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MYC rearrangement but not extra *MYC* copies is an independent prognostic factor in patients with mantle cell lymphoma

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Ferrata Storti Foundation

Haematologica 2021
Volume 106(5):1381-1389

ABSTRACT

Mantle cell lymphoma (MCL) with *MYC* rearrangement (*MYC*-R) is rare and little is known about the importance of *MYC* extra copies (EC) in the absence of *MYC*-R in MCL patients. This study includes 88 MCL patients with *MYC* tested by fluorescence *in situ* hybridization and/or conventional cytogenetics, including 27 with *MYC*-R, 21 with *MYC*-EC, and 40 with normal *MYC*-NL. MCL patients with *MYC*-R more often had blastoid/pleomorphic morphology; a higher frequency of CD10, MYC, and simultaneous MYC and BCL2 expression; a higher level of MYC; and a higher Ki67 proliferation rate (P<0.05) than those without *MYC*-R. Although patients with *MYC*-R more frequently received intensive chemotherapy (P=0.001), their overall survival (OS) was significantly shorter than those without *MYC*-R. Compared with patients with MYC/BCL2 double-hit lymphoma (DHL), patients with *MYC*-R MCL had a similar OS but more commonly had bone marrow involvement, Ann Arbor stage IV disease, and a different immunophenotype. MCL patients with *MYC*-EC showed an OS intermediate between those with *MYC*-R and *MYC*-NL, either all or only blastoid/pleomorphic MCL patients included. Multivariate analysis showed that MYC-R, but not *MYC*-EC, had an independent and negative impact on OS. In conclusion, *MYC*-R but not MYC-EC showed a higher MYC expression and is an adverse prognostic factor for MCL patients. Although the OS of MCL patients with *MYC*-R is similar to that of *MYC/BCL2* DHL patients, these groups have different clinicopathologic features supporting the retention of MCL with *MYC*-R in the category of MCL, as recommended in the revised World Health Organization classification.

Introduction

Mantle cell lymphoma (MCL) is an aggressive, incurable B-cell lymphoma characterized by t(11;14)(q13;q32) that juxtaposes the *CCND1* gene adjacent to *IGH* on the derivative chromosome 14. This translocation results in constitutive overexpression of cyclin D1 and deregulation of the cell cycle at the G1/S phase transition.[1-3] Data from mouse models and clinical studies suggest that *CCND1* is a weak oncogene and that secondary genetic aberrations likely contribute to MCL development.[4,5] Furthermore, conventional cytogenetic studies have shown that the t(11;14) is rarely an isolated genetic abnormality in MCL. The lymphoma cells display a high degree of genomic instability and tend to accumulate additional chromosomal and molecular alterations, which likely lead to clinical progression of disease.[1,6,7]

MYC is one of the most frequently deregulated oncogenes in human malignancies.[8,9] t(8;14)(q24;q32)/*MYC-IGH* was the first recurrent translocation identified in lymphomas, initially in Burkitt lymphoma (BL). Subsequently, it was learned that

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Received: November 13, 2019.
Accepted: March 27, 2020.
Pre-published: April 9, 2020.

https://doi.org/10.3324/haematol.2019.243071

MYC can partner with immunoglobulin (Ig) and non-Ig genes in multiple types of B-cell lymphoma including diffuse large B-cell lymphoma (DLBCL), high grade B-cell lymphoma (previously known as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL)[10-12] and rarely other small B-cell lymphomas, such as follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma and MCL. MYC alterations are often associated with an aggressive clinical course.[13-19]

Double-hit lymphoma (DHL) was defined broadly by Aukema *et al.*[20] as a mature B-cell lymphoma with a chromosomal breakpoint affecting the *MYC* locus combined with additional translocations involving other genes, such as *BCL2, BCL3, BCL6,* or *CCND1*. The most common genetic combination in DHL is *MYC/8q24* rearrangement and t(14;18)(q32;q21)/*IGH-BCL2*, which represents about 65% of cases.[20-22] Significant advances in the understanding of DHL have been made in recent years, and large B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements were included in the category of high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements in the 2017 World Health Organization (WHO) classification, except for cases that fulfill criteria for a follicular lymphoma, MCL, or lymphoblastic lymphoma.[23] As a result, MCL with MYC rearrangement (*MYC*-R), although fulfilling the earlier concept of DHL suggested by Aukema and colleagues, remains in the category of MCL.

MCL associated with *MYC*-R is rare and only case reports and small case series have been reported previously.[6,24-29] No study has explored the prognostic significance of *MYC*-R in MCL patients by comparing the survival of MCL patients with or without *MYC*-R. In addition, as we have studied cases of MCL by fluorescence *in situ* hybridization (FISH) to assess *MYC* we have come across cases of MCL with extra copies of *MYC* (*MYC*-EC), but without *MYC*-R. The prognostic impact of *MYC*-EC has not been well characterized previously.

In this study we had two aims. First, we addressed the prognostic impact of *MYC*-R in MCL patients, and in particular, is the prognosis more akin to that of patients with DHL with *MYC* and *BCL2* rearrangements (*MYC/BCL2* DHL). Secondly, we addressed the question of the potential prognostic impact of *MYC*-EC in the absence of *MYC* rearrangement in MCL patients.

Methods

Case selection

We searched the cytogenetic/FISH testing database of the Department of Hematopathology at The MD Anderson Cancer Center from January 1, 2004 to December 31, 2018 and identified 88 cases of MCL with *11q13/CCND1* and *8q24/MYC* tested by FISH and/or conventional karyotyping. Only three cases were before 2010 and most cases were diagnosed in recent years. *MYC*-R in MCL is rare and there are no standard rules or criteria for which MCL should be tested for *MYC* FISH, so the choice of *MYC* FISH testing for MCL was solely at the discretion of the treating oncologist and diagnosing hematopathologist. However, in general it was performed on a small subset of blastoid/pleomorphic MCL and occasional classic MCL cases. Ninety-five cases of high-grade B-cell lymphoma with concurrent *MYC* and *BCL2* rearrangements confirmed by FISH (*MYC/BCL2* DHL) from the same time period were used as a comparison group. Clinical information was obtained by review of corresponding medical records, including lymphoma history, sites of involvement, stage, treatment and overall survival (OS). Morphologic, immunophenotypic and cytogenetic data were also reviewed. The diagnosis of all cases was made according to the criteria of the current WHO classification.[10,11] The study was approved by the Institutional Review Board.

Immunophenotypic methods

Immunohistochemical stains were performed using formalin-fixed, paraffin-embedded (FFPE) tissue sections, either at the time of diagnosis or retrospectively for the purpose of this study. The monoclonal antibodies used were specific for: CD3, CD5, CD10, CD20, BCL-2, BCL-6, IRF4/MUM-1, MYC, P53, Ki67, cyclin D1, and SOX-11. The positive cutoff was ≥30% for CD10, MUM-1, and BCL6[30]; ≥20% for P53[31]; ≥40% for MYC[16]; ≥50% for BCL2[32] and >10% for SOX11[33].

Flow cytometry immunophenotypic analysis was performed using either a FACScanto II or FACSCalibur cytometer (Becton-Dickinson Biosciences, San Jose, CA, USA) as described previously.[34,35] Lymphocytes were gated for analysis using side scatter *versus* forward scatter, and CD45 *versus* side scatter. The panel of antibodies employed included CD3, CD5, CD10, CD11c, CD19, CD20, CD22, CD23, CD30, CD38, CD43, CD45, CD79b, CD200, FMC-7, and surface Ig κ and λ light chains. All antibodies were obtained from Becton-Dickinson Biosciences.

Conventional cytogenetics and fluorescence *in situ* hybridization

Conventional chromosomal analysis was performed on G-banded metaphase cells prepared from cell suspensions from tissue biopsy specimens or bone marrow aspirates using standard techniques. The karyotype was reported according to the International System for Human Cytogenetic Nomenclature (2016).[36] FISH was performed on bone marrow smears or 4-µm-thick FFPE tissue sections according to the manufacturer's instructions. A total of 200 interphase nuclei for each probe were analyzed. FISH probes used in this study included the following: locus specific identifier (LSI) *IGH/CCND1* dual-color, dual fusion translocation probe; LSI *MYC* as well as BCL6 dual-color, break-apart probe; LSI *IGH/BCL2* dual-color, dual-fusion translocation probe (Vysis/Abbott Laboratories, Des Plaines, IL, USA).

Statistical analysis

Overall survival (OS) was calculated from the date of initial diagnosis (for *de novo* cases) or the date that a *MYC* aberration was detected (for patients with *MYC* aberration detected at disease transformation or progression) to the date of death or last follow-up. Survival was analyzed using the Kaplan-Meier method and was compared by log-rank test (GraphPad Prism version 7 software). Fisher's exact test was utilized to compare the difference between groups. Multivariate Cox proportional hazard analysis was performed using SPSS 24.0 software. Differences between groups were considered statistically significant when the *P*-value is less than 0.05.

Results

Mantle cell lymphoma patients with *MYC* rearrangement

Clinical characteristics

Twenty-seven MCL patients had *MYC*-R, including 20 men and 7 women, with a median age of 63 years (range, 47 to 85). Fourteen (52%) patients with *MYC*-R presented

Table 1. Comparison of features of mantle cell lymphoma with MYC rearrangment (*MYC-R*), mantle cell lymphoma without *MYC-R* and *MYC/BCL2* double-hit lymphoma.

Features	MCL with *MYC-R* (n=27)	MCL with non-*MYC-R* (n=61)	*MYC/BCL2* DHL (n=95)	*P* value of MCL with *MYC-R* *vs.* MCL without *MYC-R*	*P* value of MCL with *MYC-R* *vs. MYC/BCL2* DHL
Age (years), Median (range)	63 (47-85)	61.5 (33-85)	60.5 (33-86)	0.25	0.21
Age ≥60 (years)	67% (18/27)	54% (33/61)	59% (56/95)	0.21	0.32
Sex (Male:Female)	20:7	44:17	64:31	0.80	1.00
Stage IV	92% (24/26)	92% (55/60)	66% (58/88)	1.00	**0.01***
BM-Positve	96% (23/24)	83%(50/60)	44% (33/75)	0.17	**0.0001***
CNS-Positve	33% (4/12)	21% (4/19)	13% (7/52)	0.68	0.20
Extranodal Sites ≥2	77% (20/26)	68% (39/57)	56% (49/88)	0.61	0.11
Elevated LDH (>618 U/L)	65% (17/26)	46% (23/50)	86% (55/64)	0.22	**0.03***
Elevated WBC(>11.0 × 10^6/μL)	40% (10/25)	44% (22/50)		0.81	
High MIPI/ High or High-Intermediate IPI	58% (15/26)	44% (22/50)	85% (60/71)	0.32	
Morphology for MCL					
Classic	11% (3/27)	46% (28/61)			
Blastic/Pleomorphic	89% (24/27)	54% (33/61)		**0.004***	
Leukemic Non-Nodal	26% (7/27)	26% (16/61)		0.88	
Immunophenotype					
SOX11$^+$	70% (7/10)	90% (28/31)		0.14	
BCL6$^+$	31% (4/13)	26% (8/31)	93% (68/73)	0.73	**0.0001***
CD5$^+$	73% (19/26)	87% (53/61)	5% (3/63)	0.11	**0.0001***
CD10$^+$	35% (9/26)	11% (6/56)	96% (87/91)	**0.01***	**0.0001***
MUM-1$^+$	50% (4/8)	67% (6/9)	31% (14/45)	0.64	0.42
BCL2 (≥50%)	86% (12/14)	97% (28/29)	94% (83/88)	0.22	0.22
MYC (≥40%)	80% (12/15)	17% (6/36)	85% (39/46)	**0.0001***	0.70
MYC/BCL2 dual-expression	69% (9/13)	9% (3/33)	78% (36/46)	**0.0001***	0.46
P53 (≥20%)	71% (12/17)	65% (13/20)	63% (12/19)	1.00	0.73
Ki67, Median(range)	90 (15-100)	60 (2-100)	85 (20-100)	**0.004***	0.53
Treatment					
Intensive chemotherapy	67% (16/24)	24% (13/54)	51% (44/86)	**0.001***	0.25
Other immuno/chemotherapy	33% (8/24)	74% (40/54)	49% (42/86)		
Initial CR	33% (8/24)	62% (29/47)	32% (27/85)	**0.03***	1.00

Blank: not available; BM: bone marrow; CNS: central nervous system; CR: complete remission; LDH: lactate dehydrogenase; MIPI: Mantle Cell Lymphoma International Prognostic Index; MCL: mantle cell lymphoma; DHL: double hit lymphoma; intensive chemotherapy (R-CHOP): rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; other immuno/chemotherapy (R-Hyper-CVAD): rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone; WBC: white blood cell; *P<0.05.

with *de novo* MCL and 13 (48%) patients acquired *MYC*-R at time of disease progression or transformation from classic to blastoid/pleomorphic MCL. There were 13 (48%) cases diagnosed initially in lymph nodes, 11 (41%) cases in bone marrow and three cases in other tissue sites. Most patients presented with high stage (Ann Arbor stage IV) disease, high frequency of involvement of bone marrow or other extranodal sites, and elevated white blood cell (WBC) count and serum lactate dehydrogenase (LDH) level (Table 1). The involved extranodal sites included the bone marrow, spleen, central nervous system, gastrointestinal tract, peripheral blood, pleural fluid, pancreas, chest wall and soft tissue. A leukemic non-nodal form of MCL, defined as MCL with peripheral blood, bone marrow and sometimes spleen involvement but without significant lymphadenopathy by WHO classification, was present in seven (26%) patients. Twenty-six patients had available clinical data to calculate the Mantle Cell Lymphoma International Prognostic Index (MIPI) score[37] and 15 (58%) patients had a high-risk MIPI score (Table 1).

Pathologic characteristics

Twenty-four (89%) cases of MCL associated with *MYC*-R cases had blastoid (n=19) (Figure 1) or pleomorphic (n=5) morphologic features and three cases were classic type. Eleven of 14 (79%) *de novo* MCL with *MYC*-R showed blastoid (n=10) or pleomorphic (n=1) morphology. All 13 patients with MCL that acquired *MYC*-R during disease progression presented with classic MCL at initial diagnosis, but had blastoid (n=9) or pleomorphic (n=4) morphology at the time of emergence of *MYC*-R.

All lymphomas were positive for one or more pan-B-cell antigens and were negative for pan-T cell antigens. As expected, all MCL with *MYC*-R cases expressed cyclin D1 (27 of 27, 100%), and most cases expressed SOX11 (7 of 10, 70%), and MYC (12 of 15, 80%). Concurrent MYC and BCL2 expression was observed in 9 of 13 (69%) MCL cases assessed. Nineteen of 26 (73%) cases were positive for CD5 (one case not assessed); the CD5-negative cases included four *de novo* MCL, two neoplasms which apparently lost CD5 at the time of detection of *MYC*-R, and one

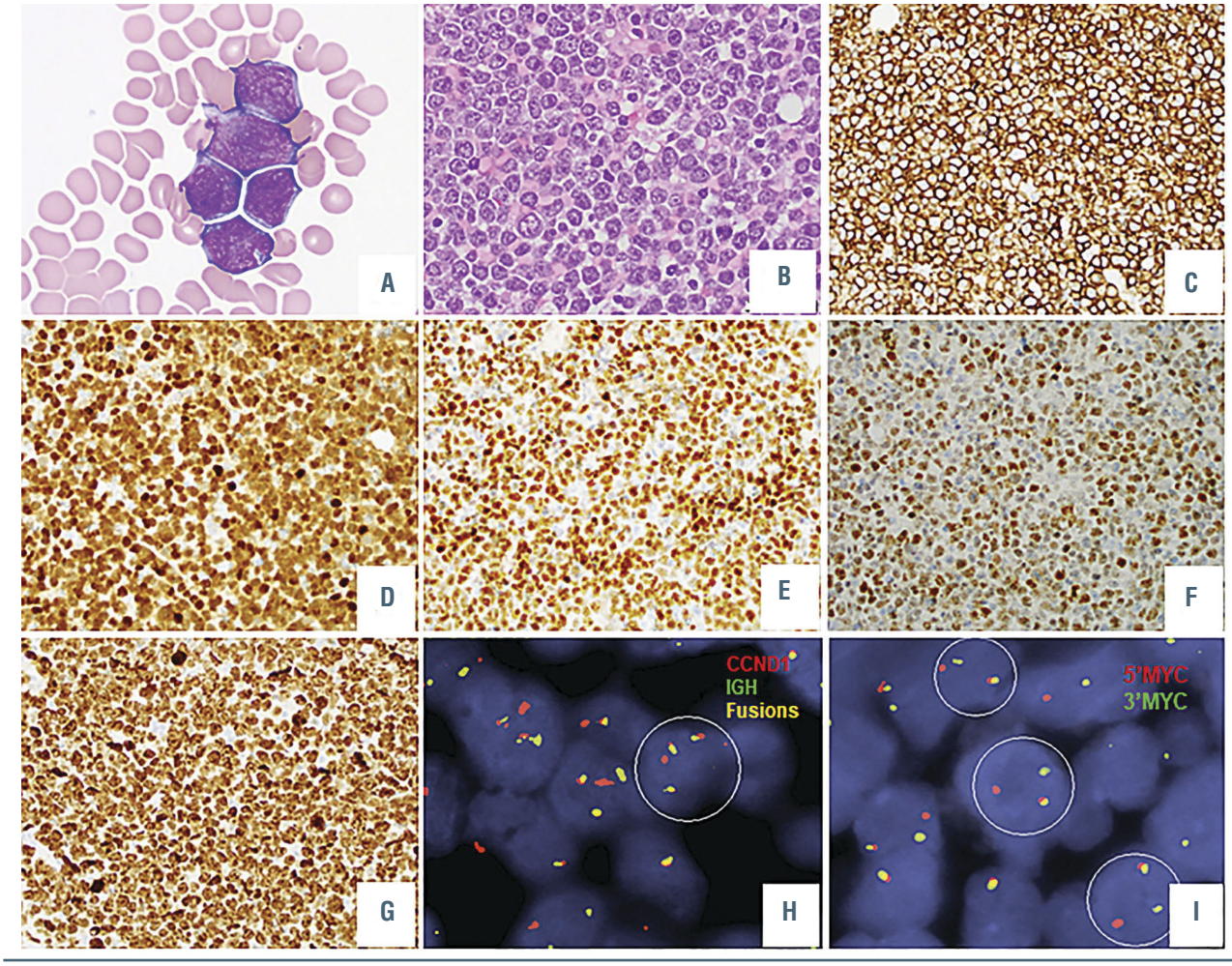

Figure 1. A representative case of mantle cell lymphoma with *MYC* rearrangement. The lymphoma cells have blastoid morphology. (A) Peripheral blood, (B) core biopsy, and express CD20 (C), cyclinD1 (D), MYC (E), BCL6 (F), and with a high Ki67 proliferation rate (G). Fluorescence *in situ* hybridization study showed *CCND1/IGH* (H) and *MYC* rearrangement (I).

case that was originally CD5-negative and developed *MYC*-R subsequently. CD10 was positive in 9 of 26 (35%) cases assessed; CD10 was acquired at the time of transformation when *MYC*-R emerged. All CD10$^+$ cases had blastoid morphology. Four CD10$^+$ MCL cases were CD5-negative. BCL-2 was positive in 12 of 14 (86%) cases of MCL with *MYC*-R. IRF4/MUM-1 and BCL-6 were positive in 4 of 8 (50%) and 4 of 13 (31%) cases assessed, respectively. Twelve of 17 (71%) cases showed P53 expression in more than 20% of cells, including all 9 cases (100%) of transformed MCL and 4 of the 8 (50%) *de novo* MCL cases tested. The Ki67 proliferation index was variable, but most cases had a high proliferation rate with a median Ki67 index of 90% (range, 15-100%; only three cases had Ki67<60%). All tested cases were negative for CD23 and CD200 (Table 1).

FISH showed *MYC*-R and *CCND1* translocation in all 27 cases. One case showed both *MYC*-R and *MYC*-EC. Since there is only one such case, it was included in the *MYC*-R group. Conventional cytogenetic analysis was available in 18 cases and all showed a complex karyotype, including t(11;14)(q13;q32) in 17 cases. By combined FISH and karyotype, 18q21/*BCL2* and 3q27/*BCL6* status were available in 19 cases and all were negative for rearrangement except one case with *BCL6*-R. Based on identifiable karyotype data, seven cases had *MYC* partner gene as *IG* gene and three with non-*IG* gene.

Treatment and prognosis

Detailed therapy data were available for 24 of 27 MCL patients with *MYC*-R. All patients were treated with combination chemotherapy: sixteen (67%) patients received intensive induction chemotherapy, mainly rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with methotrexate and cytarabine) (R-Hyper-CVAD, n=14) or rituximab, etoposide, prednisone, vincristine, and doxorubicin (R-EPOCH, n=2). Eight (33%) patients received R-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or R (rituximab) and bendamustine. Eight (33%) patients reached complete remission (CR) after induction, but six relapsed. Seven patients received a stem cell transplant (SCT), including four autologous and three allogeneic. After a median follow-up of 41.5 months, 17 of 24 (71%) patients died including 10 patients with *MYC*-R detected during disease progression/transformation. The median OS was 19.9 months and the 3-year OS rate was 33% for all 27 patients. The median OS was worse for patients with MCL in whom *MYC*-R emerged during disease progres-

Table 2. **Comparision of features of blastoid mantle cell lymphoma wih *MYC* rearrangement and without *MYC* rearrangement.**

Features	Blastoid MCL with *MYC-R* (n=24)	Blastoid MCL with Non- *MYC-R* (n=29)	*P*
Age(years), Median (range)	63 (47-82)	67 (33-85)	0.96
Age ≥60 (years)	71% (17/24)	62% (18/29)	0.76
Sex (Male:Female)	17:7	20:7	0.75
Stage IV	91% (20/22)	89% (24/27)	1.00
BM-Positve	86% (18/21)	74% (20/27)	0.72
CNS-Positve	40% (4/10)	29% (4/14)	0.67
Extranodal Sites ≥2	80% (16/20)	88% (22/25)	0.61
Elevated LDH (>618 U/L)	68% (15/22)	62% (13/21)	0.99
Elevated WBC (>11.0×10^6/μL)	40% (8/20)	30% (6/20)	0.74
High MIPI	57% (12/21)	53% (9/17)	1.00
Leukemic Non-Nodal	24% (5/21)	7.4% (2/27)	0.22
Complex Karyotype	100% (16/16)	100% (12/12)	1.00
Immunophenotype			
SOX11$^+$	73% (8/11)	88% (15/17)	0.28
BCL6$^+$	33% (4/12)	33% (7/21)	1.00
CD5$^+$	78% (18/23)	81% (22/27)	0.72
CD10$^+$	39% (9/23)	12% (3/25)	**0.046***
MUM-1$^+$	50% (4/8)	71% (5/7)	0.59
BCL2$^+$(≥50%)	86% (12/14)	95% (19/20)	0.54
MYC$^+$ (≥40%)	80% (12/15)	20% (4/20)	**0.001***
MYC/BCL2 co-express	69% (9/13)	16% (3/19)	**0.004***
P53$^+$ (≥20%)	75% (12/16)	69% (9/13)	0.97
Ki67, Median (range)	90 (15-100)	90 (30-100)	0.37
Treatment			
Aggressive chemotherapy	68% (15/22)	38% (10/26)	0.08
Other chemotherapy	32% (7/22)	62% (16/26)	0.15
Initial CR	55% (12/22)	70% (16/23)	0.34

BM: bone marrow; CNS: central nervous system; CR: complete remission; LDH: lactate dehydrogenase; MYC-R: MYC rearrangement; N: number of cases; MIPI: Mantle Cell Lymphoma International Prognostic Index; aggressive chemotherapy (R-CHOP): rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; other chemotherapy (R-Hyper-CVAD): rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone; SCT: stem cell transplant; WBC: white blood cell; *P < 0.05

sion/transformation than for patients with *de novo* MCL with *MYC*-R (Figure 2A, P=0.019).

Mantle cell lymphoma with *MYC* rearrangement *versus* non-*MYC* rearrangement

In addition to the 27 MCL patients with *MYC*-R, 21 patients had *MYC*-EC, and 40 patients had *MYC*-NL. The 21 MCL cases with *MYC*-EC showed a median MYC copy number of 3.5 (range, 3-6); most cases had 3-4 copies. OS was compared among these subgroups and showed that patients with MCL associated with *MYC*-R had the poorest OS. Patients with *MYC*-NL MCL subgroup had the best OS and patients with *MYC*-EC group had an intermediate prognosis, closer to patients in the *MYC*-NL subgroup (P=0.34) than the *MYC*-R subgroup (P=0.074) (Figure 2B, overall P=0.0007). Therefore, we combined the *MYC*-EC and *MYC*-NL patients into a non-*MYC*-R group to compare with the *MYC*-R group. Cases of MCL with *MYC*-R more frequently showed blastoid/pleomorphic morphology (89% *vs.* 54%, P=0.004), more often expressed CD10 (P=0.01), MYC (P=0.0001), and, more commonly showed coexpress of MYC and BCL-2 (P=0.0001) and also had a higher Ki67 proliferation rate (median 90% *vs.* 60%) (P<0.004). All other clinicopathologic features, including the frequency of leukemic non-nodal form MCL, were similar between the two groups (Table 1). Patients with *MYC*-R less frequently reached CR after induction chemotherapy than patients in the non-*MYC*-R group (33% *vs.* 62%, P=0.03), despite more often receiving intensive induction therapy. The median OS of MCL patients with *MYC*-R was significantly lower than that of patients in the non-*MYC*-R group with 3-year OS rates of 33% and 67%, respectively (Figure 2C, P=0.0002). This result was also true when only *de novo* cases were included in the analysis (Figure 2D, P=0.030).

Since *MYC*-R occurred predominantly in blastoid/pleomorphic variants, a comparison of MCL with *MYC*-R *versus* non-*MYC*-R was further explored in cases with only blastoid or pleomorphic morphology. There were 53 cases of MCL with blastoid/pleomorphic morphology, including 24 cases with *MYC*-R and 29 cases without *MYC*-R. As shown in Table 2, almost all clinicopathologic features, including P53 expression, frequency of a complex karyotype, and CR rate of patients with *MYC*-R were very similar to patients with non-*MYC*-R, except that the *MYC*-R group of neoplasms were more often positive for MYC and CD10 (P<0.05). The median OS of patients with *MYC*-R was significantly worse than that of patients in the non-*MYC*-R subgroup when all blastoid/pleumor-

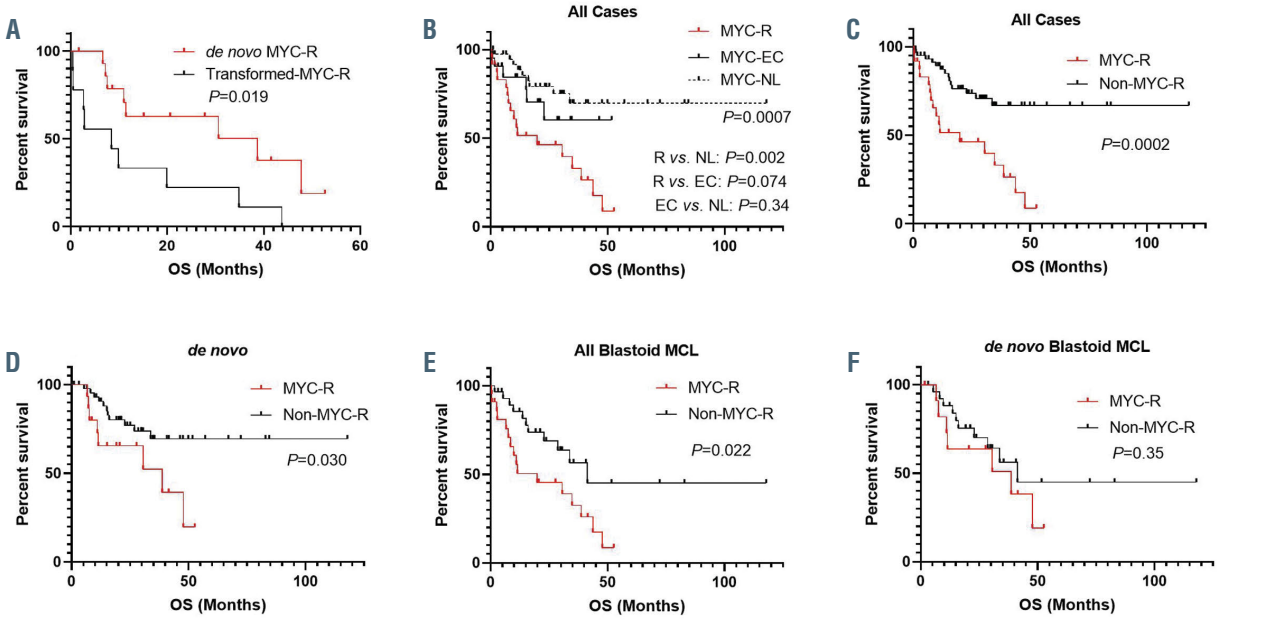

Figure 2. Comparison of median overall survival. There is a statistically significant difference in overall survival (OS) between *de novo* and transformed mantle cell lymphoma (MCL) with *MYC* rearrangement (*MYC*-R) (A); MCL with *MYC*-R and Non-*MYC*-R either all patients (C), only *de novo* cases (D), all blastoid MCL (E) or only *de novo* blastoid MCL (F) were included. In all MCL patients, *MYC*-R group had the worst OS, MYC normal (*MYC*-NL) group the best OS, and MYC extra copies (*MYC*-EC) group laid in between (B).

phic patients were included (Figure 2E, *P*=0.022). However, there was no significant difference in median OS when only *de novo* blastoid/pleumorphic MCL patients were compared (Figure 2F, *P*=0.35).

Correlation of MYC expression with MYC fluorescence *in situ* hybridization status in mantle cell lymphoma

MYC immunohistochemical stains were performed on 51 cases of MCL, including 15 with *MYC*-R, 15 with *MYC*-EC, and 21 with *MYC*-NL. MYC expression level showed a much wider distribution across samples in *MYC*-R cytogenetic subgroup than *MYC*-EC and *MYC*-R subgroups due to the higher level of expression. The mean percentage of cells expressing MYC protein was significantly higher in the *MYC*-R group than those in the *MYC*-EC and MYC-NL groups (50%, range, 1-100% in the *MYC*-R group; 13%, range, 0-55% in the *MYC*-EC group; and 15%, range, 0-60% in the *MYC*-NL group; Figure 3A, *P*<0.0001). There was no significant difference in the percentage of cells expressing MYC between the *MYC*-EC and *MYC*-NL groups (*P*=0.71). Although MCL cases with *MYC*-R demonstrated protein expression at variably high levels (≥40% in 12 of 15, 80% of cases), slightly high MYC expression could occasionally occur in MCL without *MYC*-R. By using the 40% as a cut-off value for MYC immunohistochemistry to predict *MYC*-R, the sensitivity and specificity were 80% and 83% respectively.

Multivariate analysis

In order to further explore if *MYC*-R or *MYC*-EC were independent prognostic factors in MCL patients, multivariate Cox proportional hazard analysis was performed including MYC status and other factors that often predict survival in MCL, including morphology, Ki67 rate, and MIPI score. As shown in Table 3, *MYC*-R but not *MYC*-EC was an independent prognostic factor for OS in this cohort of MCL patients.

Table 3. Multivariate analysis.

Features	HR	95% CI	P
MYC-R	3.27	1.149 - 9.306	**0.026**
MYC-EC	2.375	0.632 - 8.923	0.200
Blastoid/Pleumorphic MCL	7.038	0.767 - 64.593	0.073
Ki67≥30%	1.93	0.215 - 17.370	0.557
High MIPI	1.217	0.532 - 2.783	0.642

MYC-R: *MYC* rearrangement; *MYC*-EC: *MYC* extra copies; MCL: mantle cell lymphoma; MIPI: Mantle Cell Lymphoma International Prognostic Index; HR: hazard ratio; CI: Confidence Interval; *P*<0.05.

Patients with mantle cell lymphoma associated with *MYC* rearrangement versus *MYC/BCL2* double hit lymphoma patients

The 27 MCL patients with *MYC*-R were compared with 95 patients with *MYC/BCL2* DHL (Table 1), the latter group including 67 patients with *de novo* DHL and 28 with DHL transformed from follicular lymphoma. While many clinicopathologic features were similar between these two groups, each group had some unique features. Patients with MCL harboring *MYC*-R more often presented with bone marrow involvement (96% *vs.* 44%, *P*=0.0001), Ann Arbor stage IV disease (92% *vs.* 66%, *P*=0.01) and more frequent CD5 expression (71% *vs.* 5%, *P*=0.0001). In contrast, elevated serum LDH level and more frequent CD10 and BCL6 expression were observed more often in the *MYC/BCL2* DHL group (*P*<0.05 for all).

There was no significant difference in CR rate between MCL patients with *MYC*-R and patients with *MYC/BCL2* DHL. Patients with MCL associated with *MYC*-R had an inferior median OS compared with patients with *MYC/BCL2* DHL (Figure 3B, *P*=0.038). However, there was no significant difference in OS when patients with *de novo* MCL and *MYC*-R were compared to patients with *de novo MYC/BCL2* DHL (Figure 3C, *P*=0.83). Since prognosis

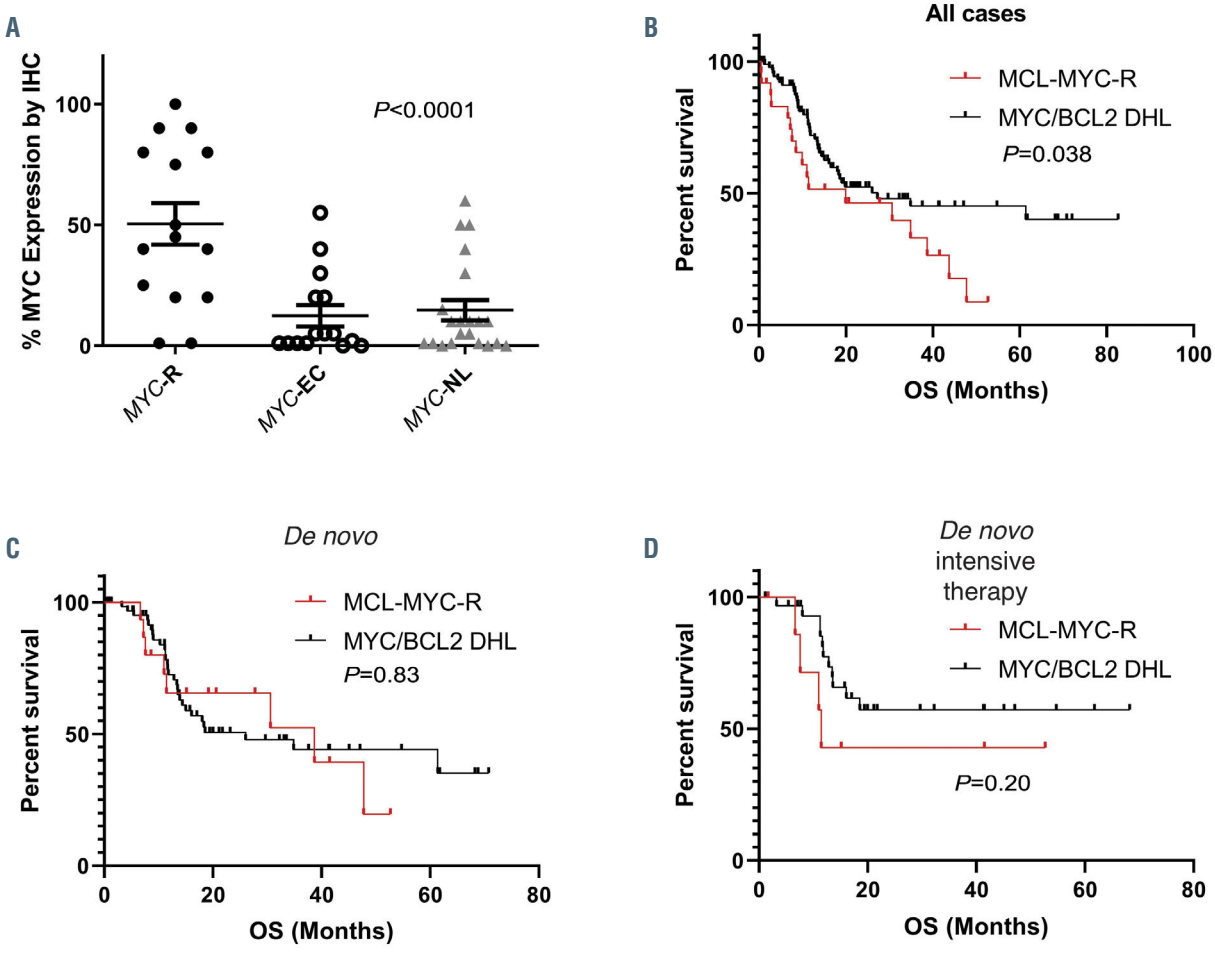

Figure 3. MYC protein expression in correlation with MYC cytogenetic status in mantle cell lymphoma. (A); Comparison of median overall survival (OS) between mantle cell lymphoma (MCL) with MYC rearrangement (MYC-R) and MYC/BCL2 double hit lymphoma (DHL): (B) All cases included; (C) Only *de novo* cases included; (D) Only patients who received intensive induction chemotherapy included.

is significantly related to the treatment regimens patients received and majority patients received intensive chemotherapy, we further compared the OS between patients who only received intensive induction immunochemotherapy (including R-Hyper-CVAD and R-EPOCH) in these two groups, and as shown in Figure 3D, there was no significant difference in OS between the two sub-groups.

Discussion

MYC aberrations can occur rarely in cases of MCL. In this study, we collected 88 MCL patients with known MYC status and explored the prognostic role of MYC aberrations. We show that *MYC*-R but not *MYC*-EC is an independent adverse prognostic factor in MCL patients. We also compared the clinicopathologic features of MCL patients with *MYC*-R, so-called double hit MCL, to a large group of patients with *MYC/BCL2* DHL and show some similarities and differences. To our knowledge, this is the largest series of MCL cases in which *MYC* status has been assessed.

MCL with *MYC*-R has been reported previously, however, most studies have been case reports or small case series that were mainly descriptive and without a *MYC*-R control group to compare for clinicopathologic features and prognosis.[6,24-29] In this study, by comparing to a control group of 61 MCL cases without *MYC*-R, MCL cases with *MYC*-R demonstrated some unique clinicopathologic features: more frequently have blastoid/pleomorphic morphology, more frequently express CD10, MYC, MYC and BCL2 co-expression, with a higher Ki67 proliferation rate and an inferior OS. It is well known that blastoid and pleomorphic variants of MCL has a poorer prognosis. In order to exclude the effect of morphology, the role of *MYC*-R was further evaluated in blastoid/pleomorphic MCL cases, which showed *MYC*-R was associated with higher MYC expression and expression of CD10 and a poorer OS, especially in transformed MCL cases. However, there are many other potential factors involved when patients with MCL undergo progression or transformation. In order to further exclude other possible confounding factors, a multivariate analysis was performed and demonstrated that *MYC*-R is an independent poor prognostic factor in MCL patients.

MYC (8q24) is an essential global transcription factor that controls 10-15% of all human genes and regulates many cellular functions including cell cycle, cell growth, metabolism, biosynthesis, survival, and apoptosis. Dysregulation of *MYC* induces lymphomagenesis. In BL, *MYC*-R is the primary event and mainly translocated with

IGH. In MCL, *CCND1* rearrangement is the primary event and *MYC*-R is likely a secondary event, which is further suggested by the more frequent translocation with IG light chain genes or non-*IG* genes in our current study. Many oncogenes function by activation mutations or forming oncogenic fusion proteins, however, *MYC* works differently by loss of tight control of intact *MYC* at both the transcriptional and translational levels. MYC protein can be upregulated by three major mechanisms, among which *MYC* translocation and amplification are two of them. This is evidenced by the current findings of significantly higher level of MYC protein expression and worse OS in the *MYC*-R MCL group than the two groups without *MYC-R*.

Fifteen patients originally diagnosed with classic variant MCL underwent disease progression/transformation to blastoid/pleomorphic variant of MCL during or after initial treatment. *MYC-R* was detected at the time of disease transformation in 13 (87%) of these patients, *MYC*-EC (4-5 copies) was detected in one patient, and no MYC aberration was detected in one patient. These data suggest that *MYC*-R is involved in MCL disease progression and transformation and also contributed to a poorer prognosis. This finding also confirmed the observation of a few case reports in the literature that described the emergence of *MYC*-R at time of MCL progression or transformation.[24,26,38] Previous studies shown *MYC* co-operated with transcriptionally activated cyclinD1 and resulted in blastoid MCL or oncogenic transformation of B-cell lymphoma in mouse models.[5,39] Studies also demonstrated that MYC plays an important role in intrinsic ibrutinib resistance in MCL, possibly by repressing miR15a and miR16-1, two tumor suppressor microRNA involved in MCL pathogenesis.[40,41] These mechanisms may explain the role of *MYC*-R in MCL progression or transformation. Of note, secondary *MYC* translocation is often associated with genomic instability and a complex karyotype. Except activating of *MYC*, many other factors may also contribute to MCL disease progression and transformation, such as inactivation of *CDKN2A* and *TP53* genes, gain or loss of other chromosomes and gene mutations. In our current study, all 18 cases of *MYC*-R MCL with karyotype available showed a complex karyotype, and all nine cases with *P53* expression data available showed an over expression of P53 (seven cases with P53 >80% and two cases 50%). These results confirmed the above points. Although only a very small number of progressed or transformed MCL cases were tested for *MYC*-R by FISH, it is reasonable to conclude that *MYC*-R is associated with MCL progression or transformation at lease in a subset of MCL patients.

In this study, the MYC protein expression level is significantly higher in MCL with *MYC-R* than those without *MYC*-R (MYC>40% in 80% *vs.* 17% of cases respectively). These findings are consistent with previously reported MYC expression in MCL and our previous study of MYC expression in DLBCL.[42-44] Our results also demonstrate that using 40% as a cut-off, MYC immunohistochemistry can predict *MYC*-R with a sensitivity of 80% and a specificity of 83%, better than those reported for DLBCL which has a similar sensitivity but much lower specificity of 61%.[44] Based on our results and the aggressiveness of MCL with *MYC*-R, we recommend using MYC immunohistochemistry of >40% as a screening tool to test MYC by FISH in all blastoid/pleomorphic MCL cases for cost effective practice.

A few cases of MCL with *MYC*-EC have been described in the literature.[6,28,45] Yi *et al.*[46] reported 14 patients with *MYC*-EC and four patients with *MYC-R* and these 18 patients had a poorer prognosis than a comparison group of MCL patients without MYC abnormalities.[46] To date, we are not aware of any prognostic studies for a pure group of MCL patients with *MYC*-EC without *MYC*-R. In this study, the prognostic effect of MYC-EC lie in between *MYC*-NL and *MYC*-R groups in patients with MCL, similar to the effect of *MYC*-EC in DLBCL patients.[47] Multivariate analysis confirmed that *MYC*-EC is not a poor prognostic factor in MCL.

MYC/BCL2 DHL is well known as a subset of large B-cell lymphoma with a poor prognosis. Although MCL with MYC-R has been originally suggested as one type of DHL (*CCND1* and *MYC*),[20] it has been excluded from the category of high-grade B-cell lymphoma with MYC and *BCL2* and/or *BCL6* rearrangements in the 2017 WHO classification, and instead retained in the MCL category. In this study, we compared of these two groups and showed both similarities and differences. Compared with patients with *MYC/BCL2* DHL, MCL patients with *MYC*-R more often presented with bone marrow involvement, Ann Arbor stage IV disease, and more frequent CD5 expression. MCL patients with *MYC*-R also had a poorer OS, however, this last finding did not hold true in patients with *de novo* disease. In contrast, elevated serum LDH level and more frequent CD10 and BCL6 expression were more often observed in the *MYC/BCL2* DHL group. Overall, these features support the position in the WHO classification that so-called double hit MCL is best kept in the MCL category.

In conclusion, *MYC*-R is significantly associated with blastoid morphology and CD10 expression in MCL. MCL patients with *MYC*-R have a very aggressive clinical course and a poor prognosis, similar to patients with *MYC/BCL2* DHL and significantly worse than MCL patients without *MYC*-R. However, the presentation of patients with MCL associated with *MYC*-R differs from patients with *MYC/BCL2* DHL supporting the exclusion of MCL with *MYC-R* from the category of high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements. MCL with *MYC*-EC has a prognostic impact intermediate between patients with *MYC*-R and patients with normal *MYC*. These results suggest that MCL patients with different *MYC* status may need different treatment strategies. We recommend using MYC immunohistochemistry as a screening tool to test *MYC*-R by FISH in blastoid/pleomorphic MCL.

Disclosures

No conflicts of interest to disclose.

Contributions

LW, GT, WH and SL performed research; LW and SL per- formed data analysis; LW, GT, LJM, JX, WH, CCY, MW, PJ, PL and SL wrote the manuscript; SL supervised the study.

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