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

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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) [1]. RS shows poor prognosis with a median survival of 2.5–8 months [2]. Recent studies demonstrated that RS is driven by two major, mutually-exclusive genetic pathways. The main pathway involves inactivation of *CDKN2A/B* and loss of *TP53*, along with *MYC* amplification or translocation, and 13q14 loss in a subset of cases [3]. The clonal relationship between CLL/SLL and the aggressive lymphoma is a critical factor influencing treatments. On the basis of direct *IGHV* gene sequencing, studies have found that 80% of RS cases featured clonal relationships between both lymphoma

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P.K. and M.U. contributed equally to writing the manuscript. J.P. and A.K. provided clinical information on the patient and helped in drafting the manuscript. W.X., A.D., M.R., and C.H. interpreted the histology, flow cytometry, and IHC of the marrow biopsies. U.A. and Y.Z. interpreted the cytogenetics testing results including FISH, karyotype and SNP array analyses. M.A., C.M., J.Y., and C.H. interpreted the molecular assays. Q.G., K.N., W.Y., and M.S. provided technical support for the flow cytometry and molecular assays. All authors reviewed and contributed to the manuscript.

Data availability statement

The complete data supporting the findings in this case report are available from the corresponding author upon reasonable request.

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 49year-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 patien'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 patien'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 patien'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 patien'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.

In summary, we describe a 49year-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

Dr. Arcila has served as a consultant and received honoraria from Invivoscribe, Inc. Dr. Ho has received honoraria from Invivoscribe, Inc. Dr. Roshal has served as a consultant for BD Biosciences, Agios, Celgene, as well as contract research funding for Agios, Roche, BMS, and Bayer. Dr. Dogan has received compensation from Roche, Novartis, Celgene, Seattle Genetics, and Corvus Pharmaceuticals for consulting/ advisory activities. Other authors declare no relevant conflict of interest.

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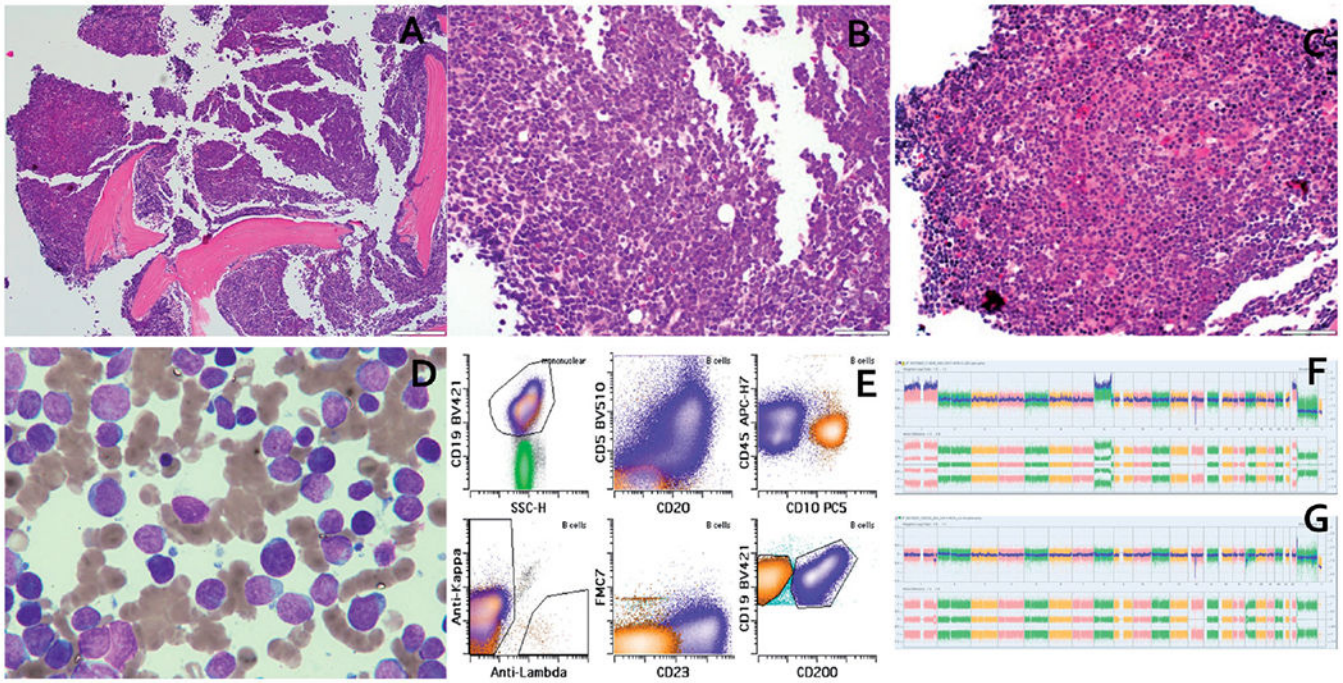

Figure 1.

(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).

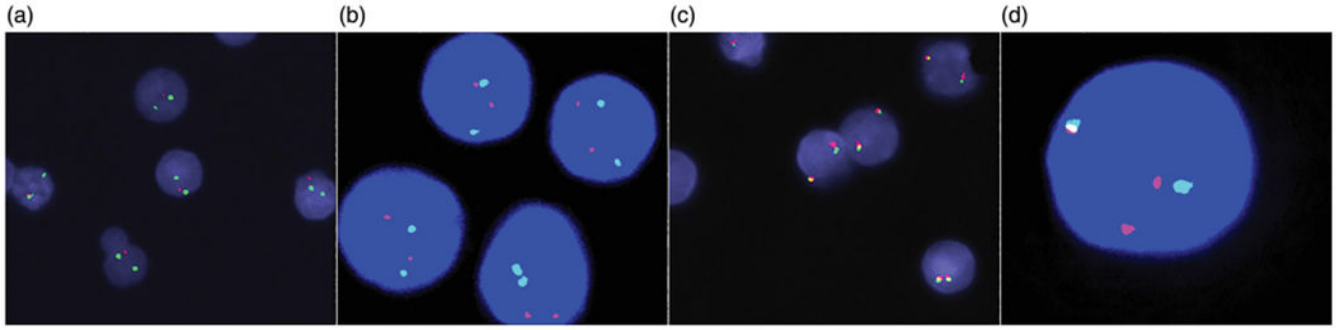

Figure 2.

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
Mutations					
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%
Copy number alterations					
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	−	+
Other chromosomal level alterations					
IGH/MYC	Translocation	Translocation	t(8;14)(q24;q32	−	+