TREATMENT UPDATE

The Role of Maintenance Therapy and Individualization of Therapy in Mantle Cell Lymphoma

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This activity is intended for physicians, registered nurses, and other healthcare professionals who wish to expand their knowledge of the evolving treatment paradigm and optimal management of patients with mantle cell lymphoma.

PURPOSE

The purpose of this activity is to provide clinicians with the latest clinical advances and practical applications for managing patients with mantle cell lymphoma.

LEARNING OBJECTIVES

- Compare agents currently used as maintenance therapy in the treatment of mantle cell lymphoma
- Describe novel agents with promise as maintenance therapy in the setting of mantle cell lymphoma
- Contrast prognostic indices and markers used in the treatment of mantle cell lymphoma
- Discuss genomic and proteomic analysis techniques of potential utility in the treatment of mantle cell lymphoma

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The Role of Maintenance Therapy and Individualization of Therapy in Mantle Cell Lymphoma

CONTENTS

Introduction to Maintenance Therapy

In the setting of mantle cell lymphoma (MCL), improvements in first-line and second-line therapies have resulted in more patients attaining a sufficiently long period of remission. This has occurred simultaneous with the development of newer, less-toxic regimens that have improved progression-free survival (PFS) and overall survival (OS) for responders.

In oncology, maintenance therapy is usually indicated when there is a potential risk of relapse or to stabilize a chronic disease considered incurable. Maintenance treatment differs from consolidation therapy, which is aimed at eliminating as much minimal residual disease as possible. Instead, the aim of maintenance therapy is to allow long-term disease control, even if not all malignant cells have been eliminated by the induction phase. Because of its anticipated chronic use, maintenance therapy must provide a demonstrable benefit for survival but also be easy to administer, be relatively nontoxic, be convenient, and not impair quality of life.[1]

Interferon Maintenance Therapy

One of the first noncytotoxic candidates for use in maintenance therapy in MCL was interferon alfa-2 (IFN). However, despite nearly a decade of trials, its utility as a maintenance therapy remains in doubt. A meta-analysis by Rohatiner and colleagues[2] of IFN use in follicular lymphoma (10 phase III studies) concluded that it improved survival when used in conjunction with chemotherapy. However, although IFN did not prolong survival when given as a maintenance therapy, it did improve 5-year and 10-year remission rates by 13% and 12%, respectively ($P < .0002$). A previous meta-analysis of 24 trials (N = 4012) concluded that in the setting of multiple myeloma, IFN maintenance resulted in extended PFS but did not improve OS—questionable advantages in view of the cost and toxicity.[3]

In the setting of MCL, there are fewer trials—with mixed results. Herold and colleagues[4] examined the effect of IFN maintenance in 164 patients with previously untreated advanced follicular lymphoma (50%), lymphoplasmocytic lymphoma/immunocytoma (24%), or MCL (26%). Patients (mean age: 58 years) were randomly assigned treatment with either bendamustine, vincristine, and prednisone (BOP) or cyclophosphamide, vincristine, and prednisone (COP). Interferon maintenance treatment (3 cycles of 4 weeks, 5 MU/week) was offered to all patients who achieved a complete response (CR) or partial response (PR) to BOP or COP. Although 115 patients qualified, only 28 responding patients (12 after BOP; 16 after COP) actually received IFN maintenance.

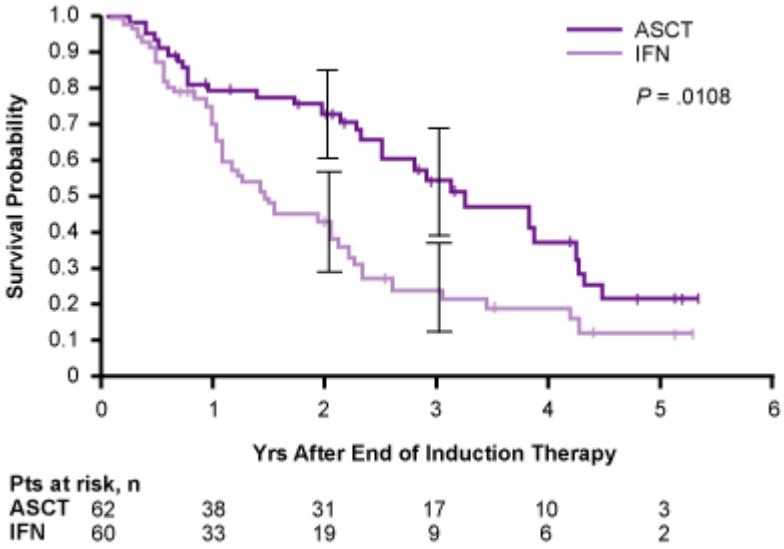
Regardless of the treatment group, event-free survival (median follow-up: 44 months) was significantly improved in responding patients who received IFN maintenance therapy ($P < .01$). For these patients, the 5-year event-free survival rate was 68% in the COP group and 75% in the BOP group. The event-free survival rate in responding patients who did not receive IFN maintenance was significantly higher after BOP than after COP (49% vs 31%, respectively; $P = .03$).

Moreover, in contrast to the meta-analysis by Rohatiner and colleagues, maintenance therapy with IFN significantly improved OS ($P = .01$), regardless of the primary treatment. For responders who received IFN, the 5-year OS rate was not significantly different between groups (91.7% for BOP recipients and 80.4% for COP recipients). In responders assigned to observation, the 5-year survival rate was significantly higher after BOP than COP (69.7% vs 47%, respectively; $P = .03$).

By contrast, results from a study by the European MCL Network indicated that maintenance therapy with IFN was inferior to stem cell transplantation following induction with a

cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)–like regimen in 230 untreated patients with advanced (stage III/IV) MCL.[5] After induction therapy, responding patients 65 years of age or younger (median age: 55 years) were randomly assigned to either myeloablative radiochemotherapy followed by autologous stem cell transplantation (ASCT) (n = 62) or IFN 6 x 10^6 U 3 times weekly until progression (n = 60). In the ASCT group, at a median follow-up of 25 months, the median PFS was 39 months and the 3-year PFS rate was 54%. In the IFN arm, the median PFS was 17 months and the 3-year PFS rate was 25% (*P* = .011) (Figure 1). The 2-year OS rates were similar between groups at a median follow-up of 34 months (86% and 82%).

Figure 1. PFS after high-dose radiochemotherapy followed by ASCT and IFN maintenance in MCL.[5]



The advent of newer treatments (eg, fludarabine-based regimens) might improve the effectiveness of IFN maintenance therapy, and the availability of pegIFN alfa could decrease toxicity.[2] Clinical trials are currently comparing IFN maintenance therapy with no maintenance in non-Hodgkin's lymphoma (NHL)[6] or to stem cell transplantation in MCL.[7]

Rituximab Maintenance Therapy

Rituximab is an anti-CD20 cytotoxic monoclonal antibody that is effective in B cell–related neoplasms such as MCL. As a single agent, rituximab has shown efficacy as both a first-line and second-line therapy and has shown utility and flexibility as an adjunct to conventional chemotherapy regimens. For maintenance therapy, where chronic use without a degradation of quality of life is desired, the high specificity and corresponding low toxicity of rituximab give it an advantage over IFN. Because relapses following therapy are believed to occur because of the persistence of lymphoma cells, the ability of rituximab to destroy residual cells offers the possibility of prolonging remission. Moreover, the antitumor activity of rituximab does not depend on cellular division, so it may be particularly useful for indolent disease.

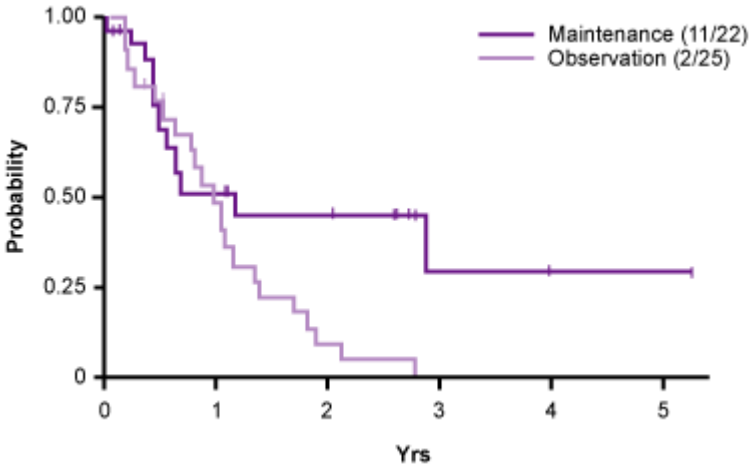
Rituximab has great promise as a maintenance therapy in MCL. Witzens-Harig and colleagues[8] evaluated quality of life in 91 NHL patients enrolled in a prospective trial comparing rituximab maintenance therapy (375 mg/m^2 every 3 months for 2 years) with observation. The mean age was approximately 55 years, most patients had received previous therapies (79%), and a range of NHLs were represented, including 8 patients (9%) with MCL. No significant differences were observed between the rituximab and observation groups regarding global, functional, and symptomatic health states, as measured by the cancer-specific questionnaire EORTC QLQ-

C30, although there was a trend toward worsening functional and symptomatic status with increasing numbers of maintenance cycles. Similar findings were seen when using the more generic EQ-5D questionnaire. Because only 1 patient in the rituximab maintenance group relapsed and, therefore, was excluded from the quality-of-life data, compared with 11 in the observation group, there may have been a selection bias for inclusion of only the healthiest observation patients. Nevertheless, the long-term administration of rituximab maintenance appears to have no adverse effects on quality of life, which is a key criterion for a viable maintenance therapy.

Forstpointner and colleagues[9] reported that rituximab maintenance after combined immunochemotherapy improved PFS in patients with advanced-stage relapsed/refractory follicular lymphoma and MCL. Patients were randomly assigned to treatment with induction chemotherapy with fludarabine, cyclophosphamide, and mitoxantrone (FCM), with or without rituximab. After treatment of the initial 147 randomized patients, the improved outcomes in the rituximab plus FCM arm resulted in all subsequent patients receiving that treatment. Patients from either primary treatment arm who attained a CR or PR (n = 195) were randomly assigned to either rituximab maintenance therapy (375 mg/m^2 weekly during Months 3 and 9 after induction) or to observation. Of 176 evaluable patients, 138 received rituximab plus FCM induction therapy.

At a median follow-up of 26 months, the median duration of response for all patients was significantly longer in the rituximab maintenance arm vs the observation arm (not yet reached vs 17 months, respectively; *P* < .001). In the MCL group specifically, the median response duration was not significantly different between rituximab and observation (14 vs 12 months, respectively), a higher proportion of patients receiving rituximab experienced remissions lasting longer than 2 years (45% vs 9%, respectively; *P* = .049) (Figure 2). For all patients, a trend was seen suggesting OS improvement after rituximab maintenance (estimated 3-year OS rate: 77% vs 57% with observation; *P* = .100).

Figure 2. Response duration after rituximab plus FCM in patients with MCL.[9]



No serious rituximab-specific toxicities were observed, and the rate of grade 3/4 infections was similar in both study arms (3% to 4%). Data from a phase III trial conducted by van Oers and colleagues[10] showed an 8% survival advantage at 3 years conferred by maintenance rituximab vs observation in follicular lymphoma patients who had received induction with R-CHOP or CHOP (*P* = .011).

However, not all studies have demonstrated an OS advantage following maintenance rituximab. Witzens-Harig and colleagues[11] reported on 162 NHL patients (18 with MCL) who were

randomized to rituximab maintenance (375 mg/m^2 every 3 months for 2 years) or observation after attaining a CR following initial standard treatment. An interim analysis showed that event-free survival was significantly prolonged in the rituximab maintenance group compared with the observation group ($P < .05$), but no OS difference was observed.

The best evidence from these randomized trials indicates that rituximab is tolerated well, does not appreciably degrade patients' quality of life, and prolongs the duration of response. Whether rituximab maintenance therapy also improves survival remains an open question.

The European Mantle Cell Lymphoma Network is currently conducting a phase III trial that will randomly assign previously untreated stage II-IV MCL patients to either IFN or rituximab maintenance, following either R-CHOP or fludarabine and cyclophosphamide (R-FC) induction therapy.[12] A phase III trial comparing rituximab maintenance with observation in patients with follicular NHL or diffuse large B-cell lymphoma is also in progress.[13] Several other groups have reported positive results with rituximab maintenance therapy in NHL patients.[14-17] One as-yet unanswered question is the incidence of infection following long-term rituximab use. There have been reports that immunoglobulin levels are depressed for a long period,[15] which could lead to the re-emergence of suppressed infections such as hepatitis C,[18] or a higher infection rate,[9] but other studies have not reported this. In addition, rituximab resistance has been reported,[19] and it is conceivable that maintenance therapy could contribute to that process.

Other Maintenance Therapy Strategies

Several other therapies are under investigation as single agents in primary or relapsed MCL. Some exhibit a low level of toxicity, which may make them candidates for longer-term use in maintenance regimens, either alone or in combination with rituximab.

Bortezomib

Blum and colleagues[20] have reported on the use of combined rituximab and bortezomib in patients with relapsed/refractory MCL (n = 13) or follicular lymphoma (n = 10). The median age of the patients was 66 years, and the median number of previous therapies received was 2 (range: 1-9). Bortezomib was originally planned to be given at 1.5 mg/m^2 for both induction and maintenance, but after 7 of the first 11 patients experienced grade 3 neurologic toxicities during induction, the dose was decreased to 1.3 mg/m^2, and patients with > grade 1 neuropathy before treatment were excluded. However, none of the patients responding to the induction phase received maintenance therapy because of grade 3/4 neurotoxicity. Ongoing phase II clinical trials examining bortezomib as maintenance therapy for MCL include:

- A study in which untreated MCL patients will receive induction with single-agent bortezomib followed by bortezomib in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R), followed by randomization to bortezomib maintenance or observation.[21]
- A study comparing bortezomib as maintenance therapy (Days 1, 8, 15, and 22 every 56 days for up to 10 courses) vs consolidation therapy (Days 1, 4, 8, and 11 every 21 days for up to 4 courses) in patients with previously untreated MCL given aggressive chemoimmunotherapy and ASCT.[22]
- A study evaluating the toxicity of bortezomib as maintenance therapy following induction with bortezomib plus R-CHOP in newly diagnosed MCL patients.[23]
- A study employing bortezomib and rituximab for both induction and maintenance in refractory/relapsed MCL and follicular NHL patients.[24]

Thalidomide and Lenalidomide

Thalidomide and lenalidomide change the micromilieu of lymphoma cells; among other effects, these related immunomodulatory agents inhibit angiogenesis and decrease the ability of cancer

cells to survive. Although not without adverse events, thalidomide and lenalidomide are generally well tolerated and are candidates for use as maintenance therapy.

In the setting of relapsed/refractory MCL, Kaufmann and colleagues[25] conducted a phase II study of rituximab and thalidomide, followed by thalidomide 50-400 mg/week alone for maintenance until relapse. The planned thalidomide dose was 400 mg/week, but fatigue, somnolence, and constipation were common, dose-dependent adverse events of thalidomide—only 6 of the 13 responding patients could tolerate that dose, which was variably reduced for the other patients. Grade 1/2 peripheral neuropathy was seen in 7 patients and was the cause of discontinuation in 1 patient. Grade 4 neutropenia was seen in 1 patient, and there were 2 venous thromboembolic events. However, despite these effects, in patients achieving a CR, PFS after rituximab/thalidomide (median: 20.4 months) was longer than PFS after the preceding chemotherapy.

Mammalian Target of Rapamycin Inhibitors

Mammalian target of rapamycin (mTOR) inhibitors, such as sirolimus, everolimus, and tacrolimus, are currently under investigation as single-line therapies in MCL and could be used for maintenance. A phase II trial is currently evaluating everolimus as maintenance therapy for older MCL patients, after either first-line or second-line chemotherapy.[26]

Whether or not rituximab is proven to improve survival—or merely lengthen the duration of response—it is likely that it will increasingly be adopted as a maintenance therapy after induction or salvage therapy. At present, candidates for maintenance therapy are drawn from the ranks of novel agents still being tested (generally in phase II trials) as single-line therapies. As the specific properties and limitations of each agent become better known, the possibilities and likelihoods of combination maintenance therapies will increase.

Maintenance Therapy and Molecular Remission

Maintenance therapy continues during remission and, therefore, ends at relapse. Although progress continues to be made in first-line treatments for MCL, it is not yet curable. Relapses are because of the posttherapy recovery of tumor cells, and detection of these cells is largely by clinical observation. Efforts are under way to develop more sensitive detection techniques, with the goals of better determining prognosis and improving outcomes following relapse. With the advent of relatively nontoxic CD20-specific therapies such as rituximab, the threshold to initiate secondary therapy earlier is greatly lowered. Therefore, in some centers, the use of genetic probes to predict relapse and remission is being explored.

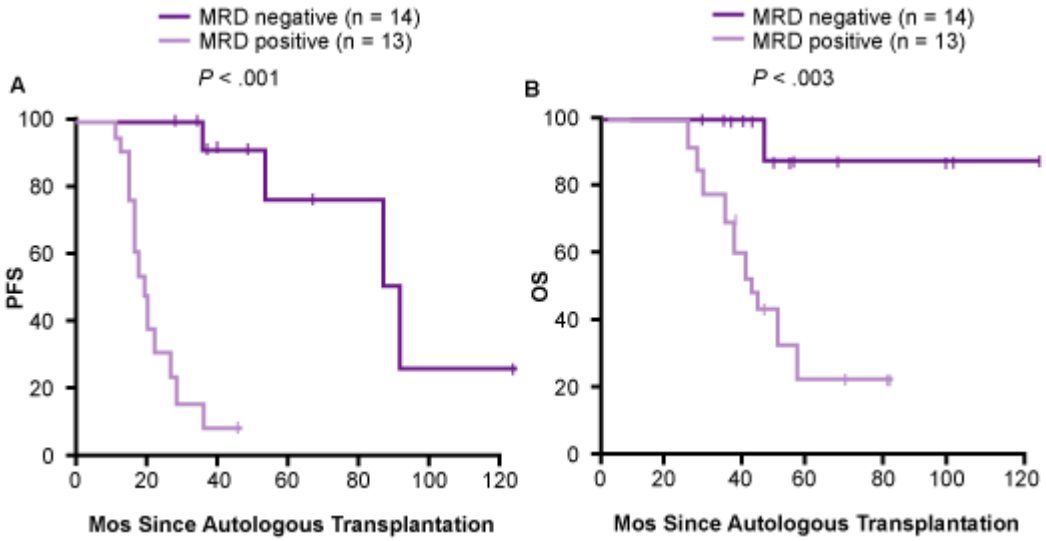
Neoplasms are cytogenetically heterogeneous, with patterns of genetic expression that differ between diseases, between patients with the same disease, and even between different times of evolution of disease in the same patient. To date, it has proven rare to find any single genetic marker that has a consistently close relationship to a single sort of cancer in the way that the Philadelphia chromosome translocation t(9;22) is associated with CML or the t(11;14) translocation in MCL. In the latter case, t(11;14) occurs in virtually all cases of MCL (but not only MCL) and results in the immunoglobulin heavy chain (IgH) enhancer, which is constitutively always active and acts on the CCND1 gene to overexpress cyclin D1.[27,28]

The close relationship of MCL to this nearly pathognomonic genetic marker theoretically simplifies the task of genetically detecting relapses and has led to the concept of molecular remission (MR); in MCL, MR is defined as the absence (by polymerase chain reaction [PCR]) of the genetic fusion sequence resulting from the t(11;14) chromosomal translocation. In fact, because clonal lines of MCL often have slightly different translocation points, an array of different genetic probes can be used to evaluate MR. IgH sequences (which are altered by the translocation) are also used to determine MR.

Early results using MR to guide therapy were positive. For example, Ladetto and colleagues[29] used MR to guide therapy for 4 MCL patients who achieved clinical remission and MR following rituximab-supplemented, high-dose sequential chemotherapy and ASCT but subsequently suffered molecular relapse. After 4-6 courses of rituximab, all patients re-entered MR.

Pott and colleagues[30] reported in 2006 that the attainment of MR following ASCT was strongly predictive of improved outcome in a study of 27 patients with stage III/IV MCL. Patients received COP, CHOP, or prednimustine and mitoxantrone (PmM) followed by dexamethasone, carmustine, etoposide, cytarabine, and melphalan (DexaBEAM), total body irradiation, cyclophosphamide, and ASCT. All patients had a PCR-detectable clonal IgH gene rearrangement in the peripheral blood, bone marrow, or lymph nodes. Complete clinical remission and MR were seen in 14 patients, whereas 13 patients (6 CRs and 7 PRs) did not have an MR in at least 1 sample. Patients with a consistently positive MR status throughout the year following transplantation had a much longer PFS (median: 92 months) than those with a molecular relapse (median: 21 months; $P < .001$). Overall survival was also longer—at a median of 48 months of observation, the median OS in the MR group was not yet reached vs 44 months in the molecular relapse group ($P < .003$). This study also found a high correlation between minimal residual disease (MRD) levels in the peripheral blood and bone marrow samples ($r^2 = 0.91$) (Figure 3).

Figure 3. PFS according to MRD status after ASCT.[30]



Somewhat different results have been reported from a recent large phase II trial reported by the Nordic Lymphoma Group.[31] In this trial, 160 MCL patients were given induction with dose-intensified R-CHOP (maxi-CHOP) alternating with rituximab plus high-dose cytarabine. Responders received high-dose chemotherapy with BEAM or carmustine, etoposide, cytarabine, and melphalan/cyclophosphamide (BEAC) plus ASCT. Patients achieving an MR who then had solely a molecular relapse were offered rituximab retreatment, and in most cases, an MR was attained again.

Of note, 84 of the 145 responding patients (58%) had a molecular marker (either t(11;14) or a clonal IgH rearrangement) that could be used to assess MR. In those who did not have such a molecular marker, neither MR nor molecular relapse could be ascertained, and therefore, they were not offered supplemental rituximab. Nonetheless, there was no PFS difference between patients with or without molecular markers. The investigators concluded that early molecular relapse is a harbinger of clinical relapse, but MR does not predict long-term remission. These

results confirm the observation of Kahl and colleagues[14] from a smaller trial that the achievement of MR did not correlate with an improved outcome.

In 2008, Pott and colleagues[32] analyzed the MR status from 2 phase III trials of MCL patients following chemotherapy and either ASCT or rituximab maintenance therapy. Of 190 evaluable patients, 106 (56%) achieved an MR. Only 2 patients achieving an MR in the bone marrow had a molecular relapse in the peripheral blood, whereas 13 patients with MRs in the peripheral blood had molecular relapses in the bone marrow. More patients achieving an MR in the bone marrow after induction remained in remission at 24 months than those showing minimal residual disease (100% vs 66%, respectively; *P* = .029).

At the present time, the usefulness of PCR to define remission and relapse in MCL at the molecular level is not yet sufficiently defined for routine clinical use. Different centers employ different genetic probes to assess MR, with potentially different results. The observation by Geisler and colleagues[31] that only approximately one half the MCL patients treated could be followed at the molecular level suggests the possibility that this and other studies may be looking only at a selected subgroup. Nonetheless, the potential exists to better define the response to therapy, to offer a more accurate prognosis, and to intervene earlier in relapsed patients.

New Developments in Prognostic Measures and Markers

Molecular analysis at the DNA level may eventually supersede the current clinical definitions of CR or PR and may allow a better definition of prognosis after therapy. However, determining the prognosis of patients before therapy is of interest as a guide to tailor therapy to specific patients. The International Prognostic Index (IPI) has often served as the benchmark for assessing a patient's status in MCL.[31,33,34] This index uses 5 independently significant factors: age (60 of age or younger vs older than 60 years of age), tumor stage (stage I/II [localized disease] vs stage III/IV [advanced disease]), number of extranodal sites of disease (≤ 1 vs > 1), performance score (0 or 1 vs ≥ 2), and serum lactate dehydrogenase level (LDH) level (≤ normal vs > normal).[35]

Despite its widespread use in MCL, the IPI was validated using a diverse population of NHL patients with diffuse mixed, diffuse large-cell, or large-cell immunoblastic lymphoma (International Working Formulation categories F, G, and H); diffuse centroblastic-centrocytic, centroblastic, immunoblastic, or unclassified high-grade lymphoma (Kiel classification[36]); or diffuse mixed lymphocytic-histiocytic or diffuse histiocytic lymphoma (Rappaport classification[37]) (MCL patients would have been termed "centrocytic" in the Kiel classification). Moreover, those patients were treated with the therapies of 15 years ago.

As NHL subsets have become better defined, it has become possible to develop more specific prognostic indices, such as the Follicular Lymphoma International Prognostic Index (FLIPI)[38] and, more recently, the MCL International Prognostic Index (MIPI).[39]

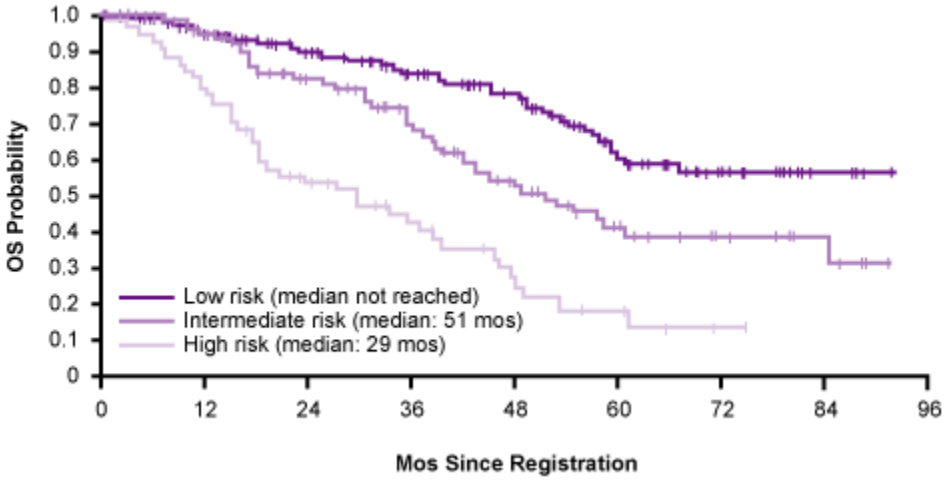
MIPI

The MIPI was validated using data from 455 patients with stage III/IV MCL who participated in 3 randomized trials.[39] The MIPI score is calculated as:

- [0.03535 x age (years)] x age (years)]
- + 0.6978 (if ECOG performance score > 1)
- + [1.367 x $\log_{10}$(LDH/upper limit of normal)]
- + [0.9393 x $\log_{10}$(white blood cell count)]

In contrast with the IPI and FLIPI, the number of extranodal sites and the number of involved nodal areas and hemoglobin levels showed no independent prognostic relevance in this MCL patient cohort, whereas the ECOG performance score showed high prognostic relevance. A simplified version of the MIPI separately classifies age, ECOG performance status, LDH, and white blood cell count on a scale from 0-3 and then sums the score overall: ≤ 3 points is low risk, 4-5 points is intermediate risk, and > 5 points is high risk. This simplified measure has high concordance with the calculated MIPI but was not independently validated. Using the full MIPI at a median follow-up of 32 months, the median OS was not reached in the low-risk group with a 5-year OS of 60%, and it was 51 months and 29 months in the intermediate-risk group and the high-risk group, respectively (Figure 4). Kaplan-Meier analyses of OS showed better separation of the risk groups using MIPI vs FLIPI or IPI.

Figure 4. OS according to the new prognostic index (MIPI).[39]



The validating population for the MIPI, like that for the IPI or FLIPI, comprised patients who could tolerate moderately intensive chemotherapy and did not include patients with stage I/II MCL, who are less frequently seen in practice and who may require a different therapeutic approach. No age adjustment was needed, and the index was accurate regardless of the initial cytoreductive therapy (CHOP, R-CHOP, or mitoxantrone, chlorambucil, and prednisone [MCP]). An early criticism of the simplified MIPI was that it did not accurately predict outcomes for patients treated with rituximab and hyper-fractionated cyclophosphamide, vincristine doxorubicin, and dexamethasone (R-hyperCVAD), but it is possible the quantitative MIPI would do better. Importantly, the MIPI continues to undergo validation for other treatments.[40] Whether there is any need for the simplified MIPI is a matter of conjecture; the “quantitative” MIPI merely requires a calculator to implement and provides a continuous linear estimate of survival time.

Ki-67 Status

Ki-67 is a protein marker for cellular proliferation. It is absent in cells in the G_0 phase but detectable in dividing cells by staining with a monoclonal antibody.[41] Ki-67 immunostaining is generally performed on lymph node or spleen samples, and the fraction of cells stained is the Ki-67 proliferation index. The ability to use a common immunohistochemical procedure, rather than more expensive and complex PCR-based or array-based genetic techniques (eg, the multigene proliferation index[28]), is a major appeal of this index. In the course of developing the MIPI,[39] the Ki-67 proliferation index was considered for inclusion as a factor. As an independent prognostic factor, > 10% Ki-67 staining had a strong prognostic relevance to OS (P < .001) and was a better predictor than cellular counts of mitoses. Ki-67 elevation was predictive of OS independent of the MIPI index, but adding Ki-67 index scores to the MIPI to make a combined biological index did not improve its predictive value.

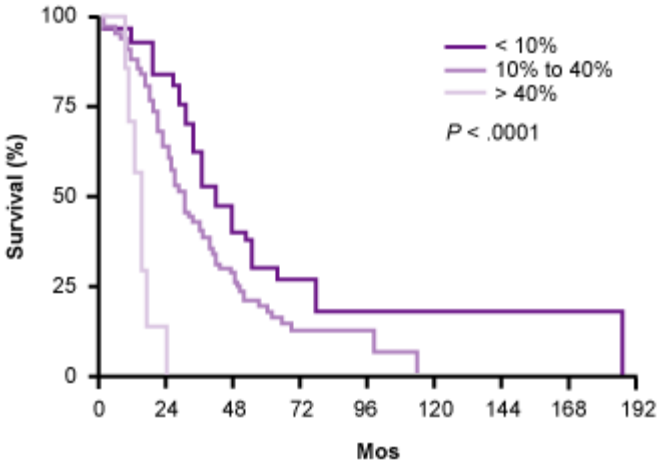
In a retrospective study (N = 127), Räty and colleagues[42] observed that MCL patients with Ki-67 expression in ≥ 26% (the upper tertile) of the lymphoma cells had a median survival time of 13 months compared with 45 months for the rest of the patients (*P* < .001; median follow-up time: 87 months). In a retrospective analysis of 21 lymph and spleen samples from primary and relapsed MCL patients, Ek and colleagues[43] found that tumors with > 30% Ki-67–positive cells (high group) also overexpressed a panoply of other genes involved in the cell cycle. Hui and colleagues[44] observed that a Ki-67 index of > 50% was significantly associated with shorter survival (*P* < .0006). These and other studies found that the Ki-67 index was independent of the IPI and often a better predictor of outcomes.

Cytopathology

In a study relating the cytopathology of MCL to outcomes, Tiemann and colleagues[45] examined 304 biopsy specimens from 351 MCL patients. The Ki-67 index was determined for 187 cases: The median Ki-67 index was 15%, 75% were < 20%, and the mean was 16.8%. Cases with classical or small-cell cytology showed a lower Ki-67 index (mean: 15.3%) than cases with pleomorphic (mean: 28.9%) or blastic cytology (mean: 28.8%; *P* < .0001). No correlation was found between the growth pattern of the lymphoma (ie, nodular or diffuse) and the Ki-67 index (*P* = .11).

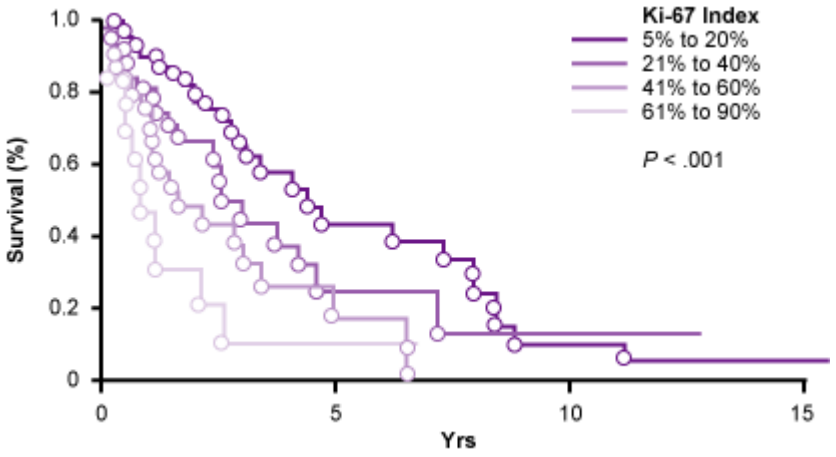
The patients' Ki-67 indices were divided into 3 groups (< 10%, 20% to 40%, and > 40%), and the corresponding OS outcomes were divided into 3 nonoverlapping groups, with median survival times of 42 months, 30 months, and 15 months, respectively (Figure 5). By univariate analysis, the Ki-67 index was significantly associated with OS (*P* < .0001) but the IPI was not. When examining only cytology, there was no significant OS difference between classic MCL and the small-cell type vs the pleomorphic and blastic variants, respectively, possibly because pleomorphic and blastic variants were rare (< 10% combined).

Figure 5. OS by Ki-67 index.[45]



A retrospective study by Katzenberger and colleagues[46] also helped place the Ki-67 index on firmer footing. The investigators divided the Ki-67 indices from 134 MCL patients into 4 groups, those with indices of ≤ 20% (n = 62), 21% to 40% (n = 32), 41% to 60% (n = 25), or > 60% (n = 15). The median survival times were 53 months, 33 months, 19 months, and 13 months, respectively (*P* < .001), demonstrating "a tight correlation between the Ki-67 index of tumor cells and survival in MCL" (Figure 6).[46]

Figure 6. Median survival by Ki-67 index.[46]



Determann and colleagues[47] tested the predictive value of the Ki-67 index in MCL patients treated with CHOP (n = 116), R-CHOP (n = 96), or MCP (n = 37). The Ki-67 index predicted OS (from time of trial entry) independent of a patient's MIPI score; a Ki-67 index score > 10% was closely comparable in prognostic relevance (relative risk: 1.27 for Ki-67 vs 1.20 for MIPI). When the patients' Ki-67 indices were stratified into 3 groups (< 10%, 10% to 29%, and ≥ 30%), each group's OS curve was distinct, regardless of treatment. These data indicate that the Ki-67 index is valid in the context of first-line rituximab. However, as the index was developed and validated on a population that was largely treated with CHOP or R-CHOP, it remains to be validated for treatment protocols that result in better OS.

Efforts to validate the Ki-67 index using other therapeutic regimens are under way. For example, Hsi and colleagues[48] examined Ki-67 expression in a population of MCL patients (n = 52) given high-dose chemotherapy (rituximab, methotrexate, cyclophosphamide, doxorubicin, vincristine, and oral prednisone) followed by ASCT. Either as a continuous variable or using a cutoff of 35%, Ki-67 was associated with shorter PFS ($P \leq .030$) and event-free survival ($P \leq .017$). The trial is ongoing and OS has not yet been reported.[49]

Garcia and colleagues[50] examined the utility of the Ki-67 index in a population of MCL patients (median age: 62 years) treated with R-hyperCVAD. Of the 71 patients included in the survival analysis, 59 had histologically classic MCL and 13 had the blastoid variant. In this study, neither age, sex, histology, serum β_2-microglobulin or LDH levels, the presence of B symptoms, the presence of peripheral blood involvement, nor IPI score (0-2 vs > 2) was correlated with either OS or failure-free survival. Of the different Ki-67 index cutoffs, a 20% division offered the highest statistical significance. By univariate analysis, the 5-year failure-free survival rate was 28.6% for patients with a high Ki-67 index (> 20%) compared with 53.1% for a low index (≤ 20%; $P = .003$). In the group with the classic MCL variant, the 5-year failure-free survival rate was 14% for patients with a high Ki-67 index vs 58% for a low index ($P = .0005$).

However, none of the cutoffs tested yielded a significant OS difference at 5 years: 51.4% for patients with a Ki-67 index > 20% vs 64.3% for a low index ($P = .19$). These data indicate the Ki-67 index may not be predictive of OS in MCL patients receiving R-hyperCVAD therapy.

The Ki-67 index is simple to perform, addresses a fundamental biological property of tumors, and appears to be fairly robust. On the other hand, Garcia and colleagues' results regarding R-hyperCVAD may be a sign that the cutoff levels may need a radical reformulation with newer therapies. In any event, further and potentially difficult standardization—for example, the cutoff levels used for grouping patients, the types and locations of tissues sampled, the criteria for

choosing fields for counting of positive cells, and how positive cells are detected—is likely to be required before the test becomes routine.

Although the IPI index remains useful, it is likely to be replaced by the Ki-67 index and/or the MIPI. This potentially opens the door for a combined index with greater power. The objective of both indices is to predict OS; both measures have been validated on patient populations that were treated with CHOP or R-CHOP, and newer therapies that could improve OS are being introduced. Therefore, these prognostic tools need further validation using more recently introduced therapies to exploit their full potential, and both will be used in the interim as guides for patient outlook.

Other Markers

The MIPI and Ki-67 index are well along the path to validation, but other markers may prove to be better prognosticators or may define additional therapeutic targets.

One new index is the 5-gene model, which is designed to circumvent the semiquantitative nature of the Ki-67 index by using PCR to analyze preserved tissue samples.[51] The test was validated only on MCL patients, most of whom were treated with CHOP. From multivariate Cox analyses of the relation of survival to the expression of 33 different genes in the samples, 5 genes were determined to offer the highest combined prognostic power: *MYC* (involved in cell cycle progression and apoptosis), *POLE2* (a DNA polymerase), *RAN* (translocates protein and RNA through the nuclear pore), *SLC29A2* (a nucleoside transporter), and *TNFRSF10B* (a receptor for tumor-necrosis factor [TNF]-family cytokine TRAIL).

By receiver operating curve analysis, the 5-gene model had better predictive power than the Ki-67 index. Validation on different paraffin-embedded samples showed that RNA from all 5 genes could be detected approximately 75% of the time; with more recent samples (1998-2003), the success rate rises to 86%. Like the MIPI, the 5-gene model is designed to provide a linear estimate of individual survival and to divide patients into different risk groups results in distinct survival curves.

One advantage of the 5-gene model is that it can be validated using stored tissue samples from patients given a wide range of treatments. The model retained its prognostic value after adjustment to take treatment into account. Although currently not all paraffin-embedded samples can be read, all frozen samples can be analyzed. Another advantage is that it appears to have higher prognostic relevance to survival than the Ki-67 index and is more quantitative. However, it requires a more sophisticated laboratory workup than the Ki-67 test, which excludes some clinics.

CD23 is a typical marker for chronic lymphocytic leukemia/small lymphocytic lymphoma and is also expressed in approximately 25% of MCL patients.[52] These patients have better event-free survival and OS; 4-year rates for 14 CD23-positive MCL patients were 45% and 75%, respectively, compared with 19% and 51% for 33 CD23-negative MCL patients. There are obstacles (phenotypic variations) to the use of CD23 as a prognostic marker for MCL[53]; its near-term importance may be as a target for immunotherapy. Clinical trials are under way using the anti-CD23 antibody lumiliximab in chronic lymphocytic leukemia.

Glutathione s-transferase π (GST-π) may be involved in detoxifying alkylating agents, and therefore, its overexpression may provide a measure of protection from chemotherapy. Overexpression of GST-π has been found to be consistently high in MCL, lower in diffuse large B-cell lymphoma, and uncommon in follicular lymphoma.[54] In diffuse large B-cell lymphoma, the degree of its overexpression can be used as a prognostic marker for survival[55]; its frequent overexpression in MCL suggests that GST-π might be a factor in MCL's relatively short survival times and that it, therefore, might be a target for therapy.

Genomic and Proteomic Analyses in Mantle Cell Lymphoma

Genomic Analysis

Before MCL was defined as a separate entity,[56] the t(11;14) translocation had already been observed in some NHL patients, the predominant translocation breakpoint in these cases had been cloned, and the affected gene had been discovered and named as a causally involved factor (BCL1, for B-cell lymphoma; later known as CCND1, for cyclin D1).[57] Therefore, the altered genetic profile has been a part of the history of MCL from its inception. Efforts to define MCL by its genetic profile have been aided in recent years by the advent of chip-based parallel gene expression.[58] Conclusions include, for example, the recognition that 8p- and 13q14-deletions are associated with worse OS, that 8p- deletions are associated with leukemic MCL, and that the genomic signature of MCL often includes deletions of 1p21, 11q22.3, 9q21-q22, and coincident 10p12-BMI1 gene amplification and 10p14 deletion.[59,60]

Specific gene arrays focused on particular genes believed to be involved in specific types of cancers (eg, Lymphochip[28] and Oncochip-CNIO[61]) have been shown to have good prognostic relevance. The advent of high-resolution, whole-genome arrays has made it feasible to scan the expression of the entire genome and find correlations with previously unknown transcripts, potentially obviating the need for the smaller, specific arrays but also increasing the number of genes to be analyzed and the scope of work needed to validate them. Many involved genetic changes have already been discovered.

Proteomic Analysis

Overexpressed, underexpressed, or misexpressed genes matter only insofar as they become overexpressed, underexpressed, or misexpressed proteins—it is the interaction between the proteins that physically manifests the disease. Importantly, genomic changes may not translate directly to changes at the protein level. For example, in hematopoietic differentiation, the correlation between the expression of mRNA and the actual protein levels is no more than 40%.[62] The physical variability of proteins makes them harder to analyze; to date, the only way to examine the changes in the proteins associated with genes of interest has been by immunohistochemistry, Western blotting, or flow cytometry, each of which is semiquantitative, hard to compare between centers, and severely impaired by formalin fixation or paraffin embedding. On the other hand, fresh tissue samples are difficult to collect and work with. Recent developments that allow custom-made antibodies to be made based on gene expression data[63,64] should greatly increase the ability to determine how changes in the genome are translated to changes in the proteome.

New “shotgun” methods of directly assessing protein expression—liquid chromatography and mass spectroscopy—have recently been applied to characterizing MCL plasma membrane proteins.[65] This methodology overcomes the limitations of 2-dimensional electrophoresis for membrane proteins, can provide information on posttranslational modifications, and allows quantitative estimation of protein abundance. In this study (N = 5 leukemic MCL patients), 423 proteins were identified (including 111 membrane proteins). CD70, PKC-β2II, and 5-LO were found to be overproduced relative to normal B cells, and each is a potential therapeutic target. Two ion channels were found that were previously unknown in B or MCL cells.

The ability to perform the kind of mass screenings for altered protein expression that can be done with gene expression, coupled with the ability to make specific antibody probes to proteins of interest (even when they cannot be physically isolated or their function is unknown), promises to rapidly and greatly expand our knowledge at the protein level in a manner analogous to what is occurring at the genomic level. The results seen with the proteomic approach may simplify our interpretation of the genomic findings, but first it will be necessary to conduct close correlative and comparative studies.

Summary and Conclusions

Improvements in first-line and second-line therapies have been furthered by the development of maintenance therapies in MCL. Although the use of interferon has extended event-free and and OS, maintenance with autologous stem cell transplantation has been shown to be superior to interferon maintenance in a large study of untreated, advanced-stage MCL. Rituximab holds promise as a maintenance therapy in MCL and has been demonstrated to improve PFS and the duration of response, but it is controversial if it also improves OS because of varying results. Novel therapies under investigation as maintenance treatment include bortezomib, thalidomide, lenalidomide, and mTOR inhibitors (eg, everolimus). Predictive factors in MCL such as molecular remission are under investigation; patients achieving a molecular remission in the bone marrow are more likely to retain their remission than those achieving molecular remission in the peripheral blood. The MCL International Prognostic Index has been developed and shown to accurately predict overall survival. The Ki-67 index has similar accuracy. Both are likely to replace the current International Prognostic Index for MCL. Genomic and proteomic analyses are being developed in this setting and may be of prognostic relevance.

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1. **Herold and colleagues studied maintenance therapy with interferon following induction chemotherapy in patients with untreated, advanced lymphomas, including mantle cell lymphoma (MCL) in 26% of the patients. All of the following results were associated with interferon maintenance EXCEPT which one?**

   A. Event-free survival was not improved in MCL but was improved in follicular lymphoma (FL) and lymphoplasmocytic lymphoma
   B. Overall survival (OS) was improved regardless of induction treatment
   C. Event-free survival was improved regardless of induction treatment
   D. OS was similar between induction therapy groups

2. **In an analysis by Witzens-Harig and colleagues of quality of life in patients with non-Hodgkin's lymphoma enrolled in a trial comparing maintenance rituximab with observation, significant differences were found between groups in which of the following areas?**

   A. Global health
   B. Functional health
   C. Symptomatic health
   D. All of the above
   E. None of the above

3. **Forstpointner and colleagues conducted a study comparing rituximab maintenance vs observation in advanced-stage, relapsed/refractory FL and MCL patients who had received induction with fludarabine, cyclophosphamide, and mitoxantrone (FCM). Which of the following results were reported from this study?**

   A. The median duration of response was significantly longer in MCL patients receiving rituximab maintenance
   B. More MCL patients receiving rituximab maintenance experienced remissions lasting longer than 2 years
   C. The median duration of response for all patients was not significantly longer with rituximab maintenance
   D. More grade 3/4 infections were seen in the rituximab maintenance arm

4. **According to results from a large phase II study by the Nordic Lymphoma Group, does achievement of a molecular remission (MR) (following rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisone [R-CHOP] and autologous stem cell transplantation [ASCT]) predict the likelihood of long-term remission?**

   A. Yes
   B. No

5. **The MCL International Prognostic Index (MIPI), developed by Hoster and colleagues, uses all of the following patient factors EXCEPT which one to determine prognosis?**

   A. Age
   B. ECOG performance score
   C. Number of extranodal sites
   D. Lactate dehydrogenase level
   E. White blood cell count

6. **The Ki-67 index defines prognoses based on percentage of Ki-67–stained cells. In a study by Determann and colleagues of MCL patients treated with chemotherapies, how did the Ki-67 index compare to MIPI?**

   A. The Ki-67 index more accurately predicted OS
   B. MIPI more accurately predicted OS
   C. Both indices were similarly able to predict OS

7. **Pott and colleagues analyzed the MR status in the bone marrow and peripheral blood from 2 phase III trials of MCL patients who had received chemotherapy maintenance therapy (ASCT or rituximab). At 24 months, which patient group had more molecular relapses?**

   A. Patients achieving MR in the bone marrow
   B. Patients achieving MR in the peripheral blood
   C. Both groups had a similar rate of relapses

8. **Which of the following results was/were seen in a phase II study by Kaufmann and colleagues of rituximab plus thalidomide, followed by thalidomide maintenance, in relapsed/refractory MCL patients?**

   A. Only one half of the patients could tolerate the planned 400-mg dose of thalidomide
   B. Grade 4 neutropenia was observed
   C. Progression-free survival (PFS) in patients achieving a complete response was longer than with previous therapy
   D. All of the above
   E. None of the above

9. **The European MCL Network conducted a study comparing maintenance with interferon to ASCT following a CHOP-like regimen in 230 untreated, advanced MCL patients. Which of the following outcomes was/were superior with ASCT?**

    A. PFS
    B. OS
    C. Both PFS and OS were superior with ASCT

10. **Rohatiner and colleagues conducted a meta-analysis of 10 phase III studies that demonstrated induction with interferon plus chemotherapy improves survival in patients with FL. Did results show that maintenance interferon also prolonged survival?**

    A. Yes
    B. No

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