The clinical significance of 8q24/*MYC* rearrangement in chronic lymphocytic leukemia

Yan Li, Shimin Hu, Sa A Wang, Shaoying Li, Yang O Huh, Zhenya Tang, L Jeffrey Medeiros and Guilin Tang

Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Chromosome 8q24/*MYC* rearrangement is associated with Burkitt lymphoma and some aggressive B-cell lymphomas, but is rare in chronic lymphocytic leukemia. We here report a cohort of 20 chronic lymphocytic leukemia patients with 8q24/*MYC* rearrangement, 3 detected at time of initial diagnosis and 17 acquired after a median interval of 48 months. At the time when 8q24/*MYC* arrangement was detected, 18 patients had B-symptoms, 17 had lymphadenopathy, and 17 had splenomegaly. Histologically, typical chronic lymphocytic leukemia morphology was seen in six patients, increased prolymphocytes in nine and Richter's transformation in five patients. Eighteen patients had karyotypic information available that showed t(8;v) in a complex karyotype in 12 patients and in a non-complex karyotype in 6 patients. Fluorescence *in situ* hybridization confirmed *MYC* rearrangement in 17/17 patients. All patients required therapy after 8q24/*MYC* rearrangement was detected. At last follow-up, five of six patients with a non-complex karyotype were alive after a median of 74 months (10~143 months) from the detection of 8q24/*MYC* rearrangement. In contrast, 10 of 12 patients with a complex karyotype died with a median survival of 5.5 months. We conclude that 8q24/*MYC* rearrangement in chronic lymphocytic leukemia is rare and often acquired during the course of disease. If it is presented in a complex karyotype, it is often associated with Richter's transformation, refractory to therapy and an aggressive clinical course; on the other hand, if it is present in a non-complex karyotype, patients often respond to risk-adapted therapies and achieve remission.

Modern Pathology (2016) 29, 444–451; doi:10.1038/modpathol.2016.35; published online 26 February 2016

Chronic lymphocytic leukemia is the most frequent type of leukemia in the Western world, characterized by overproduction of monotypic small B cells in peripheral blood and/or bone marrow with co-expression of CD5 and CD23. Patients with chronic lymphocytic leukemia follow a variable clinical course, with overall survival ranging from months to decades. About 80% of chronic lymphocytic leukemia patients have cytogenetic abnormalities detected by conventional cytogenetics and/or fluorescence *in situ* hybridization (FISH), with deletions of 6q21/*MYB*, 11q22/*ATM*, 13q14, 17p13/*TP53*, and trisomy 12 being the most common.¹

Translocations of chromosome 8q24 juxtapose *MYC* oncogene with the immunoglobulin heavy chain (14q32), or less frequently, with kappa (2p12) or lambda (22q11.2) light chain genes, leading to overexpression of *MYC* that has a role in malignant transformation of B cells.² 8q24/*MYC* rearrangement can be not only detected in over 90% patients with Burkitt lymphoma,¹ but also has been identified in other aggressive lymphomas, such as 'double hit' B-cell lymphoma,^{3,4} B-cell prolymphocytic leukemia,⁵ blastoid variant of mantle cell lymphoma,⁶ and diffuse large B-cell lymphoma transformed from follicular lymphoma.⁷ 8q24/*MYC* rearrangement is rare in chronic lymphocytic leukemia and only occasional cases have been reported.^{8–17} In most of these reports, 8q24/*MYC* rearrangement occurred as a part of a complex karyotype, often associated with increased prolymphocytes or Richter's transformation and an aggressive clinical course; only rare cases reported had typical chronic lymphocytic leukemia morphology and responded well to chemotherapy.^{5,9}

Correspondence: Dr G Tang, MD, PhD, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030-4009, USA.
E-mail: gtang@mdanderson.org

Received 7 December 2015; revised 6 January 2016; accepted 7 January 2016; published online 26 February 2016

To better characterize the clinical behavior and pathological features of chronic lymphocytic leukemia with 8q24/*MYC* rearrangement, we searched our database for all chronic lymphocytic leukemia patients at our institution in the past 15 years, and identified 20 patients with 8q24/*MYC* rearrangement who had detailed clinical, pathological, and cytogenetic information available. We show that the prognostic importance of 8q24/*MYC* rearrangement is contextual, depending in part on other cytogenetic abnormalities.

Materials and methods

Patients

We searched the pathology database of The University of Texas MD Anderson Cancer Center for all chronic lymphocytic leukemia patients diagnosed/managed at our institution between the years of 2000 to 2015. Conventional cytogenetics (karyotype) and FISH results were collected for all patients. Clinical and laboratory data, response to treatment and patient outcome were obtained through medical records for all patients with 8q24/*MYC* rearrangement. All samples were collected following institutional guidelines with informed consent in accordance with the Declaration of Helsinki.

Laboratory Data and Morphological Examination

Laboratory data including white blood cell count, hemoglobin level, platelet count, percentage of prolymphocytes, B2 microglobulin level, and lactate dehydrogenase level were collected. Peripheral blood smears, bone marrow aspirate smears, and trephine biopsy specimens were reviewed in all cases. The morphologic features and infiltration patterns, as well as the percentage of prolymphocytes in bone marrow were recorded. Cases with prolymphocytes $\geq 10\%$ but $< 55\%$ of the total lymphocytes in peripheral blood were designated as chronic lymphocytic leukemia with increased prolymphocytes, and cases with $\geq 55\%$ prolymphocytes were designated as chronic lymphocytic leukemia in prolymphocytoid transformation.

Immunophenotypic Methods

Bone marrow aspirate specimen was assessed by flow cytometric immunophenotyping for a chronic lymphocytic leukemia immunophenotype as described previously.¹⁵ For each case, the chronic lymphocytic leukemia score was calculated according to the system of Matutes *et al*,¹⁸ subsequently modified by Moreau *et al*.¹⁹ Scores were based on five variables: expression of CD5, CD23, dim surface immunoglobulin, dim or absent CD22/CD79b and absence of FMC-7. Cases with a score of 4–5 were

considered to have a typical chronic lymphocytic leukemia immunophenotype; cases that deviated from this pattern of antigen expression were considered to have an atypical immunophenotype.

Immunohistochemical analysis for ZAP-70, Ki-67, and *MYC* was performed using formalin-fixed paraffin-embedded tissue sections of bone marrow core biopsy or aspirate clot specimens in a subset of cases, as described previously.¹⁵

Somatic Mutation Analysis of Immunoglobulin Heavy Chain Variable Region Genes

Sequence analysis of the immunoglobulin heavy chain variable region genes was performed using total RNA extracted from bone marrow aspirate material or DNA extracted from paraffin-embedded bone marrow aspirate clot sections, as described previously.¹⁵ The somatic mutation status was designated as unmutated if there was $< 2\%$ deviation from germline sequences, or as mutated if there was 2% or more deviation.²⁰

Conventional Karyotyping and FISH

Conventional chromosomal analysis was performed on G-banded metaphase cells prepared from bone marrow aspirate cultured for 24 h without mitogen and 72 h with mitogens of lipopolysaccharide or a cocktail of pokeweed mitogen/phorbol ester/CpG oligodeoxynucleotide using standard techniques. Twenty metaphases were analyzed and the results were reported using the International System for Human Cytogenetic Nomenclature (2013).²¹ Cases with three or more chromosomal abnormalities were considered to have a complex karyotype.

FISH for chronic lymphocytic leukemia panel was performed on interphase nuclei obtained from cultured bone marrow cells using a multicolor probe panel designed to detect deletions of 11q22.3 (*ATM*), 13q14.3 (*D13S319*), 13q34 (*LAMP1*), 17p13.1 (*TP53*), and trisomy 12 (12p11.1-q11) according to the manufacturer's instructions (Abbott Molecular, IL, USA). FISH for *MYC* (dual-color break-part probe, Abbott Molecular) was performed on cultured bone marrow cells or bone marrow aspirate smear or paraffin-embedded clot sections. Combined morphologic-FISH analysis was performed on Wright–Giemsa stained bone marrow aspirate smears using a *MYC* probe as described previously.²²

Results

Cytogenetic Findings

Of ~4500 chronic lymphocytic leukemia patients, 8q24/*MYC* rearrangement was detected in 30 (0.7%) patients; 10 were excluded from the study owing to a lack of sufficient clinical information or follow-up data. The final study group included 20 patients,

13 men and 7 women, with a median age of 59 years (range, 43–74 years). Of note, six of these patients (cases 4–5, 14–17) have been reported previously.⁸

Seven patients had karyotypic information available prior to detection of 8q24/*MYC* rearrangement. Of those, three patients (cases 10, 13, 16) showed t(8q24;v) arrangements as a result of clonal evolution (case 10 showed a normal karyotype, but FISH detected +12 at the time of diagnosis), and two (cases 9, 17) as new clones (Table 2). Of note, patient 9 had a normal karyotype at the time of chronic lymphocytic leukemia diagnosis; however, at the 12.5-year follow-up, bone marrow showed 45,XY,-2, del(6)(q13q23), add(14)(q32) [2]/46,XY [18]. At 13.7-year follow-up, bone marrow showed 47,XY,+1, del(1)(p13p36.1), t(8;14)(q24.1;q32) [19]/46,XY [1]. Karyotype was available in 18 patients at the time of 8q24/*MYC* rearrangement detection, and all showed 8q24 translocation, including t(8;14) (*IgH/MYC*, *n* = 14), t(2;8) (*κ/MYC*, *n* = 2), and t(8;22) (*λ/MYC*, *n* = 2) (Table 2). In the remaining two patients without karyotype, *MYC* arrangement was identified by FISH performed on tissue biopsy that showed Richter's transformation.

8q24/*MYC* rearrangement was detected at time of initial diagnosis of chronic lymphocytic leukemia in three patients (case 1, 5, 8), and detected during the course of disease in 17 patients. Of the latter 17 patients, 5 were on observation only and 12 had received various treatments for chronic lymphocytic leukemia (Table 1), and 8q24/*MYC* rearrangement was detected after a median interval of 48 months (range 4–186 months) after diagnosis. T(8q24;v) was found in the context of a complex karyotype in 12 patients, 10 of whom had been treated prior to the detection of 8q24/*MYC* rearrangement; whereas t(8q24;v) was found in a non-complex karyotype in six patients, none of these patients had received prior therapy for chronic lymphocytic leukemia. Monosomy 17 was found in four patients with a complex karyotype (Table 2).

FISH for chronic lymphocytic leukemia panel was performed on 15 patients, nine cases had D13S319 deletion; three had +12; three had *TP53* deletion; two had *ATM* deletion; and three patients were negative for all probes tested. *TP53* deletion and +12 were only seen in cases with a complex karyotype (Table 2). FISH for *MYC* rearrangement was performed in 17 patients who had material available, all showed *MYC* rearrangement, and the percentage of positive cells for rearrangement is listed in Table 2.

Combined morphologic-FISH analysis was performed in two cases: case 2 (typical chronic lymphocytic leukemia) and case 1 (chronic lymphocytic leukemia with increased prolymphocytes). *MYC* rearrangement was detected in small lymphocytes (case 1 and case 2) and prolymphocytes (case 2). Granulocyte and myelocytes showed normal pattern (negative for *MYC* rearrangement (Figure 1).

Clinical and Laboratory Findings

At the time when 8q24/*MYC* rearrangement was detected, 18 patients had variable degrees of B-type symptoms, 17 had lymphadenopathy and 17 had splenomegaly. All but one patient (case 1) had leukocytosis (white blood cells: median $70 \times 10^9/l$, range $8.6\text{--}241 \times 10^9/l$); 13 patients had anemia (hemoglobin: median 11.5 g/dl, range 9–15.9 g/dl); 9 patients had thrombocytopenia (platelets: median $143 \times 10^9/l$, range $58\text{--}257 \times 10^9/l$); all patients showed absolute lymphocytosis (median $68.3 \times 10^9/l$, range $5.3\text{--}236.1 \times 10^9/l$). Prolymphocytes in peripheral blood were increased in nine patients, five with prolymphocytes of 10–34% (chronic lymphocytic leukemia with increased prolymphocytes), and four had prolymphocytes >55% (chronic lymphocytic leukemia in prolymphocytoid transformation) (Table 1). All patients showed an increased serum B2 microglobulin level (median 4.6 mg/l, range 2.1–11.2 mg/l) (reference range, 0.7–1.8 mg/l) and 17 (cases 5–20) had an increased serum lactate dehydrogenase level (median 1220 IU/l, range 507–19328 IU/l) (reference range, 313–618 IU/l). Rai stage was IV in nine patients, III in four patients, II in six patients, and 0 in one patient. All patients required treatments for chronic lymphocytic leukemia after the detection of 8q24/*MYC* rearrangement. The therapy was risk-adapted, and the detailed therapeutic regimens are listed in Table 1. Two patients also received stem cell transplant.

Morphological and Immunophenotypical Findings

At the time 8q24/*MYC* rearrangement was detected, six patients (cases 2–4, 6, 12, 17) showed predominantly small mature lymphocytes without significantly increased prolymphocytes in bone marrow and nine (patient 1, 5, 7, 8, 11, 14–16, and 18) had prolymphocytes of 10–70%. Five patients (9–10, 13, 19–20) had Richter's transformation: patient 9 had EBV-positive plasmablastic lymphoma involving bone marrow as well as left supraclavicular lymph node; patients 10 and 13 had diffuse large B-cell lymphoma in bone marrow; patient 19 had plasmablastic lymphoma in right neck mass; and patient 20 had diffuse large B-cell lymphoma in an abdominal mass. All five patients with Richter's transformation had small chronic lymphocytic leukemia cells detected concurrently in bone marrow at the time of 8q24/*MYC* rearrangement detection.

At the time of 8q24/*MYC* rearrangement detection, 13 patients (cases 1–8, 10, 12, 15, 17–18) showed a typical chronic lymphocytic leukemia immunophenotype (score 4–5); cases 9 and 19 showed two neoplastic populations: a smaller population had a typical immunophenotype for chronic lymphocytic leukemia and a larger population showed plasmacytic differentiation (CD38 bright, CD5–, CD19–) and a different light chain expression. CD20 expression

Table 1 Demographic and clinical information, treatments, and outcomes

Case no.	Sex/age	Interval ^a	Diagnosis ^b	Rai stage	ZAP-70	SHM	Prior therapy ^c	Post therapy ^c	Follow-up ^d	Outcome
1	M/70	0	CLL/PL	2	NA	NA	No	Bendamustine	10	ACR
2	F/67	22	CLL	2	Positive	Unmutated	No	Rituximab	12	ACR
3	M/63	32	CLL	4	Positive	NA	No	Revlimid	81	ACR
4	F/71	4	CLL	2	Negative	Mutated	No	R-CHOP	143	ACR
5	M/74	0	CLL/PL	3	Positive	Unmutated	No	FCR, hyper-CVAD	47	DOD, RT
6	M/66	8	CLL	3	Positive	Unmutated	No	FCR	74	ACR
7	M/64	4	CLL/PT	0	Positive	Mutated	No	FCR	81	AWD
8	M/43	0	CLL/PT	2	NA	NA	No	Hyper-CVAD, SCT	26	DOD
9	M/67	165	RT, Plasmablastic	4	Negative	Unmutated	Rituximab	FCR	0.5	DOD, RT
10	F/59	51	RT, DLBCL	4	Positive	Mutated	FCR	FCR, SCT	4	ACR
11	F/47	147	CLL/PT	4	Negative	NA	FCR	FCR	0.8	DOD
12	M/51	186	CLL	3	Positive	NA	F, C	FCR	43	DOD
13	F/46	164	RT, DLBCL	4	NA	NA	FCR	Hyper-CVAD	9	DOD, RT
14	F/71	48	CLL/PT	4	Negative	Mutated	Chlorambucil	F, C	48	DOD, RT
15	M/48	6	CLL/PL	2	Negative	Unmutated	F, C	Hyper-CVAD	2	DOD
16	F/66	114	CLL/PL	4	Positive	Unmutated	F, R, Campath	Compound 506	1	DOD
17	M/62	66	CLL	4	Positive	Unmutated	FCR, Campath	FCR	0.3	DOD
18	M/49	10	CLL/PL	4	NA	NA	Fludarabine	FCR	1	DOD
19	M/58	92	RT, Plasmablastic	2	Positive	Unmutated	FCR	FCR, hyper-CVAD	4	AWD, RT
20	M/56	133	RT, DLBCL	3	NA	Mutated	FCR	FCR, bevacizumab	1	DOD, RT

Abbreviations: ACR, alive in complete remission; AWD, alive with disease; C, cyclophosphamide; CHOP, cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone; CLL/PL, chronic lymphocytic leukemia with increased prolymphocytes (prolymphocytes 10–54%); CLL/PT, chronic lymphocytic leukemia in prolymphocytoid transformation (prolymphocytes ≥ 55%); DLBCL, diffuse large B-cell lymphoma; DOD, died of disease; F, fludarabine; F, female; hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with high dose methotrexate and cytarabine; M, male; NA, not known; R, rituximab; RT, Richter's transformation; SCT, stem cell transplant; SHM, somatic hypermutation.

^aInterval: from diagnosis of CLL to 8q24/MYC rearrangement detection (months).

^bDiagnosis at the time when 8q24/MYC rearrangement was detected.

^cTreatment received before or after the detection of 8q24/MYC rearrangement.

^dTotal follow-up time after the detection of 8q24/MYC rearrangement.

could not be assessed owing to the use of Rituximab treatment. Case 11 had an atypical immunophenotype, with loss of CD23 expression and gain of FMC-7 expression. Cases 13 and 20 were both negative for CD5 and CD23 expression. Cases 14 and 16 had no immunophenotype available at the time of 8q24/MYC rearrangement detection. All cases assessed were negative for CD10. Of note, all these patients (cases 9, 11, 13, 19, 20) with an immunophenotype different from chronic lymphocytic leukemia had a typical chronic lymphocytic leukemia immunophenotype at the initial diagnosis.

ZAP-70 was performed by immunohistochemical stain in 15 patients: positive in 10 and negative in 5 (Table 1); Ki-67 was performed in 12 patients: positivity was in 1–10% lymphocytes in cases with typical chronic lymphocytic leukemia morphology, 5–30% in cases with chronic lymphocytic leukemia with increased prolymphocytes, and 80–99% in cases with diffuse large B-cell lymphoma. MYC immunohistochemistry stain was performed in three patients (cases 13, 19, 20), and showed a positive expression of MYC in all three cases.

Somatic Hypermutation Status

Immunoglobulin heavy chain somatic hypermutation status was available in 13 patients, 8 unmutated and 5 mutated (Table 1). Somatic mutation status was concordant with expression of ZAP-70

(unmutated cases are ZAP-70 positive and mutated cases are ZAP-70 negative) in nine patients and discordant in three patients.

Follow-up

Among the six patients (cases 1–6) with a non-complex karyotype, five were alive with complete remission after a median of 74 months (range, 10–143 months) follow-up from the detection of 8q24/MYC rearrangement. One patient (case 5) developed Richter's transformation (diffuse large B-cell lymphoma in pelvic soft tissue) at 41 months and died at 47 months. Among the 12 patients with a complex karyotype (cases 7–18), 10 patients died and 2 patients were alive: patient 7 with persistent chronic lymphocytic leukemia, patient 10 with complete remission after stem cell transplant. Patient 14 developed Richter's transformation (diffuse large B-cell lymphoma in ulcerated colorectal mucosa) at 15 months and died at 48 months. The median overall survival for patients with a complex karyotype was 5.5 months. For the two patients without karyotypic information, patient 20 died at 1 month, and patient 19 was alive after 4 months of development of Richter's transformation. The overall survival of patients with a non-complex karyotype (median survival, undefined) and that of patients with a complex

Table 2 Karyotype and FISH for *MYC* and CLL panel

Case no.	Karyotype prior to t(8q24;v)	Karyotype with t(8q24;v)	FISH for <i>MYC</i>	FISH for CLL panel ^a
1	No	45,X,-Y,t(8;22)(q24.1;q11.2)[8]/46,XY[12]	24%	Negative
2	No	46,XX,t(8;14)(q24.2;q32)[5]//46,XX[14]	90%	D13S319
3	No	46,XY,t(2;8)(p12;p24.1)[1]/46,XY[15]	16%	D13S319
4	No	46,XX,t(8;14)(q24.1;q32)[10]/46,XX[22]	24%	NA
5	No	46,XY,t(8;14)(q24.1;q32)[10]/46,XY[10]	48%	D13S319
6	No	46,XY,t(8;14)(q24.1;q11.2),del(11)(q13q23)[5]/46,XY[15]	39%	ATM, D13S319
7	No	48,XY,+1,+3,t(8;14)(q24;q32)[4]/46,XY[16]	21%	D13S319
8	No	43-46,XY,-3,-8,del(12)(p13),der(14)t(8;14)(q24;q32), del(17)(p11), add(19)(p13.3),+1-3mar[15/46,XY[5]	94%	D13S319, TP53
9	45,XY,-2,del(6)(q13q23),add(14)(q32)[2] /46,XY[18]	47,XY,+1,del(1)(p13p36.1),t(8;14)(q24.1;q32)[19]/46,XY[1]	38%	D13S319
10	46,XX[20]	48,XX,t(8;14)(q24.1;q32),+12,+19[13]/48,idem,del(12)(p11.2)[6]	62%	+12
11	No	47,XX,+4,t(8;14)(q24.1;q32),i(17)(q10)[6]/47,sl,inv(7)(p13p22)[2]/45-47, sl,add(3)(p25),add(4)(p15.2),del(6)(q13q23),?t(11;11)(q23;q25),-16,-17, del(19)(p13.1p13.3),i(21)(q10),+mar[cp10]/46,XX[1]	72%	D13S319, TP53
12	No	42-45,XY,add(4)(p16),del(4)(q25),del(6)(q13),t(8;14)(q24;q32), del(11)(q13q22),add(15)(q26),+0-2mar[cp3]/45,XY,del(6)(q13),-7,-17,-19,+3mar [1]/46,XY[3]	NA	ATM, D13S319
13	50,XX,i(2)(q10),+3,+3,+13,+18 [3]/46,XX[4]	49,XX,dup(1)(q24q41),der(2)t(2;13)(p14;q13)t(2;8)(q24;q21),+3,del(3)(p14p21), t(8;14)(q24.1;q32),der(13)t(2;13),del(15)(q15q24),+18,+18[5]/46,XX[15]	10%	Negative
14	No	47,XX,t(2;8)(p12;q24.1),+12[8]/47-8,XX,t(2;8)(p12;q24),+12[cp4] / 46,XX[9]	NA	NA
15	No	42-43,XY,-2,-3,del(3)(p14),add(4)(q35),-8,t(8;14)(q24.1;q32),-15,-17,-18, add(21)(p11.2),+1-2mar[cp13]/46,XY[7]	NA	NA
16	44-46,XX,t(1;2)(q32;q12),del(15)(q11q15) [cp7]/46,XX[13]	49,XX,dup(1)(q12q44),der(2)del(2)(q13q31)del(2)(q37), +der(2)del(2)(q13q31)del(2)(q37),del(8)(q22),t(8;22)(q24;q11),del(15)(q11q15),add(16)(p13),+18,+add(20)(q13)[15]/48-50,idem,+9[cp5]	41%	NA
17	47,XY,+12[2]/46,XY[17]	47,XY,t(9;10)(q34;q24),+12[4]/45,X,-Y,-4,t(8;14)(q24.1;q32), t(9;10)(q34;q24),+add(17)(p13),add(19)(p13.3)[cp10] /46,XY[6]	8%	+12
18	No	44-46,XY,t(3;10)(p14;q26),t(8;14)(24.1;q32),-17, der(18)t(17;18)(q11.1;p11.2), del(19)(q13.2)[cp18]	39%	NA
19	46,XY[20]	NA (neck mass)	60%	+12, TP53, D13S319
20	46,XY[20]	NA (abdominal mass)	95%	Negative

Abbreviation: NA, not known.

^aListed locus showed deletions.

karyotype (median survival, 5.5 months) ($P = 0.0086$) is shown in Figure 2.

Discussion

MYC is a highly potent proto-oncogene that directs cellular functions related to growth, cell cycle progression, and survival.²³ Deregulation of the *MYC* has been shown to have a critical role in lymphomagenesis.²⁴ *MYC* rearrangement is characteristic of Burkitt lymphoma, but is also associated with a subset of other aggressive B-cell lymphomas that often demonstrate a high proliferation rate. In this study, we show that 8q24/*MYC* rearrangement is very rare in chronic lymphocytic leukemia, and like previously reported cases, *MYC* rearrangement is highly associated with disease progression and clinical aggressiveness. However, we also observed that in some chronic lymphocytic leukemia patients, especially the patients with non-complex karyotype, 8q24/*MYC* rearrangement does not interfere response to

conventional therapy. The clinical significance of *MYC* rearrangement in chronic lymphocytic leukemia appears to depend on other coexisting karyotypic abnormalities.

The clinical course of chronic lymphocytic leukemia is variable. Many patients are generally monitored without therapy unless patients have high Rai stage and the doubling time is <6 months. Progression of chronic lymphocytic leukemia to high-grade lymphoma (Richter's transformation) is an uncommon event, occurring in 2~10% of patients.¹ 8q24/*MYC* rearrangement is rare in chronic lymphocytic leukemia, was reported in ~0.2% of the patients in a study by Reddy *et al*¹⁴ and occurred in ~0.7% of the cases in the current study. Among the reported cases, most patients showed an aggressive clinical course with increased prolymphocytes or development of Richter's transformation.^{9,12-14,16} In our cohort, 14 patients (70%) showed increased prolymphocytes and/or developed Richter's transformation. Also note that almost half of the patients in our cohort had

Figure 1 Combined morphologic-FISH analysis shows *MYC* rearrangement in small mature chronic lymphocytic leukemia cells and large prolymphocytes, not in a granulocyte and myelocytes ($\times 100$ objective). (a, c) (case 2): 82% lymphocytes and 0% prolymphocytes; (b, d) (case 1): 22% small lymphocytes and 15% prolymphocytes. (a, b): Wright-Giemsa stain of bone marrow aspirate smear; (c, d): FISH analysis with *MYC* break-apart probe on the same slides as (a) and (b). Arrow head: granulocyte and myelocyte; Arrow: prolymphocytes; others are small chronic lymphocytic leukemia cells.

8q24/*MYC* rearrangement detected at time of disease relapse (including Richter's transformation) (cases 9–13, 17–20) or a suddenly progressive disease after a long period of time of observation (cases 14, 16). It seems reasonable to assume that 8q24/*MYC* rearrangement was gained during the course of disease and was a secondary event. All our patients required immediate therapy after the detection of 8q24/*MYC* rearrangement, which also suggests that the rearrangement is associated with a progressive or an advanced stage of disease.

The response to therapy and outcomes varied greatly in our patients. At time of last clinical follow-up, six patients achieved complete remission (including one with stem cell transplant), two had persistent disease and 12 died of disease. We compared the clinical, pathological, and cytogenetic features among the patients who had good response to therapy and the patients who were refractory to therapy. There was no difference regarding patients' age or gender. ZAP-70 positivity or immunoglobulin somatic hypermutation status, the two well-known prognostic factors in chronic lymphocytic leukemia,^{25,26} also did not correlate with response

to therapy. However, we found that patients who acquired 8q24/*MYC* rearrangement during the course of disease were almost always resistant to risk-adapted therapy, whereas all six patients (cases 1–4, 6–7) who had good response to therapy (not included stem cell transplant) were patients who had no prior therapy and had 8q24/*MYC* rearrangement detected at the time of first karyotyping.

Treatment response in chronic lymphocytic leukemia patients with *MYC* rearrangement was also closely correlated with the complexity of karyotype. Among the six patients with a non-complex karyotype (cases 1–6), five were alive and remained in complete remission. On the other hand, 10 of 12 patients with a complex karyotype died with a median survival of 5.5 months, the remaining two patients, one received stem cell transplant (case 10) and the other (case 7) had only two chromosome gains besides t(8;14), which may not be a 'true' complex karyotype. Interestingly, a non-complex karyotype was only seen in the untreated patients, whereas a complex karyotype was often associated with relapsed disease or in patients who after a long period of observation suddenly developed a

Figure 2 Overall survival of patients with non-complex karyotype and patients with complex karyotype after the detection of 8q24/MYC rearrangement.

progressive disease. These findings suggest that t(8q24;v) is likely a secondary event in patients with a complex karyotype and a primary event in patients with a non-complex karyotype. This phenomenon is akin to Burkitt lymphoma and diffuse large B-cell lymphoma: patients with Burkitt lymphoma often harbor a sole abnormality of t(8q24;v) or a non-complex karyotype and respond well to chemotherapy, whereas patients with 'double hit' B-cell lymphoma or other transformed high-grade lymphoma often harbor a complex karyotype and are resistant to chemotherapy.²⁷ Similar findings were described in single case reports that chronic lymphocytic leukemia patients with 8q24/MYC rearrangement in a non-complex karyotype responded well to therapy.^{5,9} Besides, we also compared the overall survival of patients (from our chronic lymphocytic leukemia database, regardless of MYC rearrangement) who had a non-complex karyotype ($n=408$) with who had a complex karyotype ($n=351$), patients with a complex karyotype had a significant inferior survival (median overall survival: 130 vs 166 months; $P<0.0001$). This finding also supports that a complex karyotype has an important role in patients' outcome.

Although 8q24/MYC rearrangement is often associated with increased prolymphocytes or Richter's transformation, six patients in this cohort showed typical small mature chronic lymphocytic leukemia morphology, including four patients with a non-complex karyotype. Ki-67 was performed in three of these patients and the proliferation rate ranged 1–10%, which is typical for chronic lymphocytic leukemia and other low-grade lymphomas. The serum lactate dehydrogenase level was normal in four of six patients with a non-complex karyotype. These findings suggest that a non-complex karyotype is often associated with low-grade morphology, low proliferation rate, low lactate dehydrogenase level, and good response to therapy.

In summary, 8q24/MYC rearrangement is very rare in chronic lymphocytic leukemia patients and in majority of the patients, 8q24/MYC rearrangement is acquired during the course of disease. Patients with a

non-complex karyotype often present with small mature chronic lymphocytic leukemia morphology, and respond well to the frontline therapies and achieve long-term complete remission. However, patients with a complex karyotype often have increased prolymphocytes and/or Richter's transformation, are refractory to treatment and have a very aggressive clinical course.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

- 1 Muller-Hermelink HK, Montserrat E, Catovsky D *et al*. Chronic lymphocytic leukemia/small lymphocytic lymphoma. In: Swerdlow SH, Campo E, Harris NL *et al*. (eds). WHO classification of tumors of hematopoietic and lymphoid tissues. Lyon, France: IARC, 2008, pp 180–182.
- 2 Cory S. Activation of cellular oncogenes in hemopoietic cells by chromosome translocation. *Adv Cancer Res* 1986;47:189–234.
- 3 Li S, Lin P, Young KH *et al*. MYC/BCL2 double-hit high-grade B-cell lymphoma. *Adv Anat Pathol* 2013;20: 315–326.
- 4 Lin P, Medeiros LJ. High-grade B-cell lymphoma/leukemia associated with t(14;18) and 8q24/MYC rearrangement: a neoplasm of germinal center immunophenotype with poor prognosis. *Haematologica* 2007;92:1297–1301.
- 5 Kuriakose P, Perveen N, Maeda K *et al*. Translocation (8;14)(q24;q32) as the sole cytogenetic abnormality in B-cell prolymphocytic leukemia. *Cancer Genet Cytogenet* 2004;150:156–158.
- 6 Hao S, Sanger W, Onciu M *et al*. Mantle cell lymphoma with 8q24 chromosomal abnormalities: a report of 5 cases with blastoid features. *Mod Pathol* 2002;15: 1266–1272.
- 7 Lossos IS, Alizadeh AA, Diehn M *et al*. Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. *Proc Natl Acad Sci USA* 2002;99:8886–8891.
- 8 Huh YO, Lin KI, Vega F *et al*. MYC translocation in chronic lymphocytic leukaemia is associated with increased prolymphocytes and a poor prognosis. *Br J Haematol* 2008;142:36–44.
- 9 Dai HP, Xue YQ, Zhang J *et al*. Translocation t(2;8)(p12;q24) in two patients with B Cell chronic lymphocytic leukemia. *Acta Haematol* 2008;120: 232–236.
- 10 Hsiao HH, Hung YH, Hsiao HP *et al*. Case of chronic lymphocytic leukemia with unusual chromosome aberrations. *Int J Hematol* 2004;80:351–353.
- 11 Mark HF, Sotomayor E, Mega A. Occurrence of both t(1;19) and t(8;14) in a patient with chronic lymphocytic leukemia. *Exp Mol Pathol* 1999;66:238–242.
- 12 Merchant S, Schlette E, Sanger W *et al*. Mature B-cell leukemias with more than 55% prolymphocytes: report of 2 cases with Burkitt lymphoma-type chromosomal translocations involving c-myc. *Arch Pathol Lab Med* 2003;127:305–309.

- 13 Pan Z, Xie Q, Repertinger S *et al*. Plasmablastic transformation of low-grade CD5+ B-cell lymphoproliferative disorder with MYC gene rearrangements. *Hum Pathol* 2013;44:2139–2148.
- 14 Reddy K, Satyadev R, Bouman D *et al*. Burkitt t(8;14)(q24;q32) and cryptic deletion in a chronic lymphocytic leukemia patient: report of a case and review of literature. *Cancer Genet Cytogenet* 2006;166:12–21.
- 15 Tang G, Banks HE, Sargent RL *et al*. Chronic lymphocytic leukemia with t(14;18)(q32;q21). *Hum Pathol* 2013;44:598–605.
- 16 Wang L, Aghel A, Peterson B *et al*. Richter transformation with c-MYC overexpression: report of three cases. *Int J Clin Exp Pathol* 2015;8:7540–7546.
- 17 Xue Y, Yu F, Zhou Z *et al*. Complex karyotypic abnormalities including t(8;22) in a patient with chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 1993;65:157–160.
- 18 Matutes E, Owusu-Ankomah K, Morilla R *et al*. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of chronic lymphocytic leukemia. *Leukemia* 1994;8:1640–1645.
- 19 Moreau EJ, Matutes E, A'Hern RP *et al*. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). *Am J Clin Pathol* 1997;108:378–382.
- 20 Fais F, Ghiotto F, Hashimoto S *et al*. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J Clin Invest* 1998;102:1515–1525.
- 21 Shaffer L, McGowan-Jordan J, Schmid M. International system for human cytogenetic nomenclature (ISCN 2013). Recommendations of the international standing committee on human cytogenetic nomenclature. Basel, Germany: Karger, 2013.
- 22 Tang G, DiNardo C, Zhang L *et al*. MLL gene amplification in acute myeloid leukemia and myelodysplastic syndromes is associated with characteristic clinicopathological findings and TP53 gene mutation. *Hum Pathol* 2015;46:65–73.
- 23 Dang CV, O'Donnell KA, Zeller KI *et al*. The c-Myc target gene network. *Semin Cancer Biol* 2006;16:253–264.
- 24 Klapproth K, Wirth T. Advances in the understanding of MYC-induced lymphomagenesis. *Br J Haematol* 2010;149:484–497.
- 25 Rassenti LZ, Huynh L, Toy TL *et al*. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med* 2004;351:893–901.
- 26 Hamblin TJ, Davis Z, Gardiner A *et al*. Unmutated Ig V (H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 1999;94:1848–1854.
- 27 Boerma EG, Siebert R, Kluin PM *et al*. Translocations involving 8q24 in Burkitt lymphoma and other malignant lymphomas: a historical review of cytogenetics in the light of today's knowledge. *Leukemia* 2009;23:225–234.