The CD38 Ectoenzyme Family: Advances in Basic Science and Clinical Practice

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One aim of this session given at the Torino CD38 Meeting in June, 2006 was to review the role of CD38 in B-cell Chronic Lymphocytic Leukemia (B-CLL), and its potential as a therapeutic target. $CD38^{high}$ B-CLL cases show activated phenotypic features as compared with $CD38^{low}$ cases. Moreover, a greater percentage of Ki-67 and telomerase activity is documented among $CD38^{high}$ cases. Also, CD38 is not merely a negative prognostic marker in B-CLL, but also a key element in the pathogenetic network underlying the disease. A large series of B-CLL cases investigating the CD38 expression on bone marrow B-cells identified CD38 value <10% as the cut-off predicting a longer time to treatment. However, neither CD38 nor ZAP-70 by themselves or in combination were able to anticipate IgVH mutational status. Transferring these findings into clinical ground, 3 groups of B-CLL cases were identified with significantly different clinical courses: i.e., low-risk (no negative prognostic factor), intermediate-risk (1 negative prognostic factor) and high-risk (2-3 negative prognostic factors) patients. Altogether these results suggest that: i) CD38-expressing cells present not only an activation status, but also a different stage differentiation with a more repeated turnover; ii) CD38 contributes to controlling a signaling pathway that confers to B-CLL cells an increased proliferative potential, enhancing aggressiveness of this variant; iii) different CD38 cut off values should be considered for peripheral blood and bone marrow; iv) CD38 seems to independently contribute to prognostic stratification of B-CLL.

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INTRODUCTION

At many stages of differentiation, B lymphocytes express surface membrane CD38. Activated mature B cells express CD38 and undergo a few rounds of replication before being recruited to sites (germinal centers, GC) within secondary follicles. These cells, now called centroblasts and centrocytes, also express CD38 and it is within the GC microenvironment that the cells undergo isotype class switching and somatic hypermutation of genes encoding variable regions of an immunoglobulin molecule (IgV). Eventually, the genetically altered cells exit the GC as either memory cells, which are $CD38^-$, or as terminally differentiated plasma cells that express CD38 at high density. Understanding the regulation of CD38 expression by clonal cells of B-CLL and the role of CD38 in B-CLL biology would shed light on the differentiation state at which the B-CLL clone is frozen and in turn yield clues to understanding the mechanisms of disease in B-CLL (1,2).

ASSOCIATIVE AND FUNCTIONAL STUDIES

Rajendra N. Damle presented data on associative and functional studies aimed at unraveling the role of CD38 in B-CLL. Damle and his co-workers have reported previously the existence of two clinically distinct subgroups of patients based on the percentage of the clonal cells expressing CD38. Specifically, B-CLL cases with ≥ 30% $CD38^+$ cells have a poor prognosis, shorter time to first treatment, repeated requirement for chemotherapy, and shorter survival post diagnosis than cases in which <30% of clonal cells express cell surface CD38 (3).

In recent studies reported during this session, Damle et al. have extended their analyses of cases with ≥30% $CD38^+$ B-CLL cells (termed $CD38^{high}$) and those with <30% $CD38^+$ B-CLL cells (termed $CD38^{low}$), determining phenotypic differences underlying their biology. Clonal cells from $CD38^{high}$ cases resemble cells that have been more recently activated than those from cells of $CD38^{low}$ cases (4). Although most members within an individual B-CLL clone are arrested in the G0/G1 phase of the cell cycle, a greater percentage of the

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clonal cells among $CD38^{high}$ cases show expression of Ki-67, marking cells that have passed the G0/G1 phase of the cell cycle. Furthermore, clonal B-CLL cells were shown to express Zeta chain-associated protein (ZAP-70), as detected by flow cytometry, irrespective of their CD38 status, especially at higher percentages by those in the bone marrow than by those in circulation.

ZAP-70, normally found in T lymphocytes, has been reported recently to be present in normal B cells upon activation and also expressed by clonal B-CLL cells. The relevance of ZAP-70 to the diagnosis and prognosis of CLL is a recently described topic. A cell's replicative history varies from that of another depending on the frequency and number of times it undergoes cell division. Every cell division is accompanied by a concomitant loss of a portion of a chromosome's telomeric region, and telomere length therefore indirectly reflects replicative history. Clonal cells from the group of $CD38^{high}$ cases showed more extensive replicative history (5) based on their significantly shorter telomere lengths than the group of $CD38^{low}$ cases. The same group of $CD38^{high}$ cases also exhibited elevated telomerase activity than the group of $CD38^{low}$ cases, possibly implying a more repeated turnover of cells among clonal cells in $CD38^{high}$ cases. Telomerase activity in individual cases correlated directly with percentage of $CD38^{+}$ cells among the clonal population.

Using a multi-color flow cytometry based approach, Damle et al. identified $CD38^{+}$ and $CD38^{-}$ cells among clonal members of individual cases, and probed these for the percentage of cells expressing the markers Ki-67 or ZAP-70. Remarkably, in each of the 20 cases studied, $CD38^{+}$ cells of a clone showed significantly higher percentages expressing Ki-67 and ZAP-70, than that within the $CD38^{-}$ subset of clonal members. In addition flow-sorted $CD38^{+}$ cells from each case also showed elevated telomerase activity than their $CD38^{-}$counterparts. Although differences were observed in Ki-67 and ZAP-70 expression and telomerase activity, the telomere lengths of these cells did not differ, suggesting that not only are the CD38-expressing cells activated cells, but many of them also are at a differentiation stage different from those clonal relatives that do not express CD38.

HUMAN CD38 IN PATHOGENETIC NETWORK UNDERLYING B-CLL

Silvia Deaglio pointed out the working hypothesis that human CD38 is not merely a negative prognostic marker in B-CLL, but also a key element in the pathogenetic network underlying the disease.

Several lines of evidence support this hypothesis. First, CD38 is a receptor that induces proliferation and increases survival of B-CLL cells (6). Second, CD38 signals upon interaction with a ligand, CD31, expressed by stromal and nurse-like cells (7). Third, CD38/CD31 contact upregulates CD100, a semaphorin involved in sustaining B-CLL growth (8). Lastly, this model of receptor/ligand crosstalk is indirectly confirmed by the finding that nurse-like cells derived from B-CLL patients express high levels of functional CD31 and plexin-B1, the high-affinity ligand for CD100 (8).

The overexpression of the tyrosine kinase ZAP-70 has recently been recognized as an independent negative prognostic marker and has been found to correlate with CD38 expression and with the absence of mutations in IgV genes. Further, several groups have shown that the simultaneous assessment of CD38 and ZAP-70 expression adds to the diagnostic power and offers a better identification of aggressive B-CLL. These findings suggest that CD38 and ZAP-70 may be functionally linked in controlling a signaling pathway that confers to B-CLL cells an increased proliferative potential. This hypothesis is currently being validated experimentally by Deaglio and coworkers. Preliminary results indicate that $CD38^{+}$/ZAP-70^{+} patients are characterized by a stronger activation and phosphorylation of ERK1/2 proteins upon CD38 ligation with an agonistic monoclonal antibody or with the CD31 ligand, as compared with $CD38^{+}$/ZAP-70^{-} B-CLL cells. Further, CD38 and ZAP-70 are part of a sequential signaling pathway: CD38 cross-linking results in the phosphorylation of activatory tyrosines in ZAP-70 in a B cell line model transfected with ZAP-70 and in selected B-CLL patients. The signaling pathway directly controlled by CD38 and ZAP-70 intertwines with the one controlled by the CXCL12/SDF-1 chemokine and its CXCR4/CD184 receptor. $CD38^{+}$/ZAP-70^{+} cells polymerize actin and migrate better than $CD38^{+}$/ZAP-70^{-} cells in response to CXCL12, even though they express comparable levels of CXCR4.

The scenario that is unraveling suggests that CXCL12 may generate a molecular gradient that guides $CD38^{+}$ cells to $CD31^{+}$ stromal cells, and thus initiate the CD38 signaling pathway. These signals would contribute to the maintenance of a tumor reservoir and to the enhanced aggressiveness of this disease variant.

CD38 EXPRESSION IN RELATIONSHIP TO OTHER PROGNOSTIC FACTORS

Michael J. Keating pointed out the relationship between CD38 expression and other prognostic factors associated with the patient (i.e., gender), with tumor burden (Rai and Binet stages and β 2 microglobulin) and with other biological characteristics of the leukemic B-CLL cell (9). In particular, high CD38 expression strongly correlated with ZAP-70 expression, IgVH gene mutation status, and certain chromosome deletions detected by FISH analysis, such as 11q-. Thirty percent threshold has been used most often in several studies to explore the power of CD38 expression in distinguishing patients requiring treatment from those with stable disease, even though some authors found prognostic impact at several different cut-off values, i.e. 7%, 10%, 20%. However, all these studies dealt with circulating neoplastic B cells. Michael Keating presented a study on a large series of B-CLL cases investigating the CD38 expression on

bone marrow B-cells and its ultimate prognostic impact on patients treatment free survival. The author clearly demonstrated that a CD38 level of <10% predicted a longer time to treatment.

DISTRIBUTION OF B-CLL MARKERS

Manlio Ferrarini presented data on the distribution of markers detected in a cohort of consecutive B-CLL patients from primary medical centers that likely reflects that found in a "general" B-CLL patient population. Ferrarini's group characterized neoplastic cells from ~500 B-CLL patients for ZAP-70 expression by western blotting (classifying cases as ZAP-70^{strong}, ZAP-70^{weak} and ZAP-70^{neg}), CD38 expression, and Ig-VH gene mutation status. They determined, by ROC curve analysis, 30% as the best cut-off value of CD38 which discriminates between mutated and unmutated cases in B-CLLs. Furthermore, they combined the value of CD38 and ZAP-70 tests to evaluate whether both variables provided more precise information in estimating VH mutational status compared with that obtained from each single test (10). In this regard, they obtained the following results: sensitivity, 42%; specificity, 97%; positive predictive value, 90%; negative predictive value, 72%; k statistic 0.43, $P < 0.001$. Moreover, ROC analysis was performed to detect the optimal percentage of IgV gene mutations capable of predicting cases with positivity of both CD38 and ZAP-70. The best cut-off value was 1.5% (AUC 0.841, $P < 0.0001$). In this first part of this study, Ferrarini demonstrated that neither CD38 nor ZAP-70 by themselves or in combination were able to anticipate IgVH mutational status, meaning that neither the single marker nor the combined use of CD38 and ZAP-70 could surrogate the IgVH mutational status.

In the second part of this study Ferrarini validated these findings on clinical grounds. Clinical information was available for 150/500 B-CLL cases investigated (11). After a median follow-up of 38 months, 83 cases remained untreated, while 67 cases received treatment. These markers predict clinical course when using time to first treatment (TTT) as a measure for disease progression. Each of the three markers was capable of discriminating two distinct groups of patients ($P < 0.0001$ for CD38, $P < 0.00001$ for ZAP-70 and IgVH mutations) with different clinical behavior, although marker combinations provided a more precise definition of prognosis. Although many patients expressed all favorable or all unfavorable markers, there also were patients with different marker combinations. Using a scoring system that subdivides patients based on the absence (score 0) or presence of 1 (score 1), 2 (score 2), or 3 (score 3) unfavorable prognostic markers, this research team identified 3 groups with significantly different clinical courses: i.e., low-risk (score 0), intermediate-risk (score 1), and high-risk (score 2-3) patients. They concluded that this scoring system has potential utility as a prognostic stratifier of B-CLL cases in designing prospective clinical trials.

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