to the common 2R/2R genotype nor with reference to a combined category of 2R/2R and 2R/3R genotypes (Table 2). These results differ from those obtained by Krajinovic *et al.* However, the settings of both studies were different. Whereas our study evaluated a small, closely matched and relatively homogeneous sample of patients with B-cell precursor childhood ALL, Krajinovic performed a study in a heterogeneous cohort of 205 patients with various types of childhood ALL. Furthermore, the application of high-dose MTX might have been important. Our patients received 4 times 5 g/m^2 MTX in the extracompartment treatment protocol M, whereas the patients analyzed by Krajinovic *et al.* received a single dose of 4g/m^2 MTX during the induction phase. Therefore, one might speculate that the prognostic impact of the genetic tandem-repeat polymorphism within the promotor region of the TS gene might be overcome by higher doses of MTX. Nevertheless, larger studies are necessary to elucidate the significance of TS genotype for outcome in childhood ALL.

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Key words: thymidylate synthase, childhood ALL, relapse, ALL-BFM.

Acknowledgments: we gratefully acknowledge the excellent co-operation of all participants of trials ALL-BFM 86 and ALL-BFM 90 as well that of all doctors and nurses at the participating medical centers.

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Dario Campana, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Campana and the Editors. Manuscript received September 18, 2002; accepted January 24, 2003.

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Simultaneous expression of CD38 and its ligand CD31 by chronic lymphocytic leukemia B-cells: anecdotal observation or pathogenetic hypothesis for the clinical outcome?

A ligand for CD38 was identified as CD31, a molecule prevalently expressed by endothelial cells and selected lymphocyte populations. The finding that chronic lymphocytic leukemia (CLL) B-cells with high CD38 expression show marked propensity to apoptosis is apparently in contrast with its negative prognostic significance. Herein, we tried to explain this discrepancy showing that CD38 is co-expressed with its ligand CD31 on B-CLL cells.

Haematologica 2003;88:354-355
(*http://www.haematologica.org/2003_3/88354.htm*)

B-cell chronic lymphocyte leukemia (B-CLL) is a disorder typically characterized by a clonal expansion of CD5$^+$ CD23$^+$ monoclonal B-lymphocytes.[1] Human CD38 is a type II transmembrane glycoprotein widely expressed by different cell types.[2] Although many hypotheses have been proposed about its *in vivo* function, it is still difficult to construct a unifying model. For instance, CD38 blocks cell differentiation in the bone marrow B-cell compartment by activating apoptosis,[3] while CD38 signaling by agonistic monoclonal antibodies prevents apoptosis in human germinal center B-cells.[4] Different studies have analyzed CD38 expression in B-CLL. In particular, the evaluation of CD38 and IgD expression has been useful in distinguishing B-cells at various stages of differentiation from naive through to memory cells.[5] Moreover, it has been demonstrated that low CD38 levels predict a longer survival in B-CLL patients[6] and a longer time to progression.[7]

A ligand for CD38 has been identified as the platelet endothelial adhesion molecule-1 or CD31.[8] Interestingly, CD38 was recently demonstrated to parallel CD31 expression in myeloma cells with the exception of the leukemic form of multiple myeloma, in which neoplastic plasma cells lost CD31.[9]

This study demonstrates that the adhesion molecule CD31 was highly expressed by B-CLL, mostly in those cases display-

Table 1. Comparison between the expression of CD38 and CD31 on peripheral blood of B-CLL cells according to a cut-off value of 30%.

CD31	*CD38*		*Total*
	<30%	*>30%*	
<30%	11	6	17
>30%	5	21	26
Total	16	27	43

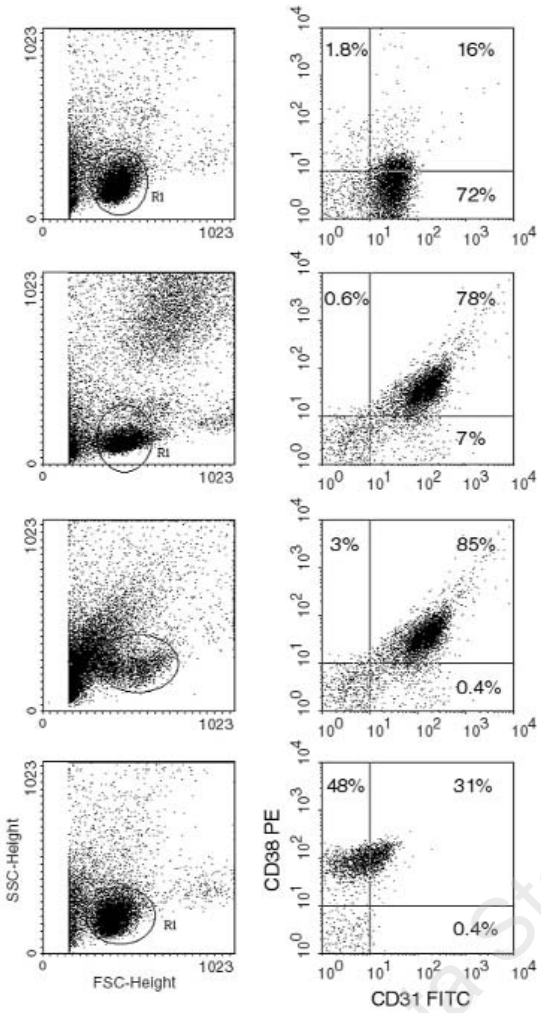

Figure 1. Flow cytometry analysis of 4 representative cases showing different phenotype patterns of CD38 and CD31 expression.

ing its receptor CD38. Forty-three B-CLL patients, 30 males and 13 females, with a median age of 64.8 years (range 34-81) entered this study. All cases presented the typical morphology of classical B-CLL and showed the sIg^{dim}, $CD5^+$, $CD19^+$, $CD23^+$ immunophenotypic pattern. Nine patients were in Rai stage 0, 19 in 1, 10 in 2, 3 in 3 and 2 in stage IV.

Fresh or cryopreserved samples were available for surface marker analyses after obtaining informed consent. Peripheral blood mononuclear cells were separated by Ficoll-Hypaque density gradient. Monoclonal antibodies (mAb) specific for CD19 and CD23 (Becton Dickinson, Mountain View, CA, USA) along with CD5- and sIg-FITC (Dako, Glostrup, Denmark) were used for the definition of a typical B-CLL phenotype. The agonist MoAb IB4 (IgG_2a) was adopted as the reagent binding CD38 at high affinity, while Moon-1 was used to detect the CD38 ligand CD31.[8] Subsequently, either FITC- or PE-labeled $F(ab')_2$ fragments of a rabbit anti-mouse Ig (Becton Dickinson) were used as the secondary reagent. Between 5,000 to 10,000 cells were examined in the tests, using an EPICS Profile II flow cytometer (Coulter Electronics, Inc., Hialeah, FL, USA). The lymphocyte population was gated on a two parameter forward angle *versus* 90° light scatter histogram.

Figure 1 shows that $CD19^+$ B-CLL cells may simultaneously express CD38 and CD31 antigen even if in variable percentages and densities. Indeed, considering a sample with >30% expression as positive, of the 43 B-CLL cases analyzed, 26 were $CD31^+$ (60.5%). Furthermore, only 6 of the 26 $CD38^+$ cases were CD31-negative (22.2%). Similarly, 5/26 (19.2%) CD38-positive cases were CD31-negative (Table 1). Conversely, out of the 17 cases showing <30% CD31 expression, 6 (35.3%) were CD38-positive. Finally, 5/16 cases with <30% CD38 expression showed CD31-positivity (Table 1). No significant association was observed between Rai stage and the CD38/CD31 phenotype.

Several reports demonstrated that B-CLL patients with higher CD38 expression had a significantly shorter overall survival.[6,7] However, this finding is apparently in contrast with the *in vitro* demonstration that highly CD38 expressing B-CLL cells show marked propensity to apoptosis. This discrepancy could be explained by considering the biological functions of CD38 and the effects elicited once the molecule is engaged by soluble ligands or surface counter-receptors.[9] This view is further supported by the finding that CD38 ligation by the agonistic mAb IB4 prevents germinal center B-cells from undergoing apoptosis.[4] Beside its involvement in the regulation of cell-to-cell contacts, the two molecules, CD31 and CD38, seem to be co-expressed in discrete cell subsets, where they display a co-ordinated regulation.[10] Our observation that CD38 is associated with CD31 gives additional explanations of the worse clinical outcome of cases that express CD38 highly on their B-CLL cells. These results prompt us to speculate that the interplay between CD38 and the surrogate of its ligand CD31 could be an important step in the regulation of cell life. Indeed, neoplastic lymphoid plasma cells of multiple myeloma patients, represent another typical example of co-expression of CD38 and its physiologic ligand CD31.[9]

In conclusion, on the basis of the above mentioned result and our data, documenting that B-CLL cells can simultaneously express CD38 and CD31, we can presume both a homotypic and heterotypic model of CD38/CD31 interactions which would give an additional explanation of the clinical outcome of patients with B-CLL with different expression of the CD38 molecule.

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Key words: B-CLL, CD38, CD31, monoclonal antibody.

Funding: supported in part by grants from the Regione Calabria and the European Community, Istituto Superiore di Sanità for the Italia-USA project in CLL, and from the AIRC (Milano, Italy), "Biotecnologia" (CNR/MURST, Roma, Italy), AIDS (Istituto Superiore di Sanità, Roma, Italy), FIRMS and Compagnia San Paolo Foundations. S.D. is a student of the Postgraduate School of Oncology, University of Torino Medical School, Torino, Italy.

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken joint-

ly by Professor Cazzola and the Editors. Manuscript received October 7, 2002; accepted January 23, 2003.

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Ultrasound-guided fine needle aspiration cytology combined with flow cytometric immunophenotyping for rapid characterization of deep-seated non-Hodgkin's lymphoma recurrence

We performed US-guided fine needle aspiration cytology combined with flow cytometry in 55 patients with deep lesions and a history of non-Hodgkin's lymphoma (NHL). Forty-seven patients were found to be affected by B-NHL, 5 by T-NHL, 3 by metastatic carcinoma. Immunocytochemistry, morphology and flow cytometry identified 58%, 77% and 100% of the 52 patients with NHL recurrence, respectively.

Haematologica 2003;88:356-358
(*http://www.haematologica.org/2003_3/88356.htm*)

We tested the role of flow cytometry compared with morphology, immunocytochemistry and cytogenetics in the assessment of deep lesions in 55 patients (median age 60 years) evaluated for possible NHL recurrence. At time of revaluation, after informed consent US-guided fine needle aspiration cytology (FNAC) of the target lesion was performed using an EUB 525 Hitachi instrument (Tokyo, Japan) with 3.5- or 7.5-MHz probe, puncture adaptor and 0.71 mm (22G) diameter modified Chiba needle 150 or 200 mm in length. Smears stained with Diff-Quick, Papanicolau and Giemsa were analyzed by two expert pathologists. The remaining cytological material was placed i) in 5 mL of isotonic saline solution and processed for flow cytometry; ii) in 15 mL of RPMI-1640 cell culture, and processed for immunocytochemistry and for cytogenetics. Flow cytometry was performed using Becton Dickinson equipment (Palo Alto, CA, USA) and a panel of fluorescein or phycoerythrin-conjugated monoclonal antibodies (MoAbs). After erythrocyte lysis, forward/side light scatter and CD45/side light scatter were used as primary gating methodologies. Normal cut off values for each lymphoid cell population were set according to standard procedures;[1] B-cell monoclonality definition required κ:λ or λ:κ ratio equal to or greater than 10:1, T-cell monoclonality was diagnosed on the basis of abnormal T-cell antigen expression.[2] Immunocytochemical and cytogenetic studies were performed according to standard procedure.[3,4] Three to 5 samples were obtained from each patient; all FNAC procedures were well tolerated. As for morphologic assessment, both pathologists agreed in diagnosing NHL in 40 patients, adenocarcinoma in 2, dysgerminoma in one and reactive lymphoid hyperplasia in 12. Immunocytochemistry, performed in 52 patients, demonstrat-

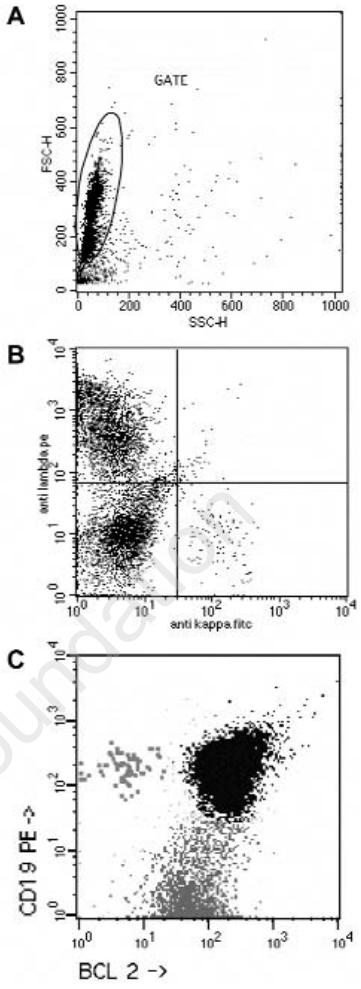

Figure 1. Flow cytometry scattergrams of a representative case of follicular center cell lymphoma (grade I). (A) Gate including low side scatter (small) cells. (B) B-cells with l restriction. (C) Neoplastic follicular B-cells with high expression of both CD19 and bcl-2 (upper-right), normal T-cells with intermediate bcl-2 expression (1 log less intensity) (down), and residual normal follicular B-cells, bcl-2 negative (upper-left).