Peripheral blood CD38 expression predicts time to progression in B-cell chronic lymphocytic leukemia after first-line therapy with high-dose chlorambucil

CD38 expression by B-cell chronic lymphocytic leukemia (B-CLL) cells has been the focus of several recent studies. The aim of this study was to evaluate the prognostic impact of CD38 expression by peripheral blood lymphocytes on progression-free survival after first-line therapy with high-dose chlorambucil in 53 previously untreated patients affected by typical $CD5^+$ $CD23^+$ B-CLL.

The use of a continuous high-dose chlorambucil schedule (HD-CLB)[1,2,3] has refueled interest in an alternative non-palliative management of B-CLL, mostly due to the high number and the quality of remissions that can be achieved. However, relapse or progression is almost the rule in B-CLL patients, even after highly efficient chemotherapeutic schedules. It has been reported that CD38 expression distinguishes two major B-CLL groups in terms of clinical course and response to therapy.[4,5,6] We recently demonstrated in a cohort of 161 previously untreated patients that peripheral blood CD38 expression is an independent factor predicting overall survival in B-CLL and we set up a score method by integrating percentage values with mean fluorescence intensity.[7] The present paper represents an expansion of our first observation and is aimed at evaluating the prognostic value of CD38 expression in terms of progression-free survival (PFS) in B-CLL patients treated with continuous HD-CLB. Out of 62 cases treated, 3 were not evaluable for response, 6 failed to respond, while 53 cases achieved either a complete remission (CR) (33 cases) or a partial response (PR) (20 cases). These patients represent the sample adopted in this study. Maintenance chemotherapy (CLB 5 mg/m^2 twice a week) was given to 43 of the 53 patients. The treatment was adjusted according to toxicity and prolonged for at least 5 years in most patients. Criteria for the definition of progression were the development of either TTM >9, anemia or thrombocytopenia.

IB4, an agonistic $IgG2_a$ monoclonal antibody, was used in indirect immunofluorescence as a reagent displaying high affinity for human CD38.[8,9] A FITC-labeled $F(ab')_2$ preparation of a rabbit anti-mouse Ig (Becton Dickinson) was used as a secondary reagent. Ten thousand events were analyzed using an EPICS Profile II flow cytometer (Coulter Electronics, Hialeah, FL, USA). Analyzing CD38 expression according PFS by referring to a CD38 cut-off value of 30%, we failed to demonstrate any significant impact. Subsequently, the patient cohort was split into 2 groups using the median values of both MFI (median value: 2.362) and percentage (median value: 58.2%) of CD38 as cut-offs. In both situations, patients with CD38 expression exceeding the median values had a trend towards a shorter PFS as compared with cases showing a CD38 expression below the cut-off values. Considering percentage and MFI together, thus splitting the patient cohort into two groups, those with both MFI and percentage of CD38 below the median values experienced a significantly longer PFS than the remaining cases (Figure 1). In this analysis, we stratified cases according to age (<60 years *versus* >60 years), sex, bone marrow histology pattern, NCI-modified Rai and Binet stages, TTM score, response to HD-CLB and the use of maintenance chemotherapy. NCI-modified Rai and Binet stages entered a Cox regression multivariate analysis along with response to HD-CLB, maintenance chemotherapy and CD38 expression. The latter three variables proved to be significantly relevant to PFS. In particular, patients achieving CR, treated with maintenance chemotherapy and with percentage and MFI values of CD38 below the median values showed a relative risk (RR) of a longer PFS of 12.2, 5.2 and 4.4, respectively.

We tried to explain the association between the lack of CD38 expression and better clinical outcome in B-CLL patients (19), considering the apparent contrast between its prognostic significance and the finding that low CD38 expression parallels a lower propensity to apoptosis.[10] It may be suggestive that B-CLL

Table 1. Main clinical and hematologic parameters of 53 B-CLL cases entered this study.

Age (<60 years/>60 years)	12/41
Sex (female/male)	18/35
NCI-revised Rai stage	
0	7
I-II	35
III-IV	11
Binet stage	
A	22
B	22
C	9
TTM score	
< 9	20
> 9	33
BM histology pattern	
non-diffuse	24
diffuse	16
not performed	13
Doubling time (months)	
>12	27
< 12	5
not valuable	21
Response to HD-CLB	
Complete	33
Partial	20

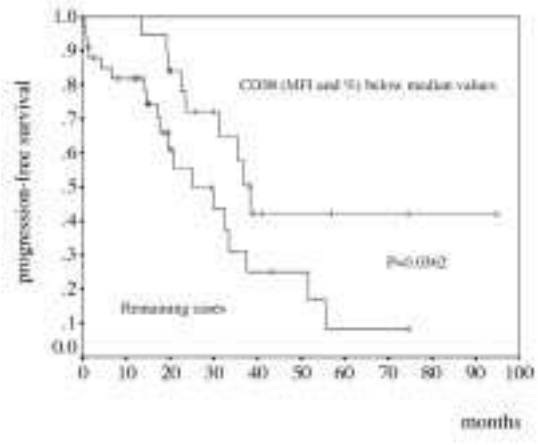

Figure 1. Progression-free survival of two B-CLL groups subdivided on the basis of concurrent evaluation of both percentage and MFI of median CD38 values.

cells of some cases selected as expressing low or high CD38 at diagnosis had increased or reduced levels of surface CD38, respectively, after *in vitro* and *in vivo* treatment with CLB (data not shown). As a consequence, the potential pivotal role which could be played by the *in vivo* CD38 cross-linking by its ligand in controlling the process of apoptosis of B-CLL cells should be re-considered in the light of CLB therapy. In conclusion, the expression of CD38 by peripheral blood lymphocytes significantly predicts PFS in typical $CD5^+$ $CD23^+$ B-CLL. In addition, it is reasonable to forecast that the prognostic penetrance of CD38 expression might be increased by its association with other biological factors and *in vitro* tests of drug resistance.

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Key words: B-CLL, CD38, progression-free survival, therapy, high-dose chlorambucil, prognosis, monoclonal antibody.

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