

## **CD 38 signaling in B-cell chronic lymphocytic leukemia (B-CLL)**

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### **Background and Aim**

CLL is a heterogeneous disease in which some patients undergo a slowly progressive clinical course, but most will eventually enter an advanced phase requiring recurrent treatment (1). The challenge is to identify molecular parameters in order to separate patients into different risk groups, so that in some cases a clinical decision for the urgency of a more aggressive therapy is justified. In the past few years, the absence of mutations in the IgV genes, the presence on the cell surface of CD38 and in the cytoplasm of ZAP-70 have emerged as markers of aggressive CLL.

The working hypothesis behind this project is that CD38 is not a mere negative prognostic marker in CLL, but also a pathogenetic agent, directly involved in determining a more severe clinical course. In this respect, initial observations made by the host laboratory were that CD38 controls a signaling pathway leading to increased survival and proliferation of the neoplastic cells (3). These effects are mediated through a direct interaction with the CD31 ligand, expressed by a population of circulating and residential cells of myeloid origin, named nurse-like according to their ability to nurture CLL cells (4,5).

To gain further insight into the mechanisms and signaling pathways controlled by CD38 and CD31, the project was articulated as follows:

#### **1) Genome wide analyses of the CD38/CD31 signaling pathway**

This part of the project is being done in collaboration with G. Chiorino (Cancer Genomics Lab, Fondo Edo Tempia, Biella, Italy), where microarray technology and relative know-how are established and running.

The aim is to obtain a general view of the genomic events implemented after exposing CD38<sup>+</sup> CLL cells to CD31<sup>+</sup> fibroblasts. To do so, we cultured purified CLL lymphocytes from 22 molecularly characterized patients with murine fibroblasts transfected with human CD31 for 5 days, previously selected as the best experimental time (ref). The 22 samples were selected for being molecularly different (IgVH mutated and unmutated, CD38 and ZAP-70 positive or negative), thus providing an internal control system. After stopping the experiment, the delivery of proliferation/survival signals was checked by examining the

morphological changes in CLL cells. An aliquot of the cells was also lysed and RNA extracted for microarray analysis. Cultures using control mock-transfected murine fibroblasts were performed as controls, as well as RNA extraction at a baseline.

At present all the RNAs have been extracted by the phenol-chloroform extraction method, retrotranscribed and hybridized with the microchips. Data analysis is ongoing but preliminary results obtained by comparing the gene profiles of molecularly different CLL samples indicate the existence of a very clear-cut signature of “resting” CLL, as characterized by presence of somatic mutations in the IgV genes and negativity for CD38 and ZAP-70. Detection of functional groups of regulated genes as far as combined regions is of current interest. The results after 5 days CD31 positive/negative fibroblasts incubation are to be analyzed.

## **2) Evaluation of the prognostic impact of CD38 polymorphism**

The gene encoding CD38 is located on chromosome 4 (4p15). *CD38* consists of eight exons, spans 70.6 kilobase pairs, and has a notably long first intron (40 kb) (ref). *CD38* has a well-characterized single nucleotide polymorphism (SNP) located at the 5' end of this intron (184 C→G) which leads to the presence or absence of a *Pvu* II restriction site (ref). The frequency of the three genotypes has been established in healthy Italian-born adults as: CC 70%, CG 26%, and GG 4%. The SNP is located in an intronic hotspot which is both part of a CpG island located at the 5' end of the gene and also contains the functional *CD38* retinoic acid responsive element. In addition, evidence based on a novel truncated *CD38* mRNA transcript, currently under study, suggests that one means of controlling *CD38* gene expression involves control of transcriptional elongation where a stop-or-go decision is taken within the 5' end of intron 1.

The frequency of the CD38 alleles has been studied in an initial cohort of xx CLL patients, resulting in a frequency of 61.9% CC, 31.7% GC and 6.3% GG in 69 patients, not significantly different from published data (ref). Dividing the patient cohort on the basis of different clinical and molecular parameters significant differences were observed considering the IgVH mutational status. A significantly higher frequency of the G allele (both homo- and heterozygous) was noted in the IgVH unmutated group as compared to the IgVH mutated sample (G alleles are present in 43% of mutated vs 65% of unmutated patients). Similar results on the G allele were observed after scoring patients on the basis of ZAP70 expression: the G allele was more frequent in the ZAP70<sup>+</sup> sample (62%), while ZAP70<sup>-</sup> patients were 31%. These results suggest an association between *CD38*

polymorphism and molecular parameters predicting poor prognosis in CLL, at least in the disease sample analyzed. These results are summarized in an abstract submitted to the 31<sup>st</sup> ESMO Congress in Istanbul 2006.

### **3) Analysis of the functional relationship between independent negative prognostic markers.**

Our working hypothesis is that all the prognostic markers so far identified are functionally linked and part of a network of signals ultimately characterizing aggressive CLL. Patients without mutations in the IgVH genes are more likely to be CD38<sup>+</sup> and - with an even more stringent correlation - ZAP70<sup>+</sup>, although exceptions clearly exist. Thus, all three markers have an independent negative prognostic value.

The aim is to isolate neoplastic B cells from CLL patients with distinct phenotypic and molecular profiles, as detailed above, and to assay the ability of CD38 to function as a receptor delivering proliferation / survival signals.

Preliminary results obtained in the Lab using a ZAP-70 transfected cell line and validated in a small cohort of CLL patients indicate that i) CD38 signaling pathway is dependent on ZAP-70 and ii) ZAP-70 is tyrosine phosphorylated upon CD38 ligation.

The final outcome of CD38 pathway could be related to the response to SDF-1/CXCL12, a chemokine secreted in high amounts by nurse-like cells and acting as a survival factor for CLL cells (ref). In order to evaluate the role of CD38 and ZAP-70 in this complex network of receptors and ligands, we are currently studying the migratory potential of a panel of molecularly characterized CLL cells. My involvement in this part of the project concerns the analysis of early (F-actin polymerization) and late (migration) responses to the chemokine. Preliminary data indicate that CD38<sup>+</sup>/ZAP-70<sup>+</sup> CLL cells migrate better in response to SDF-1.

## Relevant references

1. Caligaris-Cappio F. and T.J. Hamblin 1999. B-cell chronic lymphocytic leukemia: a bird of a different feather. *J Clin Oncol* 17:399.
2. Schroers, R., F. Giesinger, L. Trümper, D. Haase, B. Kulle, L. Klein-Hitpass, L. Sellmann, U. Dührsen and J. Dürig. 2005. Combined analysis of ZAP-70 and CD38 expression as a predictor of disease progression in B-cell chronic lymphocytic leukaemia. *Leukemia* 19:750.
3. Deaglio, S., A. Capobianco, L. Bergui, J. Durig, F. Morabito, U. Dührsen, and F. Malavasi. 2003. CD38 is a signaling molecule in B-cell chronic lymphocytic leukemia cells. *Blood* 102:2146.
4. Tsukada, N., J. A. Burger, N. J. Zvaifler, and T. J. Kipps. 2002. Distinctive features of "nurselike" cells that differentiate in the context of chronic lymphocytic leukemia. *Blood* 99:1030.
5. Deaglio, S., T. Vaisitti, L. Bergui, L. Bonello, A. L. Horenstein, L. Tamagnone, L. Boumsell, and F. Malavasi. 2005. CD38 and CD100 lead a network of surface receptors relaying positive signals for B-CLL growth and survival. *Blood* 105:3042.
6. Ferrero, E., F. Scuccia, and F. Malavasi. 1999. The human CD38 gene: polymorphism, CpG island, and linkage to the CD157 (BST-1) gene. *Immunogenetics* 49(7-8):597.
7. Gonzales-Escribano, M.F., F. Aguilar, B. Torres, Sanchez-Roman J., and A. Nunez-Roldan. 2004. CD38 polymorphisms in Spanish patients with systemic lupus erythematosus. *Human Immunol* 65(6):660.
8. Drummond, F.J., J.J. Mackrill, K. O'Sullivan, M. Daly, F. Shanahan, and M.G. Molloy. 2006. CD38 is associated with premenopausal and postmenopausal bone mineral density and postmenopausal bone loss. *J Bone Miner Metab* 24: 28.
9. Burger, J.A., N. Tsukada, M. Burger, N.J. Zvaifler, M. Dell'Aquila, and T.J. Kipps. 2000. Blood-derived nurse like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. *Blood* 96:2655.
10. Richardson, S.J., C. Matthews, M.A. Catherwood, H.D. Alexander, B.S. Carey, J. Farrugia, A. Gardiner, S. Mould, D. Oscier, J.A. Copplestone, and A.G. Prentice. 2006. ZAP-70 expression is associated with enhanced ability to respond to migratory and survival signals in B-cell chronic lymphocytic leukemia (B-CLL). *Blood* 107(9): 3584.