Table 3 [3, 7, 14-20] with corresponding references.

CONCLUSIONS

The evolution of diagnostic methodologies, risk stratification guidelines, and targeted therapies for hematologic malignancies underscores the escalating significance of thoroughly assessing an extensive array of genetic biomarkers when making informed decisions about front-line patient care. Next-generation sequencing (NGS) has emerged as a valuable tool for the prompt delivery of results across a wide spectrum of genetic targets. Moreover, the expeditious establishment of an NGS test system and its streamlined integration into clinical processes should be considered for efficient patient care.

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TP53 mutation is a high-risk factor for Richter's syndrome based on circulating tumor DNA

TO THE EDITOR: Richter's syndrome (RS) is the progression of CLL to aggressive lymphoma. It occurs in 2–10% of CLL cases and is known to involve several immune and genetic factors; however, the mechanism remains unclear [1, 2]. According to previous retrospective paired-sample studies, genetic aberrations of the *TP53* and *C-MYC* genes, chromosomal abnormalities such as non-del (13q) or del (17p), and unmuted immunoglobulin heavy chains are high-risk factors [1, 2].

When lymphoma, such as diffuse large B-cell lymphoma (DLBCL), is first diagnosed in a patient with a history of CLL, analysis of their genetic history can aid in determining whether it is *de novo* DLBCL or originated from CLL [3]. This is pertinent because some subclones of existing CLL are found in RS as the disease progresses [4]. For example, *TP53, NOTCH1*, and *BIRKC3*, which are commonly present as dominant clones in CLL, are also frequently detected in RS, and new genetic variations can occur during the progression of RS [4]. Clonal evolution and genetic diversity can be determined by detecting circulating tumor DNA (ctDNA) in peripheral blood, which plays an important role in the course of RS [5]. Here, we present a case study illustrating the clinical utility of ctDNA and *TP53* expression in CLL.

A 61-year-old female patient was diagnosed with CLL based on a bone marrow examination performed during a lymphocytosis work-up. Her total WBC count was 10.43×10^9/L (myelocytes, 1%; band neutrophils, 1%; segmental neutrophils, 5%; lymphocytes, 91%; monocytes, 1%; eosinophils, 15%), hemoglobin level was 8.9 g/dL, and platelet count was 272×10^9/L. In the bone marrow, mature small lymphocyte levels were greatly increased to 87% of the total nucleotide cells (Fig. 1A), and the CD19+/CD5+/CD23+ immunophenotypic findings were compatible with those of CLL. The karyotyping was 45–46,XX,t(1;2)(q21;q21);add(6)(q13),der(17;18)(q10;q10),i(17)(q10)[cp18]/46,XX [2]. The neck, supraclavicular, mediastinal, axillary, right cardiophrenic, left gastric, porta hepatis, perigastric, portocaval, left abdominal para-aortic, aortocaval, common iliac, and external iliac lymph nodes (LNs) were hypermetabolic. This was also observed in the inguinal area, and diffuse FDG uptake was detected in the axial and appendicular bones, with an enlarged spleen. A biopsy of the left inguinal lymph node revealed CD20+/CD5+/CD10-/CD23+/BCL2+ expression on immunohistochemistry. The patient was diagnosed with chronic lymphocytic leukemia/small lymphocyte lymphoma.

She was treated with six rounds of combination chemotherapy with rituximab, fludarabine, and cyclophosphamide (R-FC). During R-FC chemotherapy, lymphocytosis gradually improved and the size of the enlarged lymph nodes decreased. Approximately 5 months after treatment initiation, a new tumor with approximately 3 cm was observed on her left arm (Fig. 1B), at which time a histological examination was performed. Immunohistochemical analysis revealed that the expression of CD20+/CD5-/CD23-/BCL2+/BCL6+/MUM1+ cells was consistent with that of DLBCL. The blood tests showed anemia and leukopenia, but no

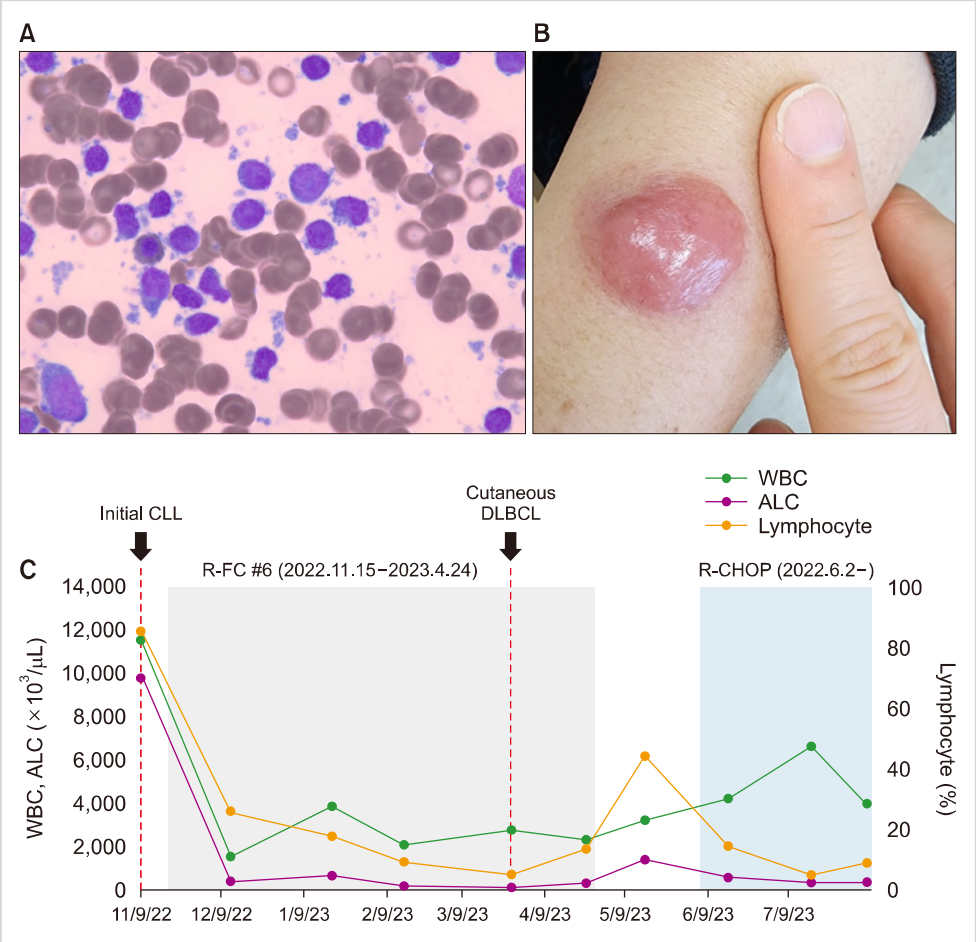

Fig. 1. Clinical assessment over treatment timeline. **(A)** Initial bone marrow aspiration showed highly increased levels of small mature lymphocytes. Initial diagnosis: CLL (BM, Wright stain, ×1,000). **(B)** New tumor lesion in left arm (cutaneous DLBCL). **(C)** Peripheral WBC count and absolute lymphocyte counts.

lymphocytosis was observed (Hb 9.5 g/dL, WBC 2.77×10^9/L, segmental neutrophils 80.1%, lymphocyte 5.1%, platelet 277×10^9/L) (Fig. 1C). Serum lactic dehydrogenase (LDH) levels were within the reference range. In addition, follow-up PET/CT revealed faint hypermetabolic LNs, and the axial and appendicular bone hypermetabolism were no longer prominent.

To determine whether cutaneous DLBCL was *de novo* or CLL-induced, the genetic profiling of initially diagnosed CLL and DLBCL was confirmed using next generation sequencing (NGS). The detection of residual circulating tumor cells was confirmed using cell-free DNA (cfDNA) in peripheral blood at the time of DLBCL occurrence. Using the initial CLL bone marrow specimen, an NGS panel test was performed with a customized kit targeting 66 genes related to lymphoma (Celemics, Seoul, Korea) and a MiseqDx platform (Illumina, San Diego, CA, USA). For the detection of cfDNA at the time of cutaneous DLBCL onset, 20 mL of peripheral blood was collected and subjected to a 55-gene Oncomine pan-cancer panel using an Ion Torrent S5 (Thermo Fisher Scientific, Waltham, MA, USA). The NGS tests, from the extraction of DNA to the data analysis, were performed by GC Labs (Youngin, Korea). We performed targeted NGS of the DLBCL tissue at our institute. DNA and RNA were extracted and libraries were prepared using an Oncomine Comprehensive Assay Plus (Thermo Fisher Scientific), and the products were sequenced using an Ion Torrent Ion S5 System (Thermo Fisher Scientific).

Table 1 shows the results of the study. The *TP53* c.566C>T, p.Ala189Val pathogenic tier 1 variant was detected in all three samples, suggesting that it may be an ancestral CLL clone. Another *TP53* c.488A>G, p.Tyr163Cys pathogenic tier 1 variant, which was only present in CLL, was the dominant CLL clone but seemed to have disappeared in the ctDNA and cutaneous DLBCL tissue. We assumed that the clones with the *TP53* c.488A>G mutation were eliminated during R-FC chemotherapy. However, in the case of the *MYD88* c.755T>C, p.Leu252Pro, and *EP300* c.4399T>A, p.Tyr1467Asn variants, we confirmed that they were newly generated over the course of RS progression. Therefore, although this patient showed an improved state after chemotherapy based on imaging and complete blood count results, we confirmed that an ancestral CLL clone remained in the peripheral blood. Additionally, newly acquired pathogenic mutations in *MYD88* and *EP300* may play a role in DLBCL progression. Tier 3 variants of *ETV6*, *NOTCH1, POT1*, and *TET2* detected at approximately 50% of the variant allele frequency in CLL and cutaneous DLBCL were germline variants.

Notably, no lymphoma lesions, other than an arm mass, were observed. The patient was switched to combination chemotherapy with rituximab, cyclophosphamide, adriamicin, vincristine, or prednisolone (R-CHOP). The patient achieved complete remission after six cycles of R-CHOP treatment.

DLBCL-type RS is generally aggressive, has a poor prognosis, and is an indication for chemotherapy for aggressive B-cell lymphoma [6]. In addition, clonally related RS to paired CLL showed shorter survival times than clonally unrelated RS [7]. Thus, it is important to monitor the disease using prognostic biomarkers, including serum LDH levels, immunophenotypic markers, cytogenetic alterations, and PET/CT imaging [8]. In our case, the residual fatal *TP53* variant, which can lead to disease progression, was only detected in the peripheral blood. With the detection of such an important genetic mutation, the use of ctDNA as a patient-specific tumor biomarker is expected to be useful for risk stratification and therapeutic targeting [5].

The clonal evolution pattern from CLL to RS shows a different genetic alteration spectrum on a case-by-case basis [5, 9]. In some cases, the dominant CLL clone is maintained in the RS, and, as in our case, the ancestral baseline clone is common to both CLL and RS. Although not all cases show the same genetic alteration pattern, ctDNA analysis seems to be useful for identifying clonally related RS. In particular, the *TP53* pathogenic variant predominates in RS, and *TP53* inactivation is considered a critical event in RS pathogenesis

Table 1. Genetic variants detected in initial CLL and cutaneous DLBCL.

Gene	Chr.	gDNA	DNA	Protein	COSMIC	Tier	Reference sequence	Initial CLL		Cutaneous DLBCL			
										ctDNA		Tissue (skin Bx)	
								Detection	VAF	Detection	VAF	Detection	VAF
TP53	17	g.7578283G>A	c.566C>T	p.Ala189Val	COSM44349	1	NM_000546	Y	(78.4)	Y	51.1	Y	(71.0)
TP53	17	g.7578442T>C	c.488A>G	p.Tyr163Cys	COSM10808	1	NM_000546	Y	(59.0)	N		N	
ETV6	12	g.12022788T>G	c.894T>G	p.His298Gln	.	3	NM_001987	Y	(50.2)	NT		Y	(50.0)
NOTCH1	9	g.139391634C>A	c.6557G>T	p.Gly2186Val	COSM95812277	3	NM_017617	Y	(43.1)	NT		Y	(49.0)
POT1	7	g.124467296C>A	c.1658G>T	p.Gly553Val	.	3	NM_015450	Y	(41.2)	NT		Y	(48.0)
TET2	4	g.106197185G>T	c.5518G>T	p.Ala1840Ser	.	3	NM_001127208	Y	(48.5)	NT		Y	(52.0)
MYD88	3	g.38182641T>C	c.755T>C	p.Leu252Pro	.	2	NM_002468.5	N		NT		Y	(37.3)
EP300	22	g.41566522T>A	c.4399T>A	p.Tyr1467Asn	COSM220521	2	NM_001429	N		NT		Y	(30.4)

Abbreviations: Bx, biopsy; Chr, chromosome; ctDNA, circulating tumor DNA; NT, not tested; VAF, variant allele frequency.

along with MYC pathway activation [10]. Our case also showed similar results to those of previous reports; therefore, molecular monitoring using ctDNA is considered necessary if a *TP53* pathogenic variant is detected at the initial diagnosis. In conclusion, this case demonstrated the clinical usefulness of ctDNA and confirmed the association between poor prognosis and expression of the *TP53* pathogenic variant in hematologic malignancies.

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T-cell large granular lymphocytic leukemia with low-grade bone marrow involvement complicated by acquired pure red cell aplasia

TO THE EDITOR: We found the comprehensive review article by Park *et al.* [1], which focused on T-cell large granular lymphocytic (T-LGL) leukemia and its typical low-grade bone marrow involvement, to be very enlightening. T-LGL leukemia can potentially lead to serious complications, such as severe infections due to neutropenia or the occurrence of pure red cell aplasia (PRCA). Herein, we present a case report of T-LGL leukemia accompanied by PRCA. Additionally, we include focused reviews on leukemic T/natural killer (NK)-cell lymphomas with frequent bone marrow infiltration and on acquired PRCA associated with different etiologies.

CASE

An 85-year-old male with unexplained transfusion-dependent macrocytic anemia prompted us to perform a bone marrow biopsy. Cytological and cytogenetic analyses did not confirm the primary differential diagnosis of suspected myelodysplastic syndrome (MDS), and next-generation sequencing of 30 genes associated with myeloid neoplasms yielded negative results. By contrast, erythroblastopenia together with the presence of atypical lymphocytes with azurophilic cytoplasmic granules was observed (Fig. 1). Immunocytological assessment revealed an aberrant T-cell profile (CD2+/CD3+/CD8++/CD57+/CD5dim/CD7-). Moreover, determination of the clonal T-cell status through T-cell receptor rearrangement analysis led to the diagnosis of T-LGL [1]. Additionally, peripheral differential blood examination revealed reticulocytopenia (regarded as a hallmark of acquired adult PRCA) in addition to erythropoietic precursor paucity (Fig. 2) [2]. Although the degree of bone marrow infiltration in our patient was low (12% lymphoma cells), concomitant PRCA was evident. Owing to his age and comorbidities, he was treated with red blood cell transfusions and corticosteroids. The patient visits the outpatient clinic weekly or biweekly, and no infections and other major complications have been reported.