CD49d Expression in Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Lymphoma: A Reference Laboratory Perspective

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Introduction
CD49d expression is an independent predictor of disease progression in chronic lymphocytic leukemia based on academic studies. However, its performance in a reference laboratory setting has not been described. Reference laboratories have unique constraints due to specimen transportation and the processing of large numbers of specimens. We sought to test whether such a setting could reproduce similar results. In addition, we hypothesize that measuring the expression of CD49d on CLL/SLL cells could replace the problematic ZAP-70 assay. This study compares CD49d expression to other prognostic indicators including ZAP-70 and Cytogenetics/FISH, which is considered a gold standard for prognosis.

Methods
- Forty-four consecutive cases of CLL/SLL were submitted to the NeoGenomics Laboratories in Aliso Viejo, CA, and evaluated for CD49d and ZAP-70 expression. Sixteen cases had concurrent Cytogenetics/FISH performed. FISH probes included: 6q [-SEC63 (6q21), MYB (6q23)], ATM [11q22.3], p53 [17p13.1], Trisomy 12 (Cen 12), 13q/-13 (13q14, 13q34), and CCND1/IgH t(11;14).
- Antibodies used for immunophenotyping are shown in Table 1. The first five were purchased from Beckman Coulter, Miami, FL: CD3-FITC, CD19-PC-Cy 5.5, CD45-Krome Or, ZAP-70-PE (Cell Signaling Technology, Beverly, MA, clone 75F12, rabbit IgG monoclonal)

Results
The cascade gating strategy for analysis of the flow cytometry data is shown in Figure 1. Starting from the upper left, then upper right, then middle-right, then left and finally bottom, each plot is gated on the region in the previous plot.

Figure 1. Sequential gating strategy

Figure 3. ZAP-70 expression in positive, negative and indeterminate cases

Both percent positive staining and the MFI ratio of CD3+ T-cells divided by CD19+5+ B-cells were measured. Either greater than 20% staining or an MFI ratio <3.0 was considered elevated ZAP-70 expression in CD19+5+ B-cells. Nineteen of the 44 cases (43%) had elevated CD49d and 12 (27%) had elevated ZAP-70 (Table 2). There was no correlation between these two variables.

Table 2: Elevations of ZAP-70 and CD49d expression are not correlated

Since the percentage of ZAP-70 expression has been shown to be unreliable, we also evaluated the ratio of the MFI for T-cell ZAP-70 to the MFI for CD19+5+ cell ZAP-70. There was still no correlation with CD49d expression. ZAP-70 and CD49d expression were evaluated in comparison to Cytogenetics/FISH testing. When analyzing these results, normal cytogenetics/FISH results and 13q deletion were considered good prognostic indicators. Other genetic changes were considered a bad prognosis. Fisher’s test of ZAP-70 percent and ratio showed no correlation with Cytogenetics/FISH indicators (Table 3).

Table 3: Lack of correlation between Cytogenetics/FISH and ZAP-70

However, CD49d expression did correlate with FISH/Cytogenetics (Table 4) as previously reported. Fisher’s exact test for the 2x2 table gave a P value of 0.035.

Table 4: CD49d expression is correlated with Cytogenetics/FISH prognosis

Conclusions
CD49d expression can easily and objectively be measured for CLL patients in a reference laboratory setting even with sample delays in testing. There is a significant correlation between CD49d results and parallel Cytogenetic/FISH testing. CD49d expression did not correlate with ZAP-70 expression. Our results are consistent with previous studies of CD49d expression in an academic setting. In spite of the constraints found in a reference lab setting, measurement of CD49d by flow cytometry can be used to provide valuable information on CLL prognosis. Variables such as delays in testing, bulk processing, and gating do not impact CD49d results. Due to the lack of reproducibility and short sample stability issues with ZAP-70, a superior and more reproducible alternative is CD49d. We conclude that CD49d is a more robust flow cytometry test which can replace the outdated and problematic ZAP-70 assay.

References