

Changes in Antigen Expression in a Follow-up of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

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Abstract

Objectives: Diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) depends on specific immunophenotype of select antigens and their characteristic density. However, reports detailing how the expression of specific antigen changes over time of disease course are sparse.

Methods: We identified 37 CLL/SLL with initial and follow-up samples studied by flow cytometry. Expression levels of a panel of antigens were compared between initial and follow-up samples from the same patients.

Results: There is a significant decrease in the CD23 density and in the percentage of CD23 positive cells, and a decrease in the density of CD5 in the follow-up samples. In contrast, a significant increase in the density of HLA-DR antigen was observed in the follow-up.

Conclusion: The expression of selected antigen changes in the course of CLL/SLL. These alterations do not impact the diagnosis of CLL/SLL based on the immunophenotype of follow-up samples and should not influence the ability to determine minimal residual disease by flow cytometry. However, they can potentially influence the responsiveness to the monoclonal antibody therapy and should be further systematically evaluated.

Keywords: Flow cytometry; CLL; Immunophenotyping

Introduction

According to the World Health Organization, the diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/ SLL) is based on the combination of morphologic, immunophenotypic, laboratory and clinical features [1]. Flow cytometry plays a paramount role in the diagnosis by establishing the characteristic immunophenotype and, in cases of primarily leukemic presentation, by defining the diagnostic threshold of peripheral blood lymphocytosis [2]. Typical CLL/SLL is characterized by the expression of B-cell markers including CD19, low-density CD20 antigen, and the positivity for CD5, CD23, CD43 and low density surface immunoglobulin light chain. The FMC7 is typically negative. These markers allow for a confident diagnosis of CLL/SLL and have been a cornerstone of the CLL/SLL scoring system [3,4]. They are also included in the automated pattern-guided principal component analysis classification of B-cell lymphoproliferative disorders [5]. Even though the majority of CLL/SLL patients present with a typical immunophenotype, cases with atypical immuno phenotypic features are not infrequently encountered. Similar to CLL/SLL with typical immunophenotype, some of these cases are also associated with presence of genetic abnormalities [6]. These cases may be challenging to diagnose since they are also more frequently associated with atypical morphological and/or clinical presentation. Due to the expansion of therapeutic options and more widespread clinical trials, patients with CLL/SLL are increasingly immunophenotyped at various time points after initial presentation either for the confirmation of the original diagnosis or to monitor residual disease. Flow cytometric immunophenotyping may also be relevant for the therapeutic decision making as the efficacy of the immunotherapy may be dependent on the level of expression of the target antigen as it has been demonstrated for rituximab and CD20 antigen [7-9].

Extensive literature describes the CLL/SLL immunophenotype at the time of initial diagnosis. However, the data on the modulation of antigen expression during the natural course of CLL/SLL and following

J Leuk ISSN: 2329-6917 JLU, an open access journal chemotherapy/immunotherapy are sparse. In a limited case series, Kusenda et al. compared the antigen expression of 13 CLL/SLL cases at the time of initial diagnosis with 10 unrelated patients with CLL/SLL following treatment [10]. Even though differences in the expression levels of select antigens were seen, small study sample size and potential variations among individuals did not allow for definitive conclusions. In the current study, we have evaluated the antigen expression in paired samples from 37 patients with CLL/SLL at the time of initial diagnosis and in the follow-up samples.

Material and Methods

Patients and samples

The review of the archives of Clinical Flow Cytometry Laboratory at Indiana University between years 2005-2011 showed more than 400 samples with the diagnosis of CLL/SLL and a detailed immunophenotypic analysis. The immunophenotypes at the time of initial diagnosis and at the follow-up of the disease were available for 37 individuals. There were 24 males and 13 females with a median age of 64 years (age range 46-84 years). The samples included 30 bone marrows (BM), 32 peripheral blood specimens (PB), 10 lymph nodes (LN), and 2 spleens. The follow-up samples were collected at least one month apart from the initial sampling (1 month to 3 year intervals). All the patients met the criteria for CLL/SLL diagnosis according to 2008 WHO classification including diagnostic threshold of $\geq 5.0 \times 10^{9}$ /L

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monoclonal lymphocytes and/or evidence of lymph node involvement. Most patients showed either lymphocytosis or lymphadenopathy at the time of initial diagnosis. The diagnosis was reviewed independently by two hematopathologists. A subset of patients was tested for cytogenetic abnormalities and 75% of tested patients showed cytogenetic alterations. A significant portion of patients (23 out of 37) were treated with chemotherapy after initial diagnosis. Seven patients did not receive any treatment. The treatment status of seven patients remains unknown after careful review of available clinical charts. When comparing the follow-up vs. initial presentation, approximately 74% of patients showed a reduction in WBC count whereas lymphadenopathy appeared to persist. The detailed clinical information is listed in Table 1.

Flow cytometric immunophenotyping

Patient samples were analyzed using four-color flow cytometry. Dependent on the sample type, the red blood cell depletion with ammonium chloride was performed. Total cell count was determined and the sample viability was analyzed with 7-AAD staining. The antibodies were directly conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-Cy5 (PC5) or PE-Texas Red (ECD). The following antibodies were analyzed: CD45-ECD, HLA-DR-PC5, CD38-PC5, CD71-FITC, CD33-PE, CD3-ECD, CD5-FITC, CD7-PE, CD4-FITC, CD8-PC5, CD56-PC5, CD19-FITC (or PE), CD20-PE (or PC5), CD10-PC5, CD22-PE, CD23-ECD, FMC7-FITC, kappa-FITC and lambda light chains-PE. When comparing geometric mean of fluorescence intensity (GMFI) of specific antigen expression, the antibody conjugated with same fluorochrome was used. All antibodies were purchased from BD Biosciences (San Jose, CA) except for kappa and lambda light chain antibodies which were obtained from DAKO (Houston, TX).

Data acquisition was performed using Coulter EPICS XL or Coulter

FC-500 flow cytometers using the Expo System II or CXP software, respectively. At least 100,000 events/cells were acquired in each sample. Data was analyzed using EXPO32 and CXP software (Beckman Coulter, Miami, FL, USA). The cellular debris was excluded before the analysis. Depending on the primary antibody combination, the cells of interest were gated on right angle light scatter vs forward scatter, or on right angle light scatter vs. CD45 display. Antigen expression was assessed quantitatively in relation to available controls. Briefly, the antigen was considered positive when the majority of cells expressed a given marker. Partial expression represented the presence of separate positive and negative populations. Heterogeneous distribution indicated variable fluorescence intensity from negative to bright positive cells. Percentages of cells positive for given antigen and/or GMFI were obtained and compared between the diagnostic and follow-up samples from the same patients. Isotype controls were used as negative controls. Frozen stock mononuclear cells with known antigen expression level were used as positive controls to ensure the consistency of antigen GMFI measurement. These controls showed no systematic bias in the antigen expression levels that could influence obtained results (data not shown). The typical CLL/SLL immunophenotype was defined as positive for CD19, CD5, CD23, CD22, low density expression of CD20, and low density expression of immunoglobin surface light chain [3,4]. CD10 and FMC7 were typically negative. The atypical immunophenotypic features included expression of FMC7, absence of CD5 or CD23 antigen expression as well as moderate to bright CD20 and/or surface immunoglobin light chain. In patient 4 and patient 8, immunohistochemical study of cyclin D1 was performed and it was negative, excluding the possibility of mantle cell lymphoma.

Statistical analysis

For all variables, their means and standard deviations were calculated. To establish the statistical significance, the paired t test was used. A p value less than 0.05 was defined as statistically significant.

| Pt # | Gender | Age at Dx | Sample | Initial FU | FU | Initial Lymphadenopathy | FU Lymphadenopathy | Treatment | Atypical Immunophenotype | ZAP-70/ CD38 | Cytogenetics |
|------|--------|--------------|---------------|-----------------|-----------------|----------------------------|-----------------------|-----------------------------|-----------------------------|-----------------|---------------------------|
| | | | | Ly # (k/ ul) | Ly # (k/ ul) | | | | | | |
| 1 | F | 55 | BM, BM | 5.3 | 2.6 | Yes | Yes | FCR | No | 20% / 4% | 46,XX |
| 2 | F | 61 | PB, BM | 3.1 | 1.5 | Yes | N/A | RCHOP | No | N/A / 12% | N/A |
| 3 | F | 62 | PB, BM | 26.2 | 20 | Yes | Yes | N/A | No | N/A / 1% | 46,XX |
| 4 | F | 59 | LN, BM | 5.4 | 0.8 | Yes | Yes | RCHOP | Negative CD5 | 16% / 42% | 47,XX,+12 |
| 5 | F | 67 | BM, PB | 215 | 149 | Yes | Yes | FCR | No | N/A / N/A | 46,XX,del(13) (q12q14) |
| 6 | М | 57 | PB, Spleen | 73.2 | 1.9 | No | Yes | FCR | No | 94% / 73% | N/A |
| 7 | М | 62 | PB, BM | 39.9 | 2.4 | No | No | FCR | No | N/A / N/A | N/A |
| 8 | F | 62 | BM, PB | N/A | N/A | Yes | Yes | FCR | Bright CD20 and FMC7+ | 2% / 23% | Del(11)(q13q23) |
| 9 | М | 54 | PB, BM | 6.3 | 2.3 | N/A | N/A | Chlorambucil and Rituxan | No | 1% / 1% | N/A |
| 10 | М | 55 | PB, PB | 195 | 41.3 | N/A | N/A | Fludarabine | No | 6% / 96% | Del11q22.3/ Del13q14.3 |
| 11 | М | 56 | BM, BM | 18.4 | 1.6 | Yes | Yes | Bendamustine | No | 40% / 80% | N/A |
| 12 | M | 84 | PB, PB | N/A | 5.8 | N/A | N/A | N/A | No | 70% / 21% | N/A |
| 13 | М | 56 | LN, BM | 2.4 | 39.8 | Yes | Yes | RCVP | No | N/A / N/A | Complex cytogenetics |
| 14 | М | 70 | PB, PB | 10.7 | 10.2 | No | No | No | No | 36% / 85% | Del(11) (?q13q23),+12 |
| 15 | М | 64 | BM, BM | 31.8 | 14.2 | No | Yes | FCR | No | N/A / N/A | Complex cytogenetics |
| 16 | F | 48 | LN, LN | 145.7 | 0.1 | Yes | Yes | RCHOP | No | N/A / 69% | N/A |
| 17 | М | 72 | PB, LN | 72.6 | N/A | No | Yes | No | No | N/A / 21% | N/A |
| 18 | М | 67 | LN, LN | N/A | N/A | Yes | Yes | FCR | Moderate light chain | N/A / 85% | N/A |
| 19 | М | 64 | PB, PB | 3.7 | 2.2 | Yes | Yes | FCR | No | N/A / 35% | N/A |
| | | | | | | | | | | | |

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| 20 | М | 66 | BM, BM | 31 | 1.7 | N/A | N/A | FCR | Moderate light chain | 61% / 38% | 47,XY Trisomy 12 |
|----|---|----|---------------|-------|-----|-----|-----|------------------------------------|----------------------|-----------|-------------------------------|
| 21 | М | 52 | PB, BM | 74.5 | 248 | N/A | N/A | N/A | Moderate light chain | N/A / N/A | N/A |
| 22 | М | 80 | LN, BM | N/A | 0.5 | Yes | N/A | Fludarabine | No | 44% / 77% | 47,XY,+12 |
| 23 | М | 64 | PB, PB | 71 | 62 | N/A | N/A | N/A | No | 1% / 1% | N/A |
| 24 | М | 68 | PB, PB | 20.6 | 3.8 | No | Yes | No | No | N/A / 81% | N/A |
| 25 | М | 81 | BM, PB | 5.2 | N/A | N/A | N/A | No | No | 2% / 9% | 46, XY. |
| 26 | М | 63 | BM, Spleen | 42.7 | 0.2 | Yes | Yes | FCR | Moderate light chain | 64% / 14% | 47,XY,t(6;7) (p23;p22),+12 |
| 27 | М | 50 | PB, BM | 20.1 | 304 | No | Yes | No | No | 3% / 3% | 13q14 deletion |
| 28 | F | 77 | PB, BM | 21 | 3 | Yes | Yes | RCVP | No | N/A / 60% | 46,XX |
| 29 | F | 81 | PB, BM | 5.8 | 2.3 | No | No | No | No | N/A / 78% | Complex cytogenetics |
| 30 | F | 73 | BM, BM | 14 | 1.7 | Yes | Yes | RCVP | No | 1% /59% | 46,XX |
| 31 | М | 71 | LN, BM | 5.5 | 29 | Yes | Yes | N/A | No | 6% / 9% | N/A |
| 32 | М | 78 | PB, LN | 24 | N/A | Yes | Yes | N/A | No | 1% / 1% | N/A |
| 33 | F | 77 | BM, PB | 29 | 24 | Yes | Yes | FCR | No | 80% / 78% | 47,XX,t(2;14) (p15;q32) |
| | | | | | | | | | | | +12 |
| 34 | М | 49 | BM, BM | 3.4 | 1.2 | Yes | Yes | FCR and stem cell transplant | No | N/A / N/A | Complex cytogenetics |
| 35 | М | 62 | BM, PB | 145.7 | 1.1 | No | Yes | FCR | No | 35% / 7% | Complex cytogenetics |
| 36 | F | 73 | PB, BM | 47 | 260 | Yes | Yes | No | No | 22% / 14% | 47,XX,+12 |
| 37 | F | 46 | PB, PB | 8.4 | 4.1 | Yes | Yes | N/A | No | 54% / 2% | N/A |

FCR: fludarabine, cyclophosphamide, and rituximab

RCHOP: rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone

RCVP: rituximab, cyclophosphamide, vincristine and prednisone

Table 1: Patient's characteristics.

Results

Immunophenotype of initial samples

The majority of the samples demonstrated a typical CLL/SLL immunophenotype: positive for CD19, CD20 (dim), CD22, HLA-DR, CD5, CD23, surface immunoglobulin light chain (dim), and negative for CD10 and FMC7 (Figure 1). Among all antigens tested, CD19 and HLA-DR demonstrated a uniformly bright expression pattern in the majority of neoplastic cells. The CD5 expression by CLL/SLL cells was evident in the majority of cases showing the CD5 expression levels slightly lower than those of CD3-positive T cells. The expression of CD22 antigen detected by flow cytometry was distinct but relatively dim compared to normal B cell population. In our study, neoplastic B cells demonstrated a dim to moderate CD23 antigen expression. In addition, there were considerable variations among individual patients in regards to the expression levels of the following antigens: CD19, CD20, CD22, CD5, CD23, HLA-DR, and immunoglobulin light chains. Although the majority of cases showed a typical CLL/ SLL immunophenotype, there were 6 cases with one or more atypical immunophenotypic features. The most common atypical finding was brighter than expected expression of immunoglobin light chains seen in 4 cases (~10% studies cases). One case was negative for CD5 antigen. One case showed simultaneous positive FMC7 and bright CD20 expression. Other markers characteristic for non-CLL type B cell lymphoproliferative disorder such as CD10 were not identified in these atypical CLL cases. Therefore, despite some atypical immunophenotypic features, the overall morphology, immunophenotypic and cytogenetics features of these cases were most compatible with CLL/SLL. These atypical immunophenotypic features remained unchanged in the follow up samples (Table 1). For example, an absence of CD5 antigen was seen in both initial and follow-up samples of patient 4.

Few patients had concurrent samples from multiple sites including

5 patients with concurrent samples from peripheral blood and bone marrow, 1 patient with concurrent samples from peripheral blood and lymph node, and 1 patient with concurrent samples from bone marrow and lymph node. The surface antigen expression pattern and expression density were compared between different anatomic sites and showed no overt differences. However, the number of the cases was too low for a statistical analysis.

Comparison of the immunophenotype of initial and followup samples

When comparing initial and follow-up samples, statistically significant differences were identified in the expression of CD23, HLA-DR and CD5 antigens. The expression of CD23 antigen was found to be decreased in the follow-up samples. The decrease in the percentages of CD23 positive B cells and the decrease in the density of CD23 antigen were both seen. Seventy-eight percent of patients showed a decrease in the percentage of CD23 positive B cells in the follow-up samples (Figure 2A, p<0.01). The extent of decrease varied among patients. The mean percentage of CD23 positive B cells decreased from 81% in initial samples to 72% in the follow up samples with the greatest reduction of approximately 33% (from 98% in the initial sample to 66% in the follow-up specimen). In addition, when comparing the initial and follow-up samples of the same patients, 72% of patients showed an average 19% decrease in CD23 antigen density in CD23-positive B cells as defined by changes in GMFI (from initial 4.6 to follow-up 3.7) (Figure 2B). Despite the variation in antigen expression among different patients, the decrease in CD23 antigen density in CD23positive B cells was statistically significant (p<0.01). Approximately 55% of patients showed both decrease in frequency of CD23-positive B cells and decreased CD23 antigen density as illustrated in Figure 2C.

Seventy seven percent of patients showed decreased CD5 antigen expression in the follow-up samples as depicted by GMFI (initial Citation: Zhou J, Tewari A, Nassiri M, Czader M (2015) Changes in Antigen Expression in a Follow-up of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. J Leuk 3: 177. doi:10.4172/2329-6917.1000177



10.6, follow-up 8.9) (Figures 3A and B). This change represented a 16% reduction in CD5 antigen expression levels and was statistically significant by paired t-test (p<0.05). In contrast, the percentages of CD5-positive cells remained stable when comparing follow-up samples and initial samples (follow-up 88% vs. initial 87%). The patient with atypical CLL/SLL immunophenotype showing negative to very dim CD5 was excluded from this analysis.

On the contrary, 81% of follow-up samples showed increased HLA-DR expression. This change was statistically significant with an average of 54% increase in GMFI (from initial 16.2 to follow-up 25.0, p<0.01, Figures 4A and B). There was no difference in the percentages of HLA-DR-positive cells as this antigen was uniformly expressed in the CLL/SLL cells from the initial and follow-up samples (follow-up 94% vs. initial 94%).

To confirm the above discussed differences in antigen expression, we have performed a separate statistical analysis in treated and untreated patients. Twenty three patients were treated with various regimens after initial diagnosis, 7 patients received no treatment and 7 patients' treatment status was unknown. The differences in the expression of CD5, CD23 and HLA-DR remained statistically significant in patients who received chemotherapy. Similar trends were seen in untreated patients. A statistical significance was achieved when comparing CD5 expression between diagnostic and follow-up samples (p=0.04). The expression of HLA-DR (p=0.19) and of CD23 (p=0.07) were not significantly different, however the numbers of untreated patients may be too low for an adequate statistical analysis.

There was no statistically significant difference between initial and follow-up samples in respect to the percentage of cells and GMFI of CD19, CD22 and surface immunoglobulin light chain. The analysis of the percentages of CD20 positive cells and GMFI for CD20 was not performed due to a significant subset of patients treated with anti-CD20 therapy.

Discussion

Typical CLL/SLL is diagnosed by a combination of clinical, laboratory and morphological features as well as characteristic flow cytometric immunophenotype: positive for CD19, CD20 (dim), CD22, CD5, CD23, HLA-DR, sIg(dim), and negative for CD10 and FMC7. The clinical course of CLL/SLL is highly heterogeneous and the patients are either treated at time of diagnosis or simply monitored depending on the severity of clinical symptoms and disease stage [11-13]. Repeated flow cytometry is sometimes performed for disease follow-up [14]. Our data demonstrated that in CLL/SLL the follow-up samples show immunophenotype similar to those seen in the initial diagnostic material, and thus allow a pathologist to render a confident diagnosis of CLL/SLL based on the follow-up specimens. Nevertheless, for select antigens significant differences occurred in the percentages of positive neoplastic cells and/or in the antigen density. The most prominent differences were seen in the expression of CD23, CD5 and HLA-DR.

CD23 is a trans-membrane glycoprotein typically present on activated mature B-lymphocytes. It functions as a B cell growth and activation factor, promoting rescue of germinal center cells and

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Figure 2A: Down-regulation of CD23 in the follow-up samples. A. Decrease in the frequency of CD23-positive B cells in the follow-up sample. The percentages of CD23-positive B cells in the initial and follow-up samples from the same patients were compared (n=36, paired t-test p<0.01).



differentiation into plasma cells. Typical CLL/SLL cells express CD23 antigen, however, Juristic et al. [15] reported that the percentage of CD23 positive cells and CD23 antigen expression level is highly variable among CLL/SLL patients. In Juristic's study, the higher percentage of CD23 positive cells was associated with longer survival and lower percentages of CD23 positive cells were seen in the advanced stages of the disease and in patients with high lymphocyte counts. Our study shows a significant decrease in percentage of CD23-positive cells and a decrease in CD23 antigen density in CLL/SLL cells in the follow-up samples. It is conceivable that the low CD23 expression seen in the advanced stages of the disease reported by Jurisic et al. is due to diagnosing these patients later in the course of the disease. In the current study, the lower CD23 expression in the follow-up CLL/SLL was not associated with increased leukocyte count as reported in Juristic's article (Table 1), which might be due to the fact that a significant portion of patients were treated. Furthermore, the changes in CD23 expression reported in our study may also have implications for therapy decision making. The humanized anti-CD23 has been developed and used in clinical trials [16]. A phase I/II clinical trial showed that the addition of anti-CD23 to fludarabine, cyclophosphamide, and rituximab (FCR) in previously treated CLL/SLL patients resulted in a higher complete response rate, and did not appear to enhance toxicity, indicating that anti-CD23 synergistically enhances antitumor activity of FCR [17]. The investigation of the relation between the levels of CD23 expression and clinical response may be beneficial to achieve the best therapeutic effect without inadvertent addition of toxicity.

CD5 is typically considered as T cell associated antigen which acts as a co-receptor in the stimulation of T cell growth [18]. The upregulation/expression of CD5 antigen in B cells is observed in a variety of B-cell lymphoproliferative disorders including CLL/SLL, mantle cell lymphoma and a subset of diffuse large B cells lymphomas. The CD5 antigen and other markers including CD23, FMC7, surface immunoglobin light chain and CD22/CD79b have also been used in the immunophenotyping scoring system to distinguish CLL/SLL from other chronic B-cell malignancies with acceptable sensitivity and specificity [19,20]. Previous studies have indicated that the



Figure 2C: Representative flow cytometry histogram of CD23 expression demonstrating a concurrent decrease in the frequency of CD23-positive B cells and decreased CD23 antigen density in the follow-up CLL/SLL samples.



Figure 3A: Downregulation of CD5 antigen expression in the follow-up CLL/ SLL. **A.** Surface CD5 expression levels denoted by GFMI were compared between initial and follow-up samples from the same subjects (n=35, paired t-test p<0.05).

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Figure 3B: Representative flow cytometry histogram of CD5 expression demonstrating a decrease of antigen density in the follow-up CLL/SLL samples.



Figure 4A: Upregulation of HLA-DR antigen expression in the follow-up CLL/ SLL samples. **A.** HLA-DR expression levels denoted by GMFI were compared between initial and follow-up samples from the same subjects (n=37, paired t-test p<0.01).

expression of CD5 in CLL/SLL is associated with chronic activation of B cell receptors, anergy and a survival of malignant B cells [21,22]. Our current study shows a statistically significant decrease in CD5 antigen density in the follow-up as compared to the initial diagnostic samples. The biological significance of this finding is not clear. The decrease in CD5 can be related to treatment since a significant portion of the patients in our study have been treated. Kusenda et al. reported a slight increase in CD5 antigen expression in CLL/SLL patients after treatment [10]. However, this previous study did not include paired, pre- and post-treatment samples and was limited by a low number of cases.

HLA-DR is a part of class II major histocompatibility complex,

normally seen on the surface of antigen presenting cells including macrophages, dendritic cells and B lymphocytes. The primary function of HLA-DR is to present peptide antigens to the immune system for the purpose of eliciting or suppressing T helper cell responses leading to the production of antibodies against the same peptide antigen. Apolizumab, a humanized anti-HLA-DR monoclonal antibody, was initially tested to treat relapsed CLL/SLL. Due to the toxicity and lack of response, the trial was discontinued [23]. Most recently, another monoclonal Ab, IMMU-114 was used in a CLL/SLL clinical trial. The IMMU-114 is a humanized IgG4 form of the murine anti-human HLA-DR. Unlike most of other therapeutic monoclonal Ab (mAbs), which exert the in vivo effects largely through immunologic effector mechanisms dependent on intact immunologic function, IMMU-114 acts through direct cytotoxicity, independent of recipient's own immunologic status. Most of mature B cell lymphomas express HLA-DR antigen, making it a potential target for anti-HLA-DR immunotherapy [24,25]. Our current study shows that CLL/SLL express bright HLA-DR and more interestingly that the HLA-DR antigen levels increase over the time of disease course. The HLA-DR expression pattern and kinetics make the anti-HLA-DR mAb a promising reagent to treat CLL/SLL at the time of initial diagnosis and later in the course of the disease.

To evaluate whether the above discussed differences in antigen expression are seen in both treated and untreated patients, we have performed a separate statistical analysis in these patient groups. The treated cohort included 23 patients. There were only 7 untreated patients. The differences in the expression of CD5, CD23 and HLA-DR antigens remained statistically significant in patients who received chemotherapy. Similar trends were seen in untreated patients, however the differences in the expression of HLA-DR and CD23 did not reach statistical significance. The low number of patients in the untreated group is a significant drawback in this analysis and the results should be confirmed in a larger cohort of untreated patients.

In conclusion, to our knowledge, this study is the first flow cytometric evaluation of changes in antigen expression during the course of CLL/ SLL in paired diagnostic and follow-up samples from the same patients. Statistically significant decrease in the expression of CD23 and CD5, as



Figure 4B: Representative flow cytometry histogram of HLA-DR expression demonstrating an increase in antigen density in the follow-up CLL/SLL sample.

well as increase in HLA-DR expression was identified. These alterations can potentially influence the responsiveness of the disease to the monoclonal antibody therapy and should be further systematically evaluated. Despite the significant changes in antigen expression seen in the follow-up samples in the majority of cases, the diagnosis of CLL/SLL can still be rendered based on the follow-up immunophenotype.

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