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In vitro drug-induced cytotoxicity predicts clinical response to high-dose chlorambucil in B-cell chronic lymphocytic leukemia

Sir,

Chlorambucil (CLB) can be considered the golden standard of front-line therapy for B-cell chronic lymphocytic leukemia (CLL).[1] In spite of its long use, the best schedule of CLB administration has not yet been established. We have demonstrated the clinical advantage of a high-dose continuous CLB schedule (HD-CLB) over standard-dose intermittent CLB[2] and the CHOP regimen.[3] In addition, according to the results of an interim analysis of the ongoing randomized EORTC study, the efficacy of HD-CLB is apparently not inferior to that of fludarabine,[4] thus confirming that we can now two efficient therapeutic options for front-line therapy of CLL.

We recenlty demonstrated that *in vitro* drug-sensitivity to fludarabine is associated with *in vivo* response.[5] This study was aimed at analyzing whether the ability of CLB to induce *in vitro* cytotoxicity in fresh isolated CLL cells might be related to the *in vivo* outcome of patients treated with the HD-CLB schedule.

Twenty-nine previously untreated CLL patients entered this study. Clinico-hematologic data, CLB-LD_{50} value and the *in vivo* clinical response to high-dose CLB are reported in Table 1. Criteria for treatment, high-dose CLB schedules and definitions of response have been described elsewhere.[2-4] The drug-induced effect on cell viability was assessed by a non-clonogenic 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay that has been repeatedly used to test *in vitro* chemosensitivity of CLL cells to CLB.[7,8] Cells were continuously exposed to the drug for 4 days. Median CLB-LD_{50} value was 25, 16 and 58 µg/mL in patients obtaining complete (CR), partial (PR) and no response (NR), respectively (p=ns for Kruskall Wallis test).

Rai and Binet stage, TTM score and age (Table 2) did not affect the response rate (CR+PR). However, a significantly higher number of responses was observed in males and in those patients showing *in vitro* CLB-LD_{50} values below 26 µg/mL. In spite of the relatively small number of cases, the relevance of this parameter was confirmed by multivariate analysis. The short median follow-up time hampers a statistically reliable analysis of survival. However, since response to therapy is a parameter well known to be associated with longer survival,[9] it is reasonable to expect that *in vitro* resistance to CLB coulde be an early indicator of an overall unfavorable outcome of patients treated with this alkylating agent.

In conclusion, in our experience the *in vitro* MTT assay is a useful tool for predicting *in vivo* treatment failure to HD-CLB in CLL. Thus, from the results obtained in cytotoxicity tests induced by different

Table 1. Main clinico-hematological data, CLB-LD_{50} value and clinical response to high-dose CLB.

Sex, male/female	13/16
Age, mean ± sem	69.9 ± 2.5
Rai stage	
0	1
I	4
II	9
III	11
IV	4
Binet stage	
A	7
B	11
C	11
TTM score, mean ± sem	12.4 ± 0.72
≤ 9	5
> 9	24
CLB-LD_{50} µg/mL (mean ± sem)	47.1 ± 7.2
Clinical response	
CR	16
PR	6
NR	7

Table 2. Univariate and logistic multivariate regression analysis of prognostic variables for clinical response to HD-CLB.

Variable	*CR+PR*	*NR*	*Uni-* variate p*	*Multi-° p*
Rai stage (0-II v III-IV)	11 v 11	3 v 4	1.0	---
Binet stage (A-B v C)	15 v 7	3 v 4	0.3	---
TTM score (≤ 11.4 v > 11.4)	11 v 11	5 v 2	0.4	---
Age, years (≤ 74 v > 74)	11 v 11	4 v 3	1.0	---
Sex (male v female)	13 v 9	0 v 7	0.008	ns
CLB-LD_{50}, µg/mL (≤ 26 v > 26)	14 v 8	1 v 6	0.035	0.05

**Fisher exact test (2-tail); °Logistic regression analysis.*

drugs, namely fludarabine and CLB, it should be possible to choose the most appropriate CLL therapeutic approach in various phases of the disease, avoiding the useless toxicity of ineffective treatments. Considering, however, that a group of patients with a high CLB-LD_{50} value do respond *in vivo* to the drug, other biological markers should be explored[10] in conjunction with the MTT assay in order to establish the real predictive power of this *in vitro* test on clinical response to CLB in CLL.

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Key words

CLL, MTT assay, chlorambucil, clinical response, cytotoxicity.

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