Clonally-Related CD5+ CLL/SLL and CD10+ high grade B-cell lymphoma suggests common neoplastic progenitor with branched disease evolution, with therapeutic implications

Priyadarshini Kumar\textsuperscript{a,*}, Manik Uppal\textsuperscript{b,*}, Wenbin Xiao\textsuperscript{a}, Ahmet Dogan\textsuperscript{a}, Mikhail Roshal\textsuperscript{a}, Qi Gao\textsuperscript{a}, Umut Aypar\textsuperscript{c}, Yanning Zhang\textsuperscript{c}, Maria E. Arcila\textsuperscript{a,d}, Christine Moung\textsuperscript{d}, Jinjuan Yao\textsuperscript{d}, Khedoudja Nafa\textsuperscript{d}, Wayne Yu\textsuperscript{d}, Mustafa H. Syed\textsuperscript{d}, Jae Park\textsuperscript{e}, Anita Kumar\textsuperscript{f,*}, Caleb Ho\textsuperscript{a,d,*}

\textsuperscript{a}Hematopathology Service, Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA
\textsuperscript{b}Weill Cornell Medical College, New York, NY, USA
\textsuperscript{c}Cytogenetics Service, Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA
\textsuperscript{d}Molecular Diagnostics, Department of Pathology, Memorial Sloan Kettering Cancer Center
\textsuperscript{e}Leukemia Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA
\textsuperscript{f}Lymphoma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Approximately 15% of chronic lymphocytic leukemia/small lymphocytic lymphomas (CLL/SLL) transform into aggressive lymphomas, often diffuse large B cell lymphomas (DLBCL), known as Richter Syndrome (RS) \cite{1}. RS shows poor prognosis with a median survival of 2.5–8 months \cite{2}. Recent studies demonstrated that RS is driven by two major, mutually-exclusive genetic pathways. The main pathway involves inactivation of \textit{CDKN2A/B} and loss of \textit{TP53}, along with \textit{MYC} amplification or translocation, and \textit{13q14} loss in a subset of cases \cite{3}. The clonal relationship between CLL/SLL and the aggressive lymphoma is a critical factor influencing treatments. On the basis of direct \textit{IGHV} gene sequencing, studies have found that 80% of RS cases featured clonal relationships between both lymphoma
populations [4]. The clonally unrelated cases had a much longer median survival (~5years) [4], despite more complex genomic alterations seen in de novo DLBCL compared to RS [5]. Therefore, the clonal relationship affects therapeutic choices and risk/benefit calculus regarding the role of autologous or allogeneic stem cell transplantation (alloSCT) consolidation.

Herein, we describe a 49-year-old male with CD5+ CLL/SLL associated with CD10+ high grade B cell lymphoma (HGBCL). We provide unequivocal molecular evidence of their clonal relationship despite stark differences in immunophenotype. Furthermore, we postulate that the CLL/SLL and HGBCL likely arose from a common neoplastic progenitor, in a branched pattern of disease evolution.

The patient presented in June 2006 with low-volume lymphadenopathy, mild splenomegaly, and lymphocytosis (WBC 17.0K/mcL). He was diagnosed with CLL/SLL and placed on observation. In November 2008, the patient presented with bulky lymphadenopathy and WBC ~40K/mcL, prompting treatment with six cycles of fludarabine, cyclophosphamide, and rituximab with good response. An end-of-treatment computed tomography (CT) scan showed resolution of lymphadenopathy with low level persistent CLL/SLL in peripheral blood (PB) (~6–7% of WBC by flow cytometry (FC)). In December 2013, the patient developed diffuse, palpable lymphadenopathy, splenomegaly, and PB absolute lymphocytosis (64.8K/mcL). Bone marrow biopsy (BMBx) in January 2014 showed marrow with extensive involvement by CLL/SLL. Fluorescence in situ hybridization (FISH) studies showed 13q and 17p deletions. In February 2014, the patient showed disease progression (WBC 115.4 K/mcL) and started on ibrutinib. Given the patient’s young age and 17p alteration, alloSCT was discussed, but the patient declined.

In August 2017, the patient was admitted with pancytopenia and febrile neutropenia. Prednisone and intravenous immunoglobulin were given for suspected autoimmune hemolytic anemia. PET/CT revealed diffuse FDG uptake throughout the marrow, splenomegaly, and diffuse lymphadenopathy. Brain MRI showed no leptomeningeal disease or intracranial abnormality, but demonstrated marrow abnormality in the left mandibular ramus. A BMBx demonstrated extensive involvement by HGBCL, coexisting with CLL/SLL. Cytogenetic and molecular studies were performed and are described below (Figure 1 and Table 1).

The patient then underwent 4 cycles of dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; rituximab (R) was omitted because of CD20 negativity in HGBCL). After cycle 2, lumbar puncture exhibited equivocal evidence of leptomeningeal disease, prompting addition of intra-thecal methotrexate. Despite an initial disappearance of circulating HGBCL, after cycle 4 he developed marked lymphocytosis and cytopenias, consistent with primary refractory disease. A subsequent BMBx demonstrated persistent disease: 70% HGBCL and 30% CLL/SLL. In November 2017, the patient was started on IVAC (etoposide, ifosfamide, cytarabine, methotrexate) with plan for alloSCT consolidation. The patient’s course after two cycles was complicated by neutropenic fever, thrombocytopenia, E. Coli bacteremia, respiratory failure with multiple viral infections, bacterial pneumonia, sepsis and subarachnoid hemorrhage, resulting in
delay in his planned alloSCT. Although he achieved remission briefly, in March 2018, the patient was diagnosed with recurrent HGBCL and was started on venetoclax, subsequently pembrolizumab without response. The patient remained refractory to therapies with disease progression and severe cytopenias, requiring daily transfusion. In April 2018, the patient died.

Pathologic examination of the August 2017 BMBx revealed 90% involvement by HGBCL coexisting with 10% CLL/SLL (Figure 1(a–d)). Immunophenotypes of the two populations by immunohistochemical (IHC) staining and FC (Figure 1(e)) demonstrated CLL/SLL: CD20+, PAX5+, CD5+, CD10−, CD23+, LEF1+, CD200+, FMC7−, and negative for BCL2, BCL6 and CMYC; HGBCL: CD20−, PAX5+, CD5−, CD10+, CD23−, LEF1−, CD200−, FMC7−, CD34−, TdT−, CMYC+ and negative for BCL2 and BCL6. The Ki67 was ~90% in the HGBCL and ~10% in CLL/SLL. FISH studies using probes flanking TP53, MYC, IGH were performed on FC-sorted cells from bone marrow aspirates (200–300 cells counted), and revealed loss of TP53 in 85% of CLL/SLL cells, but no loss of TP53 in the HGBCL cells. There was absence of MYC abnormalities in the CLL/SLL cells, but MYC rearrangement in 98% of the HGBCL cells, confirmed as t(8;14)/MYC-IGH by karyotype and IGH/MYC dual fusion probes (Figure 2(a–d) and Table 1).

SNP array-based DNA copy number analysis was performed using Affymetrix CytoScan™ HD Assay. SNP array on FC-sorted cells revealed alterations of chromosomes 13 and 17 in both lymphoma populations. The CLL/SLL population harbored 13q14.2 deletion (hemizygous deletion of RB1 involving the 3′ end of the gene only and homozygous deletion of the entire SETDB2 gene), as well as loss of 17p13.1 to 17p12, encompassing TP53. The HGBCL exhibited homozygous loss of 13q14.2 (also involving the 3′ end of the RB1 gene and the entire SETDB2 gene), and copy neutral-loss of heterozygosity (CN-LOH) of 17p terminal to 17p11.2, including TP53 (Figure 1(f–g) and Table 1).

Somatic mutation profiling was performed using a targeted, 400 gene panel NGS-based assay relevant to hematologic malignancies [6], on FC-sorted cells, with germline variant filtering using patient’s saliva. Both shared and unique mutations can be seen (Table 1). The unique mutations in each lymphoma were confirmed to be absent even at low allele frequencies in the other sample based on manual review.

IGH clonal rearrangement studies were performed using a NGS-based assay, Lymphotrack® (Invivoscribe, Inc., San Diego, CA) on FC-sorted populations and unsorted marrow samples. Clonal calling and analyses were performed as described previously [7]. All samples exhibited a single, identical clonal sequence with unmutated (0.0%) IGHV mutation status, and V-J segment usage of V3-30-J4.

CLL/SLL is characterized by CD5 expression, which is generally retained in most cases of RS [8,9]. A profound shift in immunophenotype during transformation is uncommon. CD10 expression can be seen in follicular lymphoma, DLBCL, Burkitt lymphoma, and some HGBCL, but is exceedingly rare in RS [10,11]. Adjacent lymphomas with profoundly different immunophenotypic profiles can raise the possibility of collision tumors. Nevertheless, we provided unequivocal evidence of clonally-related CD5+ CLL/SLL and
CD10+ HGBCL in the same patient, including shared mutations in SF3B1, TP53, UBR5; shared alterations in chr.13q14 and 17p; and identical clonal IGH rearrangements. Demonstration of the clonal relationship in this patient has important prognostic and therapeutic implications, as RS cases have been shown to have inferior survival rate compared to de novo DLBCL. R-EPOCH would have been potentially curative therapy for a de novo aggressive lymphoma. In contrast, R-CHOP or R-EPOCH would have suboptimal efficacy for RS. In RS cases, consolidation with autologous or alloSCT would be preferable to achieve durable remission [12]. Unfortunately, this patient’s HGBCL recurred before he proceeded with alloSCT.

Large-scale genomic studies have characterized RS as genetically distinct from CLL and DLBCL, with intermediate genomic complexity [3,5]. The HGBCL in our patient harbored a genetic signature consistent with one of the two major pathways implicated in RS [5], including MYC-IGH rearrangement, loss of 13q14 with RB1 disruption, and deleterious TP53 mutations. By immunohistochemical stains, the HGBCL showed the expected finding of high expression of C-MYC. C-MYC protein, a global transcription factor regulating an estimated 10–15% of all human genes, is essential for normal early B-cell development and maintenance of B-cell identity. However, it is repressed in germinal center dark zone to limit the numbers of cell division for normal antigen affinity-dependent maturation. During lymphomagenesis, upregulation of C-MYC protein expression, as a result of translocation or gene amplification, results in a loss of regulation of a variety of cellular pathways involved in cell cycle and growth, metabolism, biosynthesis, and mitochondrial function. It also allows the B cells to circumvent affinity-dependent maturation, increasing the chances of further oncogenic events [13]. The CLL/SLL in our patient exhibited TP53 alterations, which are found more frequently in CLL/SLL with aggressive disease courses [4,5]. Therefore, somatic mutation profiling and cytogenetics studies provided further insight into underlying disease biology.

RS transformation from CLL/SLL most commonly occurs in a linear pattern, through the CLL/SLL clone’s accumulation of additional genetic alterations [3]. Alternatively, CLL/SLL and aggressive B-cell lymphoma can rarely arise in a branched pattern of disease evolution, in which a postulated early neoplastic progenitor gives rise to two tumors. They would share some genetic abnormalities, but also harbor unique alterations through independent evolution, similar to the observation in our case. Despite the efficacy of Bruton’s tyrosine kinase (BTK) inhibitors such as ibrutinib, phosphoinositide 3-kinase inhibitors, and BCL2 inhibitors in treating CLL, a subset of patients still showed refractory disease, occasionally with the development of aggressive B-cell lymphomas [14]. Several mechanisms to BTK inhibitors resistance and progression of disease under selective pressure from therapy are known, including expansion of subclones and acquisition of additional driver mutations [15,16]. Interestingly, our patient was started on ibrutinib therapy approximately 3.5 years prior to the diagnosis of HGBCL. Although difficult to ascertain, ibrutinib might have provided selective pressure facilitating disease evolution. In the milieu of TP53 alterations and SF3B1 mutation, conferring genetic instability and chemotherapeutic insensitivity, respectively, these two related lymphomas progressed in an aggressive fashion.

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In summary, we describe a 49-year-old male with strong molecular evidence of clonally related CD5+ CLL/SLL associated with CD10+ high grade B cell lymphoma (HGBCL) after ibrutinib treatment. The similarities and differences in genetic alterations between the two suggest the presence of a common neoplastic progenitor with a branched pattern of disease evolution, rather than a linear progression of conventional Richter Syndrome (RS).

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Disclosure statement

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Figure 1.
(a) Low power view showing hypercellular marrow. (b) High power view showing “blastoid” morphology in high grade B-cell lymphoma (HGBCL). (c) High power view showing CLL/SLL. (d) Aspirate smear showing dimorphic population of small lymphocytes with mature chromatin and large lymphoid cells with dispersed chromatin and prominent nucleoli. (e) Flow cytometric plots showing immunophenotype of HGBCL (orange) and CLL/SLL (blue). (f) SNP array findings in HGBCL showing homozygous deletion at 13q14.2 (including RB1 and SETDB2) and CN-LOH of 17p terminal to 17p11.2 (including TP53). (g) SNP array findings in CLL/SLL showing a 13q14.2 hemizygous deletion for RB1 and homozygous deletion for SETDB2 and loss of 17p13.1 to 17p12 (including TP53).
Figure 2.
FISH findings on flow-sorted cells. (a) Loss of TP53 in 85% cells in CLL/SLL component. (b) No loss of TP53 in HGBCL component. (c) No MYC rearrangement in CLL/SLL component. (d) MYC rearrangement in 98% of cells in HGBCL component. (a–b) TP53 (17p13) probe (Red) and CEP17 (chr. 17 centromere) probe (Green). (c–d) MYC (8q24) break-apart probes.
**Table 1.**

Comparison of genetic alterations and Variant Allele Frequencies (VAF) between the CD5+ CLL/SLL and CD10+ High Grade B-Cell Lymphoma.

| Gene      | Type                  | Alteration                   | Location | Sorted CD5+ CLL cells | Sorted CD10+ B-Cell Lymphoma |
|-----------|-----------------------|------------------------------|----------|-----------------------|-----------------------------|
| SF3B1     | Missense mutation     | p.K666E                      | Exon 14  | 49.8%                 | 48.8%                       |
| TP53      | Missense mutation     | p.M237I                      | Exon 7   | 8.6%                  | 99.3%                       |
| UBR5      | Missense mutation     | p.Q1822E                     | Exon 39  | 19.5%                 | 62.4%                       |
| TP53      | Missense mutation     | p.G244C                      | Exon 7   | 29.9%                 | −                           |
| TP53      | Frameshift mutation   | p.L257Gfs*6                  | Exon 7   | 5.3%                  | −                           |
| TP53      | Missense mutation     | p.E271K                      | Exon 8   | 35.7%                 | −                           |
| CIITA     | Missense mutation     | p.S1095I                     | Exon 18  | 22.1%                 | −                           |
| ERG       | Missense mutation     | p.R354Q                      | Exon 10  | 21.6%                 | −                           |
| KMT2D     | Frameshift mutation   | p.G2806Cfs*43                | Exon 34  | 16.8%                 | −                           |
| KMT2D     | Frameshift mutation   | p.P2938Tfs*4                 | Exon 34  | 88.5%                 | −                           |
| KDM5C     | Missense mutation     | p.R1166L                     | Exon 23  | −                     | 97.3%                       |
| SETDB2    | Whole gene deletion   |                              | 13q14.2  | +                     | +                           |
| TP53      | Whole gene loss       |                              | 17p13.1  | +                     | −                           |
| ALOX12B   | Whole gene loss       |                              | 17p13.1  | +                     | −                           |
| RB1       | Intragenic deletion   |                              | Exons 24-25 | +             | −                           |
| RB1       | Intragenic deletion   |                              | Exons 18-27 | −             | +                           |
| TP53      | Whole gene Copy Neutral-Loss of Heterozygosity | 17p terminal to 17p11.2 | − | + |
| MYC       | Whole gene gain       |                              | 8q24.21  | −                     | +                           |
| IGH/MYC   | Translocation         | Translocation                | t(8;14)q24q32 | −             | +                           |

Note: Mutations and copy number alterations are presented with their respective locations and VAFs. Other chromosomal level alterations are also noted.