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 CD38high) and those with <30% CD38+ B-CLL cells (termed CD38low), determining phenotypic differences underlying their biology. Clonal cells from CD38high cases resemble cells that have been more recently activated than those from cells of CD38low 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
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. Moreover, clonal antibody or with the CD31 ligand, as compared with CD38−/ZAP-70− B-CLL cells. Furthermore, 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, thus initiating the CD38 signaling pathway. These signals would contribute to the maintenance of a tumor reservoir and to the enhanced aggressiveness of this disease variant.

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 mono
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-70strong, ZAP-70weak and ZAP-70weak), CD38 expression, and IgVH 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.

**REFERENCES**