 1-log increased concentrations of Mitox, FAMP and Pento, and the LD50 values were calculated. Table 1 shows the LD50 values obtained in two groups of 22 and 20 patients studied for the combinations of Mitox with either FAMP or Pento, respectively. We arbitrarily defined a sample as *sensitive* when its LD50 was below a threshold representing the drug plasma level after standard therapy, namely Mitox ≤ 0.25 µg/mL,[22] Pento ≤ 1.6 µg/mL[23] and FAMP ≤ 1 µg/mL.[24] Thus, 11 out of 22 and 8 out of 20 samples were sensitive to Mitox in the FAMP and Pento groups, respectively; on the other hand, 2 out of 22 and 0 out of 20 samples appeared sensitive to FAMP and Pento, respectively.

In vitro cytotoxicity induced by Mitox+FAMP and Mitox+Pento combinations and drug interaction evaluation

In the combination study, both FAMP- (Figure 2A) and Pento-LD50 (Figure 2B) values were significantly reduced by the addition of Mitox 2.5 µg/mL (p=0.0007 and p=0.0001, respectively) and Mitox 0.25 µg/mL (p=0.0010 and p=0.0015, respectively).

Table 2 shows the *in vitro* effect of drug interactions in CLL samples. The interactions between Mitox and FAMP or Pento were tested in 20 different combinations in 22 and 20 samples, respectively, resulting in 440 and 400 interactions for Mitox+FAMP and Mitox+Pento, respectively. In particular, 124 combinations (28.2% of the total) and 157 combinations (39.3%) were synergistic in the FAMP and Pento group, respectively. A comparable percentage of antagonistic interactions (46.1% versus 41.2%) was observed in the two groups, while a slightly higher percentage of additive interactions was detected in the Mitox+FAMP interactions (25.7% versus 19.5%). Lastly, the synergistic interactions at LCS ≤ 50% were 11.1% and 11.7% in the FAMP and Pento groups, respectively.

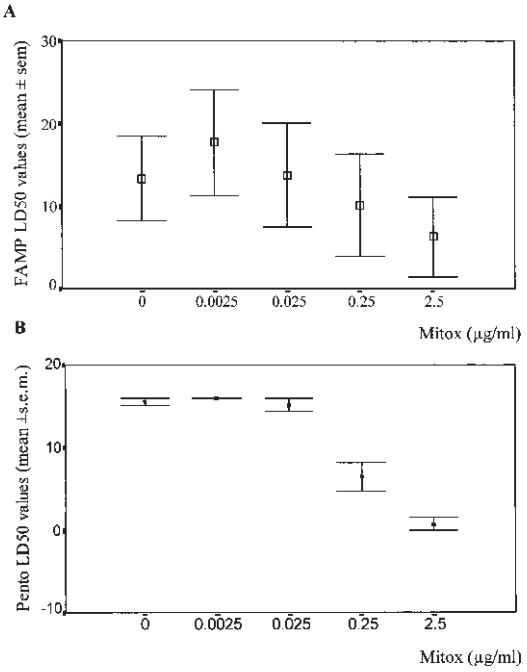

Figure 2. *In vitro* effect of several Mitox concentrations on FAMP- (A) and Pento- (B) LD50 values at MTT assay. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test. The difference of the mean values was significant between Mitox = 0 mg/mL and Mitox = 0.25 mg/mL (p=0.0010) and between Mitox = 0 mg/mL and Mitox = 2.5 mg/mL (p=0.0007) in the FAMP group (A), between Mitox= 0 mg/mL and Mitox = 0.25 mg/mL (p=0.0015) and between Mitox = 0 mg/mL and Mitox = 2.5 mg/mL (p=0.0001) in the Pento group (B).

Table 1. Mitoxantrone (Mitox)-, fludarabine (FAMP)- and pentostatin (Pento)-induced cytotoxicity in samples from 24 CLL cases; 18 samples were included in both groups. Data are expressed as LD50 values (μg/mL).

Sample	*Mitox*	*FAMP*	*Sample*	*Mitox*	*Pento*
1	0.13	2.18	1	0.13	>16
2	1.55	3.20	2	1.55	>16
3	1.70	6.44	3	1.70	>16
4	0.15	55.9	4	0.15	>16
5	0.20	1.70	5	0.20	>16
6	0.20	0.77	6	0.20	>16
7	0.21	2.00	7	0.21	>16
8	0.37	2.84	8	0.37	>16
9	0.66	3.69	9	0.66	>16
10	0.18	0.47	10	0.18	>16
11	1.58	9.04	11	1.58	>16
12	>2.5	1.76	12	>2.5	>16
13	0.12	2.24	13	0.12	>16
14	1.57	3.22	14	1.57	>16
15	1.68	2.38	15	1.68	>16
16	0.34	1.26	16	0.17	>16
17	2.23	9.70	17	0.34	>16
18	0.17	3.13	18	0.17	7.31
19	>2.5	>100	19	1.54	>16
20	2.09	27.1	20	2.05	>16
21	1.71	14.7			
22	>2.5	40.1			

Table 2. *In vitro* effect of drug interactions between Mitox and FAMP in 22 CLL samples and between Mitox and Pento in 20 samples; 18 samples were included in both groups. The number of combinations in which each specific interaction was detected is indicated.

		Mitox+FAMP		
	Total # of interactions	*Sy*	*Ad*	*An*
At any LCS	440	124	113	203
At LCS ≤ 50%	266	49	88	129
		Mitox+Pento		
	Total # of interactions	*Sy*	*Ad*	*An*
At any LCS	400	157	78	165
At LCS ≤ 50%	148	47	39	62

Sy: synergistic; Ad: additive; An: antagonist; LCS: leukemic cell survival.

Table 3. Number of antagonistic, additive and synergistic interactions for Mitox+FAMP and Mitox+Pento, at Mitox ≤ 0.25 μg/mL, FAMP ≤ 1 μg/mL and Pento ≤ 1.6 μg/mL, corresponding to patient plasma concentrations after standard dose therapy.

	FAMP ≤ 1 μg/mL			*Pento ≤ 1.6 μg/mL*		
Mitox (μg/mL)	*AN*	*Ad*	*Sy*	*An*	*Ad*	*Sy*
0.25	29	21	16	73	35	36
0.025	26	11	29	53	28	63
0.025	25	4	37	53	18	73

Sy: synergistic; Ad: additive; An: antagonist.

Enumeration and efficacy of synergistic interactions

The distribution of drug interactions of Mitox with Pento and FAMP, at dose combinations below the patients' plasma concentrations achieved after standard dose therapy,[22-24] are shown in Table 3. It is worth noting that the number of synergistic interactions inversely correlates with the Mitox concentrations. Table 4 shows the correlation between the Mitox-, FAMP- and Pento-LD50 values with the number of synergistic, additive and antagonistic interactions. A significant direct correlation was demonstrated between the number of synergistic interactions and the Mitox-LD50 values in the Mitox+FAMP group, while the number of additive interactions inversely correlated with the Mitox-LD50 values in both drug combination groups.

Discussion

Treatment strategies for CLL vary from the *watch-and-wait* theory to aggressive combination chemotherapy.[6] Standard-dose chlorambucil has long been considered the treatment of choice in CLL.

Table 4. Pearson correlation between the nuimber of synergistic, additive and antagonistic interactions of Mitox+FAMP and Mitox+Pento drug combinations and the Mitox-, FAMP- and Pento-LD50 values.

		Mitox+FAMP	
Drug-LD50	*Sy*	*Ad*	*An*
FAMP	r=0.3824 p=0.079	NS	NS
Mitox	r=0.6005 p=0.003	r=–0.4853 p=0.022	NS
		Mitox+Pento	
Drug-LD50	*Sy*	*Ad*	*An*
Pento	NS	NS	NS
Mitox	NS p=0.003	r=–0.5960 p=0.006	NS

Subsequent clinical trials demonstrated enhanced response rates and a significant improvement in survival with high-dose chlorambucil in comparison with anthracycline-containing regimens.[8] The standard treatment recommendations were again challenged with the advent of the nucleoside analogs.[6,9] In CLL, FAMP is apparently the most promising and the most extensively used purine analog, considering that several randomized trials comparing this agent with chlorambucil are still ongoing.[25,26] The remaining analogs, 2'-chlorodeoxyadenosine and Pento, although significantly fewer investigations have been done on them, are able to produce responses in patients with CLL.[27] Considering that few new agents with *in vitro* evidence of a potential anti-leukemic effect for CLL are under investigation,[28] as an alternative to experimenting with new drugs, new combinations of effective drugs could have an important role in the therapeutic strategy of CLL. In this respect, the therapeutic option of purine analogs in combination with Mitox is more and more frequent for the treatment of lymphoproliferative disorders.[4,5]

Few *in vitro* drug combination studies have been reported in CLL.[13,15,29] Synergy was observed between chlorambucil and Pento in CLL cells,[29] while an additive effect on B neoplastic cell apoptosis was described with a combination of chlorambucil and FAMP.[30] More recently, the phenomenon of synergism between methylxanthine derivatives and chlorambucil was proven *in vitro*.[31] Furthermore, we recently demonstrated that the *in vitro* combination between chlorambucil and either methylprednisone or deflazacort resulted in a synergistic effect.[17] Finally, we demonstrated an *in vitro* synergism of chlorambucil with FAMP and 2'-chlorodeoxyadenosine in inducing CLL cell cytotoxicity (in preparation). In the present *in vitro* study, the MTT assay results, evaluated by the *maximum* and *multiplicative* models,[17,19,20] indicated that the phenomena of additivity and synergism, when pooled together, were found in 53.9% and 58.8% of cases for the Mitox+FAMP and Mitox+Pento groups, respectively. It is worth noting that evaluating synergistic and additive interactions at the therapeutically achievable plasma concentration of each drug, the percentage reduced to 21.1% and 21.4% for the Pento and FAMP group, respectively. It should also be noted that the number of synergistic interactions increased with the lowering of Mitox concentrations. Lastly, a correlation analysis demonstrated that the lower the drug cytotoxicity induced by Mitox, the higher the probability obtaining synergism between the anthracenedione and the purine analog. Considering that the concern for *in vivo* clinical toxicity may represent the major reason for combining Mitox with FAMP or Pento, these results should be considered in view of the optimal drug interaction employing the concentration of the two drugs useful in achieving a synergistic effect. However, it is also evident that synergistic interactions are effective if the LCS values achieved by the combinations of the drugs are at least less than 50%. Clinically, preliminary data demonstrated that Mitox may be successfully associated with acceptable toxicity to purine analogs.[4]

At flow-cytometry analysis, the Mitox+Pento combination was able to significantly improve each drug cytotoxic effect, while the Mitox+FAMP interaction was unable to increase any further the apoptotic effect of Mitox and FAMP taken as single drugs. This latter report is apparently in contrast with the MTT assay results. It should be emphasized, however, that a single drug concentration was used in the flow cytometry test while dose-response curves were performed in the MTT assay. In addition, the apoptotic phenomenon explored by flow cytometry does not provide a real-time analysis of the apoptotic process, but merely uses an end-point analysis. Lastly, assays of apoptosis do not take into consideration the overall cell survival which is a crucial point in determining drug efficacy. Considering that the MTT assay is an easy, semi-automated method for analyzing multiple dose-response curves and defining synergism and additivity by the *multiplicative* and *additive* models, it could be a better choice than flow cytometry in evaluating drug synergism.

This study was performed in both treated and untreated cases. Although the aim was not the comparison of the two groups of patients, we found that there were no statistically significant differences in terms of drug interactions, while the mean values of FAMP-LD50 significantly differed

between the group of untreated (7.2±3.6 s.e.m) and treated (21.9±11.2 s.e.m) (p=0.023) cases, but no difference was found for the other drugs. On the other hand, flow cytometry results showed that significantly higher mean values of apoptotic cells were counted among samples obtained from treated patients, as compared to untreated cases, both in the absence of drugs (27.8±4.3 s.e.m. versus 17.9±3.7 s.e.m., p=0.0317) and after incubation with the combination Mitox+FAMP (39.6±5.6 s.e.m versus 27.3±3.3 s.e.m., p=0.0475). However, the relatively low number of cases compared in each group requires further specific studies on this aspect.

In conclusion, these *in vitro* results support the clinical finding that the combination of Mitox with FAMP or Pento is an effective treatment in CLL. Further clinical investigations are required to optimize the concentration of each single drug in order to obtain *in vivo* synergistic effects with the lowest clinical toxicity. We demonstrated that the *in vitro* MTT assay substantiated with models measuring the effect of interactions of drugs[19,20] is a useful method in studying synergism in CLL. An *in vitro/in vivo* controlled study on the use of the therapeutic combination of Mitox with FAMP or Pento, considering also biological parameters of disease,[32] would be of interest.

References

1. Bowden GT, Roberts R, Alberts DS, et al. Comparative molecular pharmacology in leukemic L1210 cells of the anthracene anticancer drugs mitoxantrone and bisantrene. Cancer Res 1985; 45:4915-20.
2. Paciucci PA, Davis RB, Holland JF et al. Mitoxantrone and costant infusion etoposide for relapsed and refractory acute myelocytic leukemia. Am J Clin Oncol Cancer 1990; 13:516-9.
3. Bajetta E, Buzzoni R, Valagussa P, et al. Mitoxantrone: an active agent in refractory non-Hodgkins lymphomas. Am J Clin Oncol 1988; 11:100-3.
4. McLaughlin P, Hagemeister FB, Swan F, et al. Phase I study of the combination of fludarabine, mitoxantrone, and dexamethasone in low-grade lymphoma. J Clin Oncol 1994; 12:1343-8.
5. Saven A, Lee T, Kosty M, Piro L. Cladribine and mitoxantrone dose escalation in indolent non-Hodgkins lymphoma. J Clin Oncol 1996; 14:2139-44.
6. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N Engl J Med 1995; 19:1052-7.
7. 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.
8. Jaksic B, Brugiatelli M, Krc I, et al. Comparison of high-dose chlorambucil versus Binets modified CHOP regimen in B-cell chronic lymphocytic leukemia in advanced phase: results of an international multicentric randomized trial. Cancer 1997; 79:2107-14.
9. The French Cooperative Group on CLL. Multicentre prospective randomised trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukaemia. Lancet 1996; 347:1432-8.
10. Molica S, De Rossi G, Luciani M, Levato D. Prognostic features and therapeutical approaches in B-cell chronic lymphocyitc leukemia: an update. Haematologica 1995; 80:176-93.
11. Kerr JFR, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. Cancer 1994; 73:2013-26.
12. Di Simone D, Testi R, Caracciolo F, et al. Glutathione-S-transferase activity and multidrug resistance phenotype in chronic lymphocytic leukemia: do they have any clinical relevance? Haematologica 1995; 80:103-7.
13. 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.
14. 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: 3440-6.
15. Morabito F, Callea I, Rodin 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.
16. Morabito F, Stelitano C, Callea I, et al. In vitro chemosensitivity of chronic lymphocytic leukemia B-cells to fludarabine, 2'-chlorodeoxyadenosine and chlorambucil: correlation with clinico-hematological and immunophenotypic features. Haematologica 1996; 81:224-31.
17. Morabito F, Callea I, Irrera G, et al. In vitro improvement of chlorambucil-induced cytotoxicity by deflazacort and 6-methylprednisolone in B-cell chronic lymphocytic leukemia. Eur J Haematol 1997; (in press).
18. 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; 87:4990-7.
19. Valeriote F, Lin HS. Synergistic interaction of anticancer agents: a cellular prospective. Cancer Chemother Rep 1975; 59: 895-9.
20. Sondak OK, Korn EL, Kern DH. In vitro testing of chemotherapeutic combinations in a rapid thymidine incorporation assay. Int J Cell Cloning 1988; 6:378-81.
21. Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Methods 1991; 139:271-9.
22. Gruber A, Liliemark J, Tidefelt U et al. Pharmacokinetics of mitoxantrone in plasma and leukemic cells during treatment of patients with acute non-lymphocytic leukemia. Leuk Lymphoma 1992; 6: 493-6.
23. Smyth JF, Paine RM, Jackman AL, et al. The clinical pharmacology of the adenosine deaminase inhibitor 2-deoxycoformycin. Cancer Chemother Pharmacol 1980; 5:93-101.
24. 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.
25. Rai KR, Peterson B, Elias L, et al. A randomized comparison of fludarabine and chlorambucil for patients with previously untreated chronic lymphocytic leukemia. A CALGB, SWOG, CTG/NCI-C and ECOG inter-group study. Blood 1996; 88(suppl 1):141a.
26. Jaksic B, Delmer A, Brugiatelli M, et al. Interim analysis of a randomised EORTC study comparing high dose chlorambucil (HD-CLB) vs fludarabine (FAMP) in untreated B-cell chronic lymphocytic leukaemia (CLL) [abstract]. VII International Workshop on CLL 1997; 064a.
27. Tallman MS, Hakimian D. Purine nucleoside analogs: emerging roles in indolente lymphoproliferative disorders. Blood 1995; 86: 2463-74.
28. O'Brien S, Kantarjian H, Ellis A, Zwelling L, Estey E, Keating M. Topotecan in chronic lymphocytic leukemia. Cancer 1995; 75: 1104-8.
29. Johnston JB, Verburg L, Shore T, Williams M, Israels LG, Begleiter A. Combination therapy with nucleoside analogs and alkylating. Leukemia 1994; 8(suppl 1):140-3.
30. Frankfurt OS, Byrnes JJ, Seckinger D, Sugarbaker EV. Apoptosis (programmed cell death) and the evaluation of chemosensitivity in chronic lymphocytic leukemia and lymphoma. Oncol Res 1993; 5: 37-42.
31. Ments F, Mossalayi D, Quaaz F, et al. Theophylline synergizes with chlorambucil in inducing apoptosis of B-chronic lymphocytic leukemia cells. Blood 1996; 88:2172-82.
32. Callea V, Morabito F, Luise F, et al. Clinical significance of sIL2R, sCD23, sICAM-1, IL6 and sCD14 serum levels in B-cell chronic lymphocytic leukemia. Haematologica 1996; 81:310-5.