




ORIGINAL ARTICLE

Mutations found in cell-free DNAs of patients with malignant lymphoma at remission can derive from clonal hematopoiesis

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Abstract

Cell-free DNA (cfDNA) analysis to detect circulating tumor DNA has been focused on monitoring malignant lymphomas. However, clonal hematopoiesis of indeterminate potential (CHIP)-associated mutations can also be detected by cfDNA analysis. Our aim is to investigate the origin of mutations detected in cfDNA among B-cell lymphoma patients. *MYD88/CD79B*, *DNMT3A*, and *TP53* were chosen as genes of interest, representing each of the following categories: lymphoma driver genes, CHIP-related genes, and genes shared between lymphoma and CHIP. Seventy-five B-cell lymphoma patients were included in this retrospective study. Serum cfDNAs at time of complete metabolic response (CMR) were sequenced for *TP53* (N = 75) and *DNMT3A* (N = 49). *MYD88* p.L265P and *CD79B* p.Y196C/H mutations were analyzed in diffuse large B-cell lymphoma (DLBCL) patients whose tumor samples were available (N = 29). Two and seven mutations in *TP53* and *DNMT3A*, respectively, were detected in cfDNA at CMR. These mutations were detected in either bone marrow mononuclear cells (BMMC) or PBMC. Although four *DNMT3A* mutations were also detected in tumors, median variant allele frequencies in the tumors (<1.0%) were significantly lower than those in both BMMC (6.1%) and serum (5.2%) obtained before the therapy. Conversely, five *MYD88* and three *CD79B* mutations detected in tumors were confirmed in cfDNA before therapy, but not in BMMC nor in cfDNA at CMR. Thus, all *TP53* and *DNMT3A* mutations detected in cfDNA at remission seemed to originate from CHIP rather than from residual disease. Results of liquid biopsy should be carefully interpreted, especially in genes shared between lymphomas and CHIP.

KEYWORDS

B-cell lymphoma, cell-free DNA, circulating tumor DNA, clonal hematopoiesis of indeterminate potential, *TP53*

1 | INTRODUCTION

Cell-free DNA (cfDNA) analysis to detect circulating tumor-derived DNA (ctDNA) has recently been used to focus on disease monitoring of malignancies, including malignant lymphomas (ML).¹ It enables us to track minimal residual disease (MRD) and clonal evolution, with minimal invasiveness. *TP53*, a tumor suppressor gene, is occasionally mutated in ML as well as in other hematological malignancies, albeit less frequently than in many solid tumors; for example, 17%, 5%-12%, and 9% in newly diagnosed diffuse large B-cell lymphoma (DLBCL),² follicular lymphoma (FL),³ and Hodgkin lymphoma (HL),⁴ respectively. *TP53* mutations have been reported as a poor prognostic indicator of DLBCL in the R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) therapy era,⁵ and are more prevalent in refractory/relapsed DLBCL than in newly diagnosed DLBCL.^{6,7} Similarly, it has been reported that *TP53* mutations are more prevalent in transformed follicular lymphoma than in untransformed FL.⁸ Accordingly, detecting *TP53* mutations by cfDNA analysis in a longitudinal method may be informative.

Recent studies have shown that one or more somatic mutations could be detected in the peripheral blood cells of elderly individuals without any history of hematological malignancies.^{9,10} This condition has been termed clonal hematopoiesis of indeterminate potential (CHIP).^{9,10} It has been reported that relative risk of developing hematological malignancies is greater in individuals with detectable CHIP than in those without.^{9,10} Mutations in CHIP are most frequently found in *DNMT3A* (50%-60%),^{9,10} followed by some cancer-associated genes including *TP53*. Retrospective studies have shown a higher risk of developing therapy-related myeloid neoplasms (t-MN) in cancer patients with *TP53* CHIP mutations at initial cancer diagnosis.¹¹⁻¹³

Tumor cells and blood cells are thought to be major sources of cfDNAs.^{14,15} Hence, mutations detected in cfDNAs of patients with malignancies can derive from either or both tumor cells and CHIP cells. Our aim is to investigate the origin of CHIP-related gene mutations detected in serum cfDNAs from patients with B-cell lymphoma (including HL). For this purpose, *TP53*, which is found in both B-cell lymphomas and CHIP, *DNMT3A*, which is the most frequent CHIP gene and is rare in B-cell lymphomas, and *CD79B/MYD88*, which are recurrently mutated in 14% and 18%, respectively, of non-selective DLBCL¹⁶ and are rarely present in CHIP, were chosen as genes of interest.

2 | MATERIALS AND METHODS

Seventy-five patients with a diagnosis of B-cell lymphoma whose archived serum samples at the time of complete metabolic response (CMR) were available were included in this retrospective study. Serum cfDNAs obtained at the time of CMR from all patients were sequenced for *TP53* (N = 75) and *DNMT3A* (N = 49) (Figure S1). Among the patients who showed mutations in serum cfDNAs at the time of CMR, the following samples were sequenced by targeted

amplicon-based deep sequencing: (i) bone marrow mononuclear cells (BMMC), serum, and tumors, all of which had been obtained before therapy; and (ii) BMMC or PBMC obtained at the time of CMR (Figure S1). Only BMMC and PBMC with undetectable lymphoma cells were analyzed. For patients with DLBCL whose tumor samples were available (N = 29), L265P MYD88 and Y196C/H CD79B mutations were analyzed using droplet digital PCR (ddPCR) (Figure S2). Among the patients who showed mutations in the tumors, the following samples were also analyzed: (i) BMMC and serum obtained before therapy; and (ii) serum obtained at the time of CMR. Variant allele frequencies (VAF) were assessed to compare mutations between mononuclear cells, serum and tumors. Haploid genome equivalents per milliliter (hGE/mL), which is determined as the product of VAF and total cell-free DNA concentration, was assessed to quantify levels of mutations in cfDNA before and after therapy. The study was approved by the review board of University of Tsukuba Hospital, and was conducted in accordance with the Declaration of Helsinki.

2.1 | DNA extraction

The cfDNAs were extracted from 1 mL serum using a QIAamp circulating nucleic acid kit (Qiagen). Serum was separated within 6 hours, respectively, after blood collection. Blood samples were collected into tubes with serum separator or tubes with heparin and centrifuged at 1750 \times g for 15 minutes at room temperature. Serum was transferred into cryogenic vials (Corning) and stored at -80°C until DNA extraction. Tumor DNAs were extracted from archived formalin-fixed paraffin-embedded (FFPE) specimens using a GeneRead FFPE kit (Qiagen). Tumor purity of the specimen was evaluated by a pathologist. DNA was extracted from cryopreserved BMMC and PBMC samples using a DNA mini kit (Qiagen). Extracted DNAs were stored at -20°C until analysis.

2.2 | Primer designs, constructing libraries, and variant calling pipelines in sequencing

Sequencing for *TP53* and *DNMT3A* was carried out using Ion Torrent Personal Genome Machine (Thermo Fisher Scientific). Ion Ampliseq Custom Panels for *DNMT3A* and *TP53* were designed using online Ion Ampliseq Designer (Thermo Fisher Scientific). The gene panel for *DNMT3A* was composed of 75 amplicons, sizes of which were 125 to 140 base pairs (bp), totaling 6.09 kilobases. That for *TP53* was composed of 38 amplicons, sizes of which were 125 to 140 bp, totaling 2.97 kilobases. Libraries were prepared using an Ion Ampliseq Library Kit 2.0 (Thermo Fisher Scientific). For targeted amplicon-based deep sequencing, each primer was designed individually using Primer 3 and Primer-BLAST (Table S1). Torrent Suite version 5.8 software (Thermo Fisher Scientific) was used to carry out signal processing, base calling, and sequence alignment to the reference genome (hg19). Variant caller plugin was used with the following settings: (i) minimum allele frequency = 0.02 for screening, 0.01 for targeted amplicon-based deep sequencing; (ii)

minimum Phred quality score = 6; (iii) minimum coverage = 100; (iv) minimum coverage on either strand = 4; (v) maximum strand bias = 0.95; (vi) maximum common signal shift = 0.12. Synonymous variants, intronic and UTR variants, variants curated in dbSNP, and variants whose allele frequency was 40.0-60.0 or 80.0-100.0% were filtered out. Variants detected in cfDNAs but not in tumor DNAs nor in BMMC/PBMC-derived DNAs were also filtered out. All variants were inspected manually using an integrated genome viewer.

2.3 | Droplet digital PCR

MYD88 p.L265P, CD79B p.Y196C, and CD79B p.Y196H were analyzed using ddPCR. Predesigned primers and FAM- and HEX-labeled TaqMan probes were used for the detection of the following variants: c.794T>C (L265P) MYD88 mutation (Bio-Rad, Assay ID: dHsaMDS2516944); c.587A>G (Y196C) CD79B mutation (Bio-Rad, Assay ID: dHsaMDS717317229); and c.586T>C (Y196H) CD79B mutation (Bio-Rad, Assay ID: dHsaMDS540972798).

Distilled water controls, negative and positive controls were included in each run. The assay was carried out using the QX200 ddPCR system (Bio-Rad). PCR amplification was carried out as follows: initial enzyme activation at 95°C for 10 minutes, 40 cycles of denaturation and annealing/extension at 94°C for 30 seconds, hold at 56°C for 1 minute, and then enzyme deactivation at 98°C for 10 minutes. The ramp rate was at 2°C/second throughout the entire amplification process. Results were analyzed using QuantaSoft version 1.7.4. (Bio-Rad). Lower limit of detection (LOD) for L265P MYD88 was fractional abundance of 0.1% as previously described.¹⁷ LOD for Y196H and Y196C CD79B were determined as 0.12% and 0.037% each by a dilution study (Figure S3).

2.4 | Statistical analysis

Statistical analysis was carried out using R version 3.5.2. Categorical and continuous data were analyzed using Fisher's exact test and Wilcoxon rank sum test. Kruskal-Wallis one-way analysis of variance (Kruskal-Wallis ANOVA) and post-hoc test with Bonferroni correction were used to compare VAF of cfDNA, tumor, PBMC/BMMC.

3 | RESULTS

3.1 | Patient characteristics

Details of patient characteristics are provided in Table 1. Median age was 65 (range: 25-89). Forty-five (60.0%), 20 (26.7%), six (8.0%), and four (5.3%) patients were diagnosed with DLBCL, FL, B-cell lymphomas other than DLBCL or FL, and HL, respectively. All patients except for one (1.3%) who received radiation therapy alone received chemotherapy; 43 (57.3%), 14 (18.7%), four (5.3%), two (2.7%) and 11 (14.7%) patients received R-CHOP, R-bendamustine, ABVD (doxorubicin, bleomycin, vinblastine, and

TABLE 1 Characteristics of patients in the present study

	Liquid biopsy at complete metabolic response		P value
	Negative	Positive	
No. of patients	66	9	
Median age, y (range)	63.5 (25-89)	73 (65-78)	0.03
Male, n (%)	36 (54.5)	7 (77.8)	0.29
Histological diagnosis, n (%)			
DLBCL	37 (56.1)	8 (88.9)	0.45
Follicular lymphoma	19 (28.8)	1 (11.1)	
Other B-cell lymphomas	6 (9.1)	0 (0.0)	
Hodgkin lymphoma	4 (6.1)	0 (0.0)	
Advanced clinical stage, n (%)	38 (57.6)	5 (55.6)	1.00
Bone marrow invasion, n (%)	11 (16.7)	0 (0.0)	0.34
LDH > upper normal limit, n (%)	39 (59.1)	6 (66.7)	0.35
Chemotherapy, n (%)			
R-CHOP	36 (54.5)	7 (77.8)	0.72
R-Bendamustine	12 (18.2)	2 (22.2)	
ABVD	4 (6.1)	0 (0.0)	
Other	2 (3.0)	0 (0.0)	
More than 2 regimens	11 (16.7)	0 (0.0)	
Never (radiation alone)	1 (1.5)	0 (0.0)	
Radiation therapy, n (%)	9 (13.6)	2 (22.2)	0.61
Median follow up, months (range)	31.3 (6.3-82.6)	25.6 (14.1-48.2)	0.65
Secondary malignancies, n (%)			
Aggressive NK cell leukemia	0 (0.0)	1 (11.1)	0.12

Significant *P* values are shown in bold text. Abbreviations: ABVD, doxorubicin, bleomycin, vinblastine, dacarbazine; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; DLBCL, diffuse large B-cell lymphoma; LDH, lactate dehydrogenase; NK, natural killer; R, rituximab.

dacarbazine), other regimens, and more than two regimens, respectively. Eleven patients received chemoradiotherapy. During the median follow up of 30.9 months (range, 6.3-82.6), one patient developed a secondary malignancy (aggressive natural killer cell leukemia, ANKL).

3.2 | Sequencing depth

Average sequencing depths were 8009 and 3783 for *TP53* and *DNMT3A*, respectively, in multiplex PCR-based sequencing. That of targeted amplicon-based sequencing, which was designed for each specific variant detected through *TP53* and *DNMT3A* sequencing, was 58 073. Median target base coverages with at least 500 reads were 100.0%, 94.1%, and 100.0% for target regions of *DNMT3A*, *TP53*, and each amplicon.

3.3 | Mutations and variant allele frequencies before therapy and at the time of complete metabolic response

Two mutations in *TP53* (two missense mutations: p.A119D and p.C275Y) and seven in *DNMT3A* (four missense mutations, two nonsense mutations, one frameshift deletion; p.W860R in two patients, and p.V563M, p.R882H, p.E392*, p.W601*, and p.E565 fs in one each) were detected in serum obtained at the time of CMR (Table S2). All these mutations were detected in either BMMC before therapy or in PBMC/BMMC at the time of CMR. For the seven patients whose tumor samples before the therapy were available, four of five *DNMT3A*, but none of two *TP53* mutations, were also detected in paired tumors obtained before the therapy (Table S2). However, VAF in the tumors were significantly lower than those in both serum and BMMC obtained before the therapy. Median (range) VAF of mutations were 6.1% (<1.0%-13.8%), 5.2% (<1.0%-11.4%), and <1.0% (<1.0%-1.6%) in BMMC, serum, and tumor, respectively (Kruskal-Wallis ANOVA, $P = .01$; Wilcoxon rank-sum test with Bonferroni correction, BMMC vs serum, $P = 1.00$; BMMC vs tumor, $P = .02$; serum vs tumor, $P = .03$; Figure 1A, Table S2). This finding suggested that the mutations detected in cfDNAs may have derived from CHIP rather than from the tumors. Moreover, this notion was strengthened in all of the nine patients by the following observations: (i) patients' age was significantly higher in those with *TP53* or *DNMT3A* mutation than in those without (median, 73.0 vs 63.5 years, $P = .03$, Table 1). This was compatible with the fact that CHIP was more prevalent with older age;^{9,10} (ii) median (range) VAF of PBMC/BMMC and serum were 4.6% (1.3%-15.3%) and 6.0% (3.2%-23.8%), respectively, at CMR, where residual tumor burden was extremely low (Figure 1B, Table S2).

Furthermore, we surveyed *MYD88* p.L265P (L265P *MYD88*) and *CD79B* p.Y196C and *CD79B* p.Y196H (Y196C/H *CD79B*) mutations, which were prevalent in DLBCL as driver mutations and rare in CHIP, to examine whether MRD-derived mutation could be detected at CMR. Five L265P *MYD88*, one Y196C *CD79B*, and two Y196H *CD79B* mutations were confirmed in six of 29 DLBCL tumor samples. These mutations were also detected in serum cfDNA before therapy, but not in BMMC nor serum cfDNA at CMR. Median (range) fractional abundance of mutations were <0.1 (<0.1-<0.12)%, 7.4 (0.7-20.6)%, and 41.1 (20.5-82)% in BMMC, serum, and tumor, respectively (Kruskal-Wallis ANOVA, $P = .0004$; Wilcoxon rank sum test with

Bonferroni correction, BMMC vs serum, $P = .012$; BMMC vs tumor, $P = .0048$; serum vs tumor, $P = .0093$; Figure 2, Table S3). This result decreased the possibility that MRD-derived mutations could be detected at CMR with the thresholds of ddPCR, which was more sensitive than that of targeted sequencing. Quantitative levels of *TP53* and *DNMT3A* mutations in serum were within 1.15 log-fold, except for W860R *DNMT3A* mutation which was undetectable before therapy (Figure 3A). In contrast, quantitative levels of L265P *MYD88* and Y196C/H *CD79B* mutations decreased more than 3 log-fold and were below the threshold of detection (Figure 3B).

3.4 | Longitudinal analysis of a patient with DLBCL who developed aggressive NK cell leukemia

One DLBCL patient with the *TP53* p.A119D mutation in cfDNAs (on day 253) showed relapse on day 962 and later developed ANKL on day 1296. VAF of the *TP53* p.A119D were 23.8% and 15.3% in lymphoma-free BMMC and PBMC, respectively (Table 2). They were, however, as low as 1.8% in the DLBCL tumor sample at relapse, and undetectable in the DLBCL tumor sample at diagnosis and in the leukemic cells of ANKL with a threshold of 1% (611 altered reads per total 66 086 reads) (Table 2). This implied that both DLBCL and ANKL did not derive from a *TP53* p.A119D-CHIP-carrying clone. Concentrations of *TP53* p.A119D in serum ranged within 1.3-log fold change throughout the entire course.

4 | DISCUSSION

Liquid biopsy sometimes enables us to detect relapse of tumors and development of drug-resistant clones by longitudinal sampling during the clinical course. However, our data suggested that *TP53* and *DNMT3A* mutations detected in cfDNAs at the time of CMR originated from CHIP rather than from tumors, judging from the presence of mutations in mononuclear cells and the absence or minimal presence of mutations in tumors (Figure 1 and Table S2). Very low variant load found in tumors may represent intervening CHIP-derived non-tumor cells. This phenomenon has also been reported in next-generation sequencing without matched controls.^{18,19} Similarly, *TP53* mutations detected by liquid biopsy were also recently reported to be attributed to CHIP in 15% (5/33) of non-small cell lung carcinoma patients.²⁰ *DNMT3A* and *TP53* mutations, probably considered as CHIP, were observed in seven (14.3%) of 49 and in two

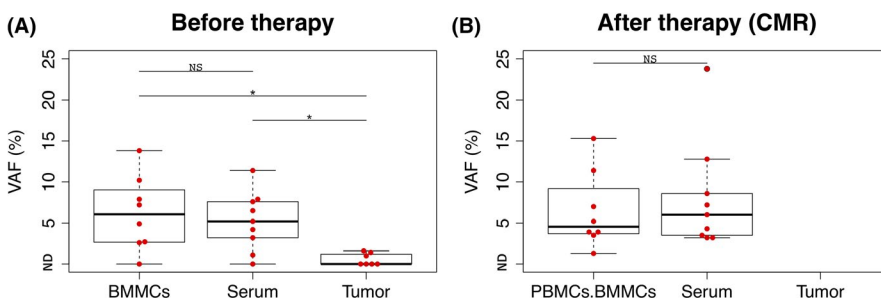


FIGURE 1 Comparison of variant allele frequencies (VAF) among *TP53* and *DNMT3A* mutations detected in each sample before and after therapy. BMMC, bone marrow mononuclear cells; CMR, complete metabolic response; ND, not detectable; NS, not significant. * $P < .05$

FIGURE 2 Comparison of fractional abundance of *MYD88* and *CD79B* mutations detected in each sample before and after therapy. BMMC, bone marrow mononuclear cells; CMR, complete metabolic response; ND, not detectable. * $P < .05$; ** $P < .01$; *** $P < .005$

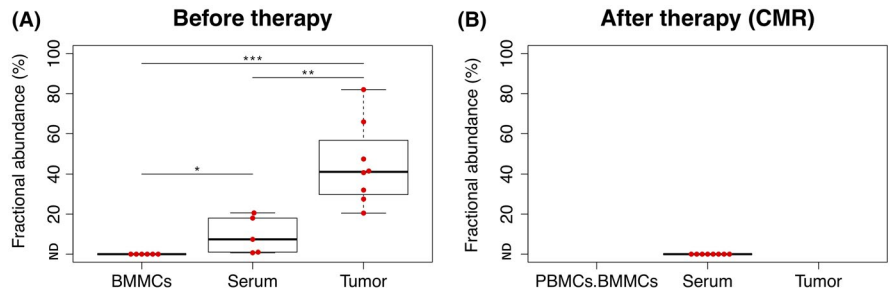
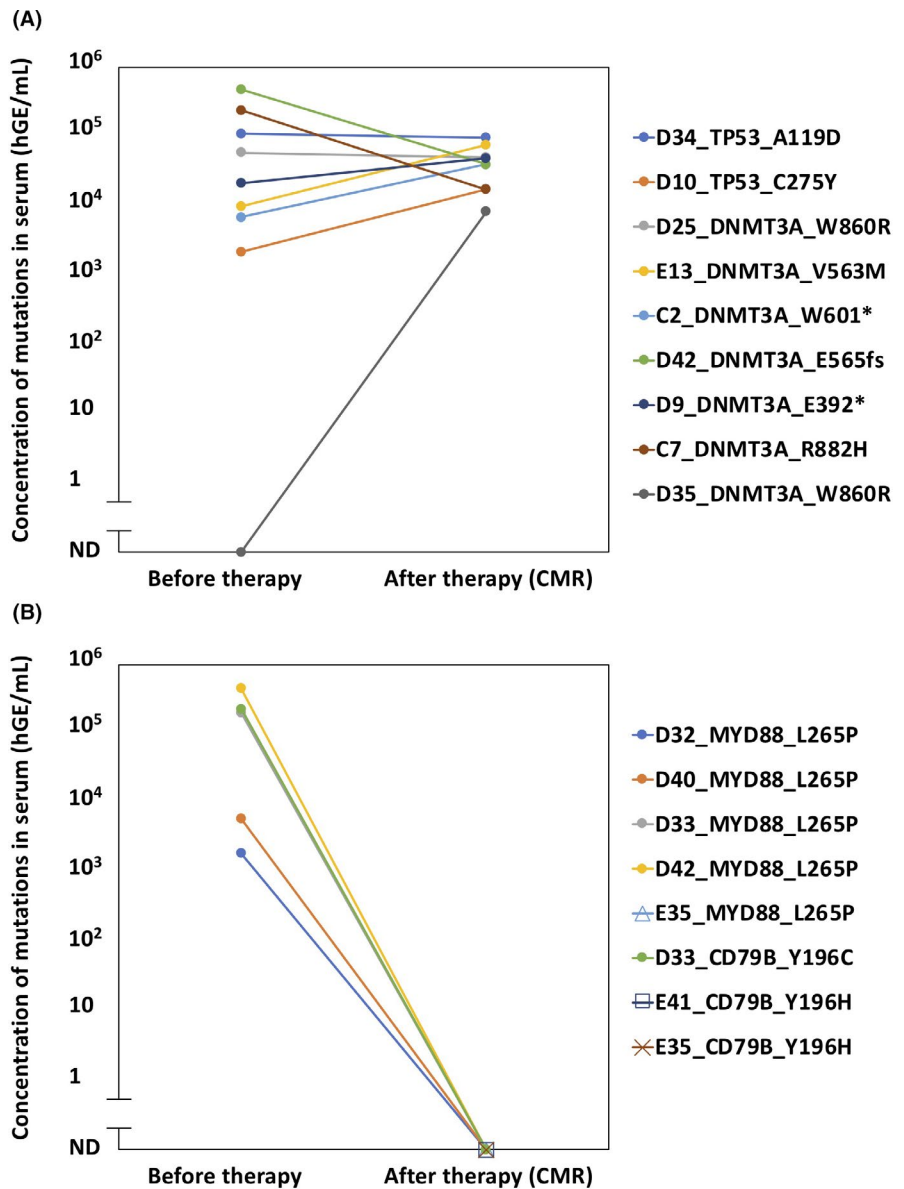


FIGURE 3 Longitudinal assessment of concentrations in cell-free DNA. CMR, complete metabolic response; hGE/mL, haploid genome equivalents per milliliter; ND, not detectable



(2.7%) of 75 patients, respectively. Prevalence of CHIP in the current study was compatible with that of the previous literature, in which the cohort was patients with lymphoma and the detection threshold of VAF was 2%.²¹

There are several arguments against the interpretation that mutations detected in cfDNA originated only from CHIP. As a first

argument, *TP53* mutations originated from the B-cell lymphomas. Because regional heterogeneity is not uncommon, biopsy from different sites might cause false-negativity of *TP53* mutations in the tumor-derived DNA. Nevertheless, ≥ 3 -log decrease of the *MYD88* or *CD79B* mutational burden was seen in cfDNA of six patients, whereas *TP53* mutational burdens were stably high in two out of

TABLE 2 Longitudinal analysis of *TP53* p.A119D mutation in the patient who developed ANKL

	Before therapy	After therapy (CMR)	Relapse of DLBCL	After therapy (2nd CMR)	Diagnosis of ANKL
Concentration of <i>TP53</i> p.A119D mutation in serum (hGE/mL)	1.1×10^5	9.6×10^4	NA	NA	2.1×10^6
VAF_serum (%)	7.6	23.8	NA	NA	3
VAF_DLBCL (%)	ND	NA	1.8	NA	NA
VAF_PBMC/BMMC (%)	13.8 ^a	NA	23.8 ^a	15.3	NA ^b
VAF_ANKL (%)	NA	NA	NA	NA	ND (611/66 086 reads)

Abbreviations: ANKL, aggressive NK cell leukemia; BMMC, bone marrow mononuclear cells; CMR, complete metabolic response; DLBCL, diffuse large B-cell lymphoma; NA, not available; ND, not detectable; VAF, variant allele frequency.

^aBone marrow invasion was not observed in these samples.

^bNearly 100% of peripheral blood cells and bone marrow cells were leukemic cells at the time of developing ANKL.

two. Therefore, the possibility that the *TP53* mutations are from non-fluorodeoxyglucose (FDG)-avid MRD lesions at the time of CMR should be negligible. As a second argument, non-FDG-avid occult cancers other than B-cell lymphomas could be a source of *TP53* mutations in cfDNA. Even if there was imaging-study-occult stage 1 cancer, in a previous report, mean VAF of ctDNA detected in stage 1 cancers was 0.05%.²² Therefore, given that the lower detection limit of VAF in the current study was 1%, variants were reasonably under this level.

We expected that *DNMT3A* mutations could be evidence for CHIP-associated mutations in B-cell lymphomas because of their rarity in this category of tumor at study commencement. However, it was recently reported that *DNMT3A* and *TET2* mutations were recurrently mutated in patients with Epstein-Barr virus (EBV)-positive DLBCL.²³ EBV-positive DLBCL is an EBV-positive clonal large B-cell proliferation, with the exclusion of several specific types of EBV-positive lymphomas, such as plasmablastic lymphoma.²⁴ It is unclear whether *DNMT3A* and *TET2* mutations detected in lymphomas were derived from CHIP, likewise in the pathogenesis of angioimmunoblastic T-cell lymphoma,²⁵ as targeted sequencing analysis of lymphoma specimens in the study was carried out in a non-paired method.²³ We must pay attention to *DNMT3A* mutations detected in cfDNA of patients with EBV-positive DLBCL unlike other subtypes of DLBCL because their origin can be either or possibly both CHIP and lymphoma.

Kurtz et al²⁶ reported a 2.5-log decrease of ctDNA after two cycles of R-CHOP therapy in patients with DLBCL who were stratified responders compared with non-responders. More than a 3-log decrease of all *MYD88* and *CD79B* mutations in cfDNA was compatible with this previous literature because all patients with DLBCL in this cohort had achieved CMR. On the contrary, changes of *TP53* and *DNMT3A* mutations detected in cfDNA were within 1.3-log except for the *DNMT3A* W860R mutation which became detectable after therapy. Stable or increasing burden of mutations detected in cfDNA at CMR implies that their origins are CHIP as far as the mutations are known to be commonly present in CHIP.

As for dynamics of a CHIP-carrying clone, Wong et al. reported that 33.3% of *DNMT3A* mutations and, in particular, all of p.R882H,

showed a significant increase, which was defined as more than twofold with a *P* value <.05, after autologous stem cell transplantation.²⁷ Only the *TP53* p.C275Y mutation and none of seven *DNMT3A* mutations including one p.R882H fulfilled these criteria in the current study. Coombs et al. also reported that *PPM1D* and *TP53* clonal hematopoiesis was detected in a mobilized stem-cell product of patients with lymphomas at a higher frequency compared with the healthy population.¹³ In the current study, *TP53* CHIP-carrying clone in one patient with DLBCL decreased in size at the time of developing ANKL. *TP53* mutation was reportedly observed in only one of 14 ANKL patients in an exome-wide sequencing study.²⁸ Although detection of *TP53*-CHIP has been reported as a risk factor for developing t-MN,¹¹⁻¹³ some *TP53*-carrying CHIP clones themselves are not founding leukemic clones.¹¹ The significance of monitoring a single specific *TP53*-CHIP-carrying clone and its predictive value for t-MN remains uncertain.

Herein, we raised important concerns regarding careful evaluation of liquid biopsy in B-cell lymphomas. For simplicity, in the present study, screening of mutations using liquid biopsy was carried out at the time of CMR. If the screening is carried out at the time of diagnosis, relapse, or refractory disease, not only CHIP-derived, but also tumor-derived *TP53* mutations, would be detected (Figure S4). In such settings, simultaneous sequencing of PBMC/BMMC, and lymphoma samples, if available, may be required to determine the origins of mutations detected by liquid biopsy and to interpret their significance appropriately.

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CONFLICTS OF INTEREST

Mamiko Sakata-Yanagimoto received a research fund from Celgene corporation. Shigeru Chiba received scholarship endowments from Kyowa Hakko Kirin corporation, Astellas Pharma corporation, and Bristol-Myers Squibb corporation. The other authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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