Ki-67 predicts outcome in advanced stage mantle cell lymphoma patients treated with anti-CD20 immunochemo-therapy: results from randomized trials of the European MCL Network and the German Low Grade Lymphoma Study Group

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Abstract
Clinical outcome of mantle cell lymphoma (MCL) is highly heterogeneous. Tumor cell proliferation as assessed by the Ki-67 index has been shown to yield prognostic information on MCL in many studies using heterogeneously treated patient cohorts. The prognostic value of the Ki-67 index in patients treated with anti-CD20 therapy has not been studied so far. We analyzed the Ki-67 index at primary diagnosis in 249 advanced stage MCL patients treated within randomized trials. Ki-67 showed high prognostic relevance for overall survival (relative risk 1.27 for 10% higher Ki-67, \( p < 0.0001 \)), also independently from clinical prognostic factors. The three groups with different Ki-67 index of <10%, \( \geq 10 \) to <30%, and \( \geq 30\% \) showed significantly different overall survival in patients treated with CHOP \( (p = 0.0002) \) as well as in patients treated with CHOP in combination with anti-CD20 therapy (R-CHOP, \( p = 0.0126 \)). Thus, the Ki-67 index remains an important prognostic marker in the era of anti-CD20 therapy. The European MCL study is registered at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) as #NCT00016887.
Introduction
Mantle cell lymphoma (MCL) is an aggressive B-cell Non-Hodgkin Lymphoma (B-NHL) with a median overall survival (OS) of the patients of 3-5 years \(^1\). Clinical outcome of this disease is highly heterogeneous \(^2\). Since MCL patients often present at an advanced age and treatment strategies differ in terms of their potential side effects, several attempts have been made to identify high- and low-risk patients for a risk adapted therapy \(^2\)-\(^5\). In contrast to many other B-NHL, increased proliferation of the tumor cells has been shown to be distinctly correlated with shorter survival in MCL. Thus, the proliferation index as assessed by the percentage of Ki-67 positive cells has turned out to represent an important prognostic marker in several studies \(^2\); \(^6\); \(^7\). However, the studies published so far aiming at the prognostic value of Ki-67 analyzed patients with heterogeneous therapeutic regiments that were not treated within prospective randomized trials \(^2\); \(^6\); \(^7\). The addition of anti-CD20 (rituximab) to the chemotherapy protocols improved the outcome and may have changed the risk factor profile of diffuse large B-cell lymphomas (DLBCL). Thus, BCL2 expression, a marker for unfavorable outcome in DLBCL may lose its predictive value in DLBCL patients treated with rituximab \(^8\). The introduction of rituximab into the treatment protocols for MCL has improved the outcome substantially \(^9\). However, to date it is uncertain, whether proliferation can predict outcome in MCL treated with rituximab. We studied the tumor cell proliferation rate in 249 newly diagnosed patients with advanced stage MCL treated within the randomized trials of the European MCL Network and the German Low Grade Lymphoma Study Group.

Material and Methods
Local ethics committees of the participating centers approved the study protocol, and all patients gave written informed consent in accordance with the Declaration of Helsinki.

Patients
Patient specimen were collected from three randomized trials of the German Low Grade Lymphoma Study Group (GLSG) \(^9\); \(^10\) and the European MCL Network \(^11\). 116 patients in our cohort received CHOP therapy and 96 patients CHOP in combination with rituximab (R-CHOP). Additional 37 patients had received MCP without rituximab.

Ki-67 index
Specimen were analyzed in agreement with the local ethical guidelines as well as the approved study protocols. Staining for Ki-67 was done on lymph node biopsies using the antibody Mib-1 or Ki-S5 as described before. The proliferation index (percentage of Ki-67 positive lymphoma cells) was assessed by counting 1000 cells in at least 2 representative areas of the lymphoma and the average of both values was used for further analysis.

Statistical methods
The outcome parameter was OS calculated from the day of trial recruitment to death from any cause or the latest follow-up date. The prognostic relevance of Ki-67 as continuous parameter was tested by univariate Cox regression in the entire cohort and then adjusted for the MIPI prognostic score using multiple Cox regression. If Ki-67 was identified as independent prognostic factor, two optimal cut-off points were searched maximizing the log rank statistic for overall survival and defining three reasonably sized risk groups. The candidate cut-off points were 10%, 20%, 30%, and 40%. Using the identified cut-off points we then calculated Kaplan-Meier-Plots and performed log rank tests for the subgroups of CHOP and R-CHOP treated patients separately. In addition, for each subcohort we adjusted the statistical significance of the categorized Ki-67 index for the MIPI prognostic score using the likelihood ratio test in multiple Cox regression.

Results and Discussion
As a continuous parameter, Ki-67 showed strong prognostic relevance for OS with a relative risk (RR) of 1.27 for 10% higher Ki-67 (95% CI 1.15 to 1.39, p < 0.0001) in the entire cohort. This was also true independently from the MIPI prognostic score (adjusted RR 1.20, 95% CI 1.09 to 1.33, p = 0.0004). All combinations of two cut-off points yielded significantly different OS curves. Taking also group size into account, the combination of 10% and 30% showed highest statistical significance (log rank-$\chi^2$ 32.70, p < 0.0001) with 32%, 52%, and 16% of the patients having a Ki-67 index <10%, >= 10 to <30%, and >=30%.

The statistically significant difference in OS between the groups of <10%, >=10 to <30% and >=30% Ki-67 index was also seen in the subgroups of CHOP (median OS 112, 59, and 30 months, 3 years OS 81%, 75%, 46%, p=0.0002) and R-CHOP treated patients (median OS not reached, not reached, 52 months, 3 years OS 93%,
74%, and 66%, \( p=0.0126 \) for R-CHOP, Figure 1). After adjusting for the MIPI score\(^{12}\) the \( p \)-values were \( p=0.0025 \) for CHOP and \( p=0.14 \) for R-CHOP treated patients. The median follow-up was 63 for CHOP and 39 months for R-CHOP treated patients, respectively. The follow-up in the R-CHOP treated cohort was shorter because randomization between CHOP and R-CHOP was started in 2000\(^9\), whereas in the preceding trial CHOP was randomly compared to MCP\(^{10}\). However, the cohort of CHOP and R-CHOP patients did not differ in respect of clinical parameters like age, stage, LDH, ECOG or bone marrow involvement (data not shown).

We have recently shown, that Ki-67 index shows strong prognostic relevance in combination with the clinical prognostic index for advanced stage MCL\(^{12}\). The data presented herein indicate, that the Ki-67 index might be a valuable prognostic parameter for MCL patients treated with immuno-chemotherapy including rituximab. However, compared to CHOP therapy, R-CHOP immuno-chemotherapy improves response and time to treatment failure of MCL but does not improve OS in our cohort (data not shown) and the randomized comparison\(^9\). Therefore, the prognostic value of the Ki-67 index has to be validated in treatment protocols that improve overall survival of MCL patients.

In contrast to PCR- or array-based techniques for prediction of prognosis, the Ki-67 index can be cost-effectively evaluated on paraffin embedded tissue. The Ki-67 index thus might be the first biological parameter that can be included for further risk stratification of MCL. However, standardized methods and guidelines to assess Ki-67 in MCL are needed for future use.

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**Conflict of Interest Disclosure:** The authors declare no competing financial interests.
References


Figure Legend:
Kaplan-Meier plot for overall survival of patients treated with CHOP (A) and R-CHOP (B) stratified in three groups according to the Ki-67 index of <10% (<10), >=10 to <30% (>=10), and >=30% (>=30) Ki-67 positive cells.

A.

B.
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