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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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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 (NL) MYC. 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, stage 4 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 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 al20 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 DHLs
have been made in recent years, and large B-cell lymphomas with \textit{MYC} and \textit{BCL2} and/or \textit{BCL6}
rearrangements were included in the category of high-grade B-cell lymphoma with \textit{MYC} and
\textit{BCL2} and/or \textit{BCL6} rearrangements in the 2017 World Health Organization (WHO) classification,
except for cases that fulfill criteria for a follicular lymphoma, MCL, or lymphoblastic
lymphoma.\textsuperscript{23} As a result, MCL with \textit{MYC} rearrangement (\textit{MYC-R}), although fulfilling the
earlier concept of DHL suggested by Aukema and colleagues, remains in the category of MCL.
MCL associated with \textit{MYC-R} is rare and only case reports and small case series have been
reported previously.\textsuperscript{6,24-29} No study has explored the prognostic significance of \textit{MYC-R} in MCL
patients by comparing the survival of MCL patients with or without \textit{MYC-R}. In addition, as we
have studied cases of MCL by fluorescence in situ hybridization (FISH) to assess \textit{MYC} we have
come across cases of MCL with extra copies (EC) of \textit{MYC}, but without \textit{MYC-R}. The prognostic
impact of \textit{MYC-EC} has not been well characterized previously.

In this study we had two aims. First, we address the prognostic impact of \textit{MYC-R} in MCL
patients, and in particular, is the prognosis more akin to that of patients with double hit
lymphoma with \textit{MYC} and \textit{BCL2} rearrangements (\textit{MYC/BCL2} DHL). Secondly, we address the
question of the potential prognostic impact of \textit{MYC-EC} in the absence of \textit{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 3 cases were before 2010 and most cases were diagnosed in recent years. MYC-R in MCL is rare and there is 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. But 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 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, TP53, Ki67, cyclin D1, and SOX-11. The positive cutoff was ≥30%
for CD10, MUM-1, and BCL6; ≥20% for P53; ≥40% for Myc; ≥50% for BCL2 and >10% for SOX11.

Flow cytometry immunophenotypic analysis was performed using either a FACScanto II or FACSCalibur cytometer (Becton-Dickinson Biosciences, San Jose, CA, USA) as described previously. 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 immunoglobulin kappa and lambda light chains. All antibodies were obtained from Becton-Dickinson Biosciences.

Conventional Cytogenetics and FISH

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). 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: 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 *P*-values is less than 0.05.
RESULTS

MCL Patients with MYC-R

Clinical Characteristics

Twenty-seven MCL patients had MYC-R, including 20 men and 7 women, with median age of 63 years (range, 47 to 85). Fourteen (52%) patients with MYC-R presented 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 3 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 7 (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 3 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 (27/27, 100%), and most cases expressed SOX11 (7/10, 70%), and c-MYC (12/15, 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 4 de novo MCL, 2 neoplasms which apparently lost CD5 at the time of detection of MYC-R, and 1 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% to 100%; only 3 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, 7 cases had MYC partner gene as Immunoglobulin (IG) gene and 3 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 and bendamustine. Eight (33%) patients reached complete remission (CR) after induction, but 6 relapsed. Seven patients received stem cell transplant (SCT), including 4 autologous and 3 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 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 progression/transformation than for patients with de novo MCL with MYC-R (Figure 2A, p=0.019).

**MCL with MYC-R versus Non-MYC-R**

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% versus 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/pleomorphic 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 FISH Status in MCL**

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-NL 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% with a range of 1% to 100% in the MYC-R group; 13% with a range of 0% to 55% in the MYC-EC group; and 15% with a range of 0% to 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 cutoff value for MYC immunohistochemistry to predict MYC-R, the sensitivity and specificity were 80% and 83% respectively.

**Multivariate analysis**

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.
Patients with MCL associated with MYC-R versus MYC/BCL2 DHL 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 BM involvement (96% versus 44%, \(P=0.0001\)), stage 4 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 is significantly related to the treatment regimens patients received and majority patients received intensive chemotherapy, we further compared the OS between patients 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 non-MYC-R control group to compare for clinicopathologic features and prognosis. 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 BCL-2 coexpression, 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. 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. 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 regulate many cellular functions including cell cycle, cell growth, metabolism, biosynthesis, survival, and apoptosis. Dysregulation of *MYC* induces lymphomagenesis. In Burkitt lymphoma, *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. Previous studies shown *MYC* cooperated with transcriptionally activated cyclinD1 and resulted in blastoid MCL or oncogenic transformation of B cell lymphoma in mouse models. 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 microRNAs involved in MCL pathogenesis. 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 9 cases with P53 expression data available showed an over expression of P53 (7 cases with P53>80% and 2 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 least 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. Our result also demonstrate that using 40% as a cutoff, 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%. 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. Yi et al reported 14 patients with MYC-EC and 4 patients with MYC-R and these 18 patients had a poorer prognosis than a comparison group of MCL patients without MYC abnormalities. 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. 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), 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 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 BM involvement, stage 4 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.
REFERENCES


## TABLE 1. Comparison of features of MCL with MYC-R, MCL without MYC-R and MYC/BCL2 DHL

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

Blank, not available; BM, bone marrow; CNS, central nervous system; CR, complete remission; LDH, lactate dehydrogenase; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; R-hyper-CVID, rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone; SCT, stem cell transplant; WBC, white blood cell; *, P<0.05
<table>
<thead>
<tr>
<th>Features</th>
<th>Blastoid MCL with MYC-R (n=24)</th>
<th>Blastoid MCL with Non-MYC-R (n=29)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years), Median (range)</td>
<td>63 (47-82)</td>
<td>67 (33-85)</td>
<td>0.96</td>
</tr>
<tr>
<td>Age ≥60 (years)</td>
<td>71% (17/24)</td>
<td>62% (18/29)</td>
<td>0.76</td>
</tr>
<tr>
<td>Gender (Male:Female)</td>
<td>17:7</td>
<td>20:7</td>
<td>0.75</td>
</tr>
<tr>
<td>Stage IV</td>
<td>91% (20/22)</td>
<td>89% (24/27)</td>
<td>1.00</td>
</tr>
<tr>
<td>BM-Positive</td>
<td>86% (18/21)</td>
<td>74% (20/27)</td>
<td>0.72</td>
</tr>
<tr>
<td>CNS-Positive</td>
<td>40% (4/10)</td>
<td>29% (4/14)</td>
<td>0.67</td>
</tr>
<tr>
<td>Extranodal Sites ≥2</td>
<td>80% (16/20)</td>
<td>88% (22/25)</td>
<td>0.61</td>
</tr>
<tr>
<td>Elevated LDH (&gt;618)</td>
<td>68% (15/22)</td>
<td>62% (13/21)</td>
<td>0.99</td>
</tr>
<tr>
<td>Elevated WBC (&gt;11.0)</td>
<td>40% (8/20)</td>
<td>30% (6/20)</td>
<td>0.74</td>
</tr>
</tbody>
</table>
| BM, bone marrow; CNS, central nervous system; CR, complete remission; LDH, lactate dehydrogenase; MYC-R, MYC gene translation; N, number of cases; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; R-hyper-CVAD, rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone; SCT, stem cell transplant; WBC, white blood cell; *, P < 0.05

<table>
<thead>
<tr>
<th>Immunophenotype:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX11+</td>
<td>73% (8/11)</td>
<td>88% (15/17)</td>
<td>0.28</td>
</tr>
<tr>
<td>BCL6+</td>
<td>33% (4/12)</td>
<td>33% (7/21)</td>
<td>1.00</td>
</tr>
<tr>
<td>CD5+</td>
<td>78% (18/23)</td>
<td>81% (22/27)</td>
<td>0.72</td>
</tr>
<tr>
<td>CD10+</td>
<td>39% (9/23)</td>
<td>12% (3/25)</td>
<td>0.046*</td>
</tr>
<tr>
<td>MUM-1+</td>
<td>50% (4/8)</td>
<td>71% (5/7)</td>
<td>0.59</td>
</tr>
<tr>
<td>BCL2+ (≥50%)</td>
<td>86% (12/14)</td>
<td>95% (19/20)</td>
<td>0.54</td>
</tr>
<tr>
<td>MYC+ (≥40%)</td>
<td>80% (12/15)</td>
<td>20% (4/20)</td>
<td>0.001*</td>
</tr>
<tr>
<td>MYC/Bcl-2 co-express</td>
<td>69% (9/13)</td>
<td>16% (3/19)</td>
<td>0.004*</td>
</tr>
<tr>
<td>PS3+ (≥20%)</td>
<td>75% (12/16)</td>
<td>69% (9/13)</td>
<td>0.97</td>
</tr>
<tr>
<td>Ki67, Median (range)</td>
<td>90 (15-100)</td>
<td>90 (30-100)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment:</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Aggressive Chemotherapy</td>
<td>68% (15/22)</td>
<td>38% (10/26)</td>
<td>0.08</td>
</tr>
<tr>
<td>Other chemotherapy</td>
<td>32% (7/22)</td>
<td>62% (16/26)</td>
<td>0.15</td>
</tr>
<tr>
<td>Initial CR</td>
<td>55% (12/22)</td>
<td>70% (16/23)</td>
<td>0.34</td>
</tr>
<tr>
<td>Features</td>
<td>HR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------</td>
<td>-----------------</td>
<td>-------</td>
</tr>
<tr>
<td>MYC-R</td>
<td>3.27</td>
<td>1.149 - 9.306</td>
<td>0.026</td>
</tr>
<tr>
<td>MYC-EC</td>
<td>2.375</td>
<td>0.632 - 8.923</td>
<td>0.200</td>
</tr>
<tr>
<td>Blastoid/Pleomorphic MCL</td>
<td>7.038</td>
<td>0.767 - 64.593</td>
<td>0.073</td>
</tr>
<tr>
<td>Ki67≥30%</td>
<td>1.93</td>
<td>0.215 - 17.370</td>
<td>0.557</td>
</tr>
<tr>
<td>High MIPI</td>
<td>1.217</td>
<td>0.532 - 2.783</td>
<td>0.642</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS:

Figure 1. A representative case of mantle cell lymphoma (MCL) with MYC-R. 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). FISH study showed CCND1/IGH (H) and MYC-R (I).

Figure 2. Comparison of median overall survival (OS). There is statistically significant difference in OS between de novo and transformed MCL with 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-NL group the best OS, and MYC-EC group laid in between (B).

Figure 3. MYC protein expression in correlation with MYC cytogenetic status in MCL (A); Comparison of median overall survival (OS) between MCL with MYC-R and MYC/BCL2 DHL: (B) All cases included; (C) Only de novo cases included; (D) Only patients received intensive induction chemotherapy included.
A. % MYC Expression by IHC

B. All cases

   - MCL-MYC-R
   - MYC/BCL2 DHL

   Percent survival

   p = 0.038

   OS (Months)

C. De Novo

   - MCL-MYC-R
   - MYC/BCL2 DHL

   Percent survival

   p = 0.83

   OS (Months)

D. De Novo-Intensive therapy

   - MCL-MYC-R
   - MYC/BCL2 DHL

   Percent survival

   p = 0.20

   OS (Months)