Regular Article

blood

LYMPHOID NEOPLASIA

TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy

Christian W. Eskelund,[1,2] Christina Dahl,[3] Jakob W. Hansen,[1,2] Maj Westman,[4] Arne Kolstad,[5] Lone B. Pedersen,[1] Carmen P. Montano-Almendras,[1,2] Simon Husby,[1,2] Catja Freiburghaus,[6] Sara Ek,[6] Anja Pedersen,[1,2] Carsten Niemann,[1] Riikka Räty,[7] Peter Brown,[1] Christian H. Geisler,[1] Mette K. Andersen,[4] Per Guldberg,[3] Mats Jerkeman,[8] and Kirsten Grønbæk[1,2]

[1]Department of Hematology, Rigshospitalet, Copenhagen, Denmark; [2]Biotech Research and Innovation Centre, Copenhagen, Denmark; [3]Danish Cancer Society Research Center, Copenhagen, Denmark; [4]Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; [5]Department of Oncology, Oslo University Hospital, Oslo, Norway; [6]Department of Immunotechnology, Lund University, Lund, Sweden; [7]Department of Hematology, Helsinki University Hospital, Helsinki, Finland; and [8]Department of Oncology, Lund University Hospital, Lund, Sweden

---

Key Points

- The intensified standard-of-care regimens for younger patients with MCL do not overcome the deleterious effects of *TP53* mutations.

- MCLs with *TP53* mutations should be considered for alternative frontline treatment.

Despite recent advances in lymphoma treatment, mantle cell lymphoma (MCL) remains incurable, and we are still unable to identify patients who will not benefit from the current standard of care. Here, we explore the prognostic value of recurrent genetic aberrations in diagnostic bone marrow (BM) specimens from 183 younger patients with MCL from the Nordic MCL2 and MCL3 trials, which represent current standard-of-care regimens. In the univariate model, mutations of *TP53* (11%) and *NOTCH1* (4%), and deletions of *TP53* (16%) and *CDKN2A* (20%), were significantly associated with inferior outcomes (together with MIPI, MIPI-c, blastoid morphology, and Ki67 > 30%); however, in multivariate analyses, only *TP53* mutations (HR, 6.2; *P* < .0001) retained prognostic impact for overall survival (OS), whereas *TP53* mutations (HR, 6.9; *P* < .0001) and MIPI-c high-risk (HR, 2.6; *P* = .003) had independent prognostic impact on time to relapse. *TP53*-mutated cases had a dismal outcome, with a median OS of 1.8 years, and 50% relapsed at 1.0 years, compared to a median OS of 12.7 years for *TP53*-unmutated cases (*P* < .0001). *TP53* mutations were significantly associated with Ki67 > 30%, blastoid morphology, MIPI high-risk, and inferior responses to both induction- and high-dose chemotherapy. In conclusion, we show that *TP53* mutations identify a phenotypically distinct and highly aggressive form of MCL with poor or no response to regimens including cytarabine, rituximab, and autologous stem-cell transplant (ASCT). We suggest patients with MCL should be stratified according to *TP53* status, and that patients with *TP53* mutations should be considered for experimental frontline trials exploring novel agents. (*Blood*. 2017;130(17):1903-1910)

Introduction

Mantle cell lymphoma (MCL) is a rare type of non-Hodgkin lymphoma (NHL), which accounts for 5% to 8% of all NHLs. Historically, MCL was associated with a dismal outcome, with a median overall survival (OS) of only 3 to 5 years[1,2]; however, during the past decades, the outcome, especially for younger patients, has improved substantially by an intensified frontline regimen including cytarabine, rituximab, and consolidation with high-dose therapy and autologous stem-cell transplant (ASCT).[1,3,4] Hence, in the recent long-term follow-up of such a regimen, the Nordic MCL2 trial, we observed a median OS and progression-free survival (PFS) of 12.7 and 8.5 years, respectively.[5] Despite this marked improvement, MCL remains an incurable, albeit heterogeneous, disease with a wide span of early and late relapses. However, none of the existing risk stratification systems has yet been incorporated into clinical decision making.[4,5]

The most frequently used clinical prognosticator, the MCL International Prognostic Index (MIPI), has been thoroughly validated to define 3 groups of patients with diverse prognoses, and has recently been refined to include Ki67 expression (then called MIPI-c) with the 10% to 13% of patients who are high-risk, showing an exceedingly poor outcome.[6-8] Also, blastoid cases of MCL have recurrently been associated with more aggressive disease and poorer outcome.[7,9]

Molecular studies of MCL demonstrate recurrent aberrations in genes involved in the regulation of the cell cycle, DNA repair, and epigenetics.[10-13] In recent years, next-generation sequencing has led to comprehensive mutational characterization of MCL; however, most studies have been performed in either small or diverse patient cohorts, limiting the translational impact of the findings.[10,14,15] Nonetheless, *TP53* mutations have recurrently demonstrated negative prognostic impact for the outcome of patients with MCL, and more recently, *NOTCH1* and *NOTCH2* mutations have been associated with inferior outcomes.[14-18] Furthermore, del(17p13) and del(9p21) (harboring

---

TP53 and *CDKN2A*, respectively) have recurrently been associated with poor outcome.[12,17,19-21] Recently, Delfau-Larue et al[22] validated this in a large homogenous cohort of patients from the European-MCL Younger trial, but with no information about *TP53* mutations.

Here we aim to describe the prognostic effect of the most common genomic alterations of MCLs in the large, homogenously treated cohorts from 2 Nordic trials, MCL2 and MCL3. Both regimens represent current standard-of-care regimens for younger patients, and thus hold important information for the daily handling of patients with MCL.

Materials and methods

Patients

Three hundred twenty patients were included in the Nordic MCL2 and MCL3 trials from 2000 to 2005 and 2005 to 2009, respectively.[3,23] Patients were younger than 66 years, had stage II-IV MCL, and were considered fit for ASCT. Diagnostic specimens underwent central pathological review according to World Health Organization criteria. All samples were required to express Cyclin-D1 or to carry the t(11;14) translocation. Both of the MCL2 and MCL3 regimens consisted of an induction phase of alternating R-maxi-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) and R-high-dose cytarabine, followed by high-dose chemotherapy with BEAM (bis-chloroethylnitrosourea, etoposide, cytarabine, and melphalan) or BEAC (bis-chloroethylnitrosourea, etoposide, cytarabine, and cyclophosphamide) and ASCT.[3,23] Patients with an available minimal residual disease (MRD) marker by nested polymerase chain reaction (PCR) for translocation t(11;14) or clonal immunoglobulin heavy chain rearrangement were monitored during follow-up and treated preemptively with rituximab on MRD-positivity without concurrent clinical relapse.[24] Patients from the MCL3 protocol in complete remission unconfirmed (CRu)/partial remission pre-ASCT received Zevalin to optimize the induction response; however, this did not alter the outcome, and thus the combined cohorts are here considered as one.[3,23] The studies were approved by local ethical committees, and all patients signed informed consent.

One hundred eighty-three patients from the combined MCL2 and MCL3 cohort, with available DNA from diagnostic bone marrow (BM) samples, were included in the genomic studies. Of these, deletions were analyzed in 177 patients, and mutations in 176. Both were analyzed in 170 patients.

Next-generation sequencing

DNA was extracted from fresh frozen pretreatment BM specimens using QIAprep Miniprep (Qiagen, Valencia, CA). Samples were analyzed using a custom-designed multiplex Ion Ampliseq panel (Ampliseq designer, Thermo Fischer Scientific, Waltham, MA) including selected coding regions, splice sites, and untranslated regions of the following 8 genes: *ATM*, *KMT2D*, *CCND1*, *TP53*, *WHSC1*, *BIRC3*, *NOTCH1*, and *NOTCH2* (supplemental Table 1, available on the *Blood* Web site). The gene panel was constructed on the basis of previous whole-exome and whole-genome studies.[10,14] Initial DNA quantification was carried out using Qubit broad range assay. Ion Ampliseq technology was used for library construction, and quantification of the final library was performed by qPCR, using a TaqMan Ion library quantification kit. Template preparation was automatically carried out on the Ion Chef Instrument, using Hi-Q technology and reagents. Seven samples with unique barcodes were simultaneously loaded on a 318 Ion Chip. Subsequent sequencing was carried out on the Ion PGM System using Hi-Q technology. All steps were carried out according to manufacturer's instructions, and reagents and equipment were manufactured by Thermo Fischer Scientific.

Variant calling

Cutoff for calling a somatic variant was 5%. Median coverage for all samples was 2900×, and the limit for calling a variant was set to 400×. Variants were carefully investigated using the IGV software (Broad Institute). Common single nucleotide polymorphisms (SNPs; as reported >1% by dbSNP database) were excluded from analyses. We report only nonsynonymous mutations and mutations in splice site region or untranslated regions that were not reported in the SNP databases. Eight variants have been reported both as rare SNPs and as missense mutations, and are reported here as mutations, but with references to both COSMIC-ID and dbSNP-ID shown in supplemental Table 2. Seven mutations were verified by Sanger sequencing because of coverage below 400×.

Three *TP53* mutations with variant allele frequencies of 3% to 5% were detected. Because of the apparent effect of *TP53* mutations, these 3 mutations were validated in a specific *TP53* panel on the Ion Torrent platform, and hence were included in all subsequent analyses (supplemental Table 2).

Deletion analysis

TP53 and *CDKN2A* deletions were identified by droplet digital PCR, using the QX200 system (Bio-Rad Laboratories, Hercules, CA). Fifty nanograms of DNA were analyzed using PrimePCR droplet digital PCR copy number variation (CNV) assays with *RPP30* as reference locus, according to the manufacturer's instructions (Bio-Rad). CNs were calculated using the QuantaSoft software. The dynamic range of the instrument and threshold settings were determined by analysis of blood DNA from 6 healthy donors. CN loss was defined as CN < 1.95. Each sample was analyzed at least twice and scored manually. The person who performed the data analysis and evaluation was blinded for patient characteristics.

Statistics

Statistical analyses were performed in SPSS 22.0 for Windows and GraphPad Prism 7.02 for Windows (GraphPad). To compare demographics, laboratory tests, and mutational status between the 2 groups, we used Pearson's χ-squared test or Fischer's exact test for dichotomous variables. Overall survival (OS), progression-free survival (PFS), and cumulative incidence of relapse (CIR) were used as outcomes for all prognostic analyses. Starting point for all 3 was date of treatment start, and endpoint for OS was date of death of any cause, endpoint for PFS was date of documented relapse or progression or death of any cause, and endpoint for CIR was date of documented relapse or progression of MCL. The log-rank test was used to compare the outcome of groups in univariate analyses, and Cox regression analysis was used for multivariate analyses. Differences were considered statistically significant when $P < .05$.

Results

Patient characteristics

Three hundred twenty patients were enrolled in the MCL2 and MCL3 trials.[3,23] One patient from the MCL2 trial has since been withdrawn because of a change of diagnosis.[5] Of the remaining 319 patients, the median age was 57 years (range 29-65), 76% were male, 51% were MIPI high- or intermediate-risk, 18% had blastoid morphology, and 41% had Ki67 ≥ 30%.

With a median follow-up time of 9.2 years for all 319 patients, median OS and PFS were 12.5 and 8.2 years, respectively, and 50% of the patients had relapsed at 10.2 years. In line with previous reports, blastoid morphology, Ki67 ≥ 30%, and higher MIPI and MIPI-c risk groups were significantly associated with poorer outcomes (supplemental Figure 1; supplemental Table 3). Importantly for the subsequent genetic analyses, we did not observe any differences in outcome among patients with or without BM involvement by morphological review (supplemental Table 3).

Genetic findings

Of the combined cohort of 319 patients, DNA was available for 191, and 183 samples were of sufficient quality for the subsequent genetic analyses. The study cohort was selected only by availability of DNA and did not differ from the entire MCL2 and MCL3

Table 1. Patient characteristics of the combined cohorts of MCL2 and MCL3

	No available DNA (N = 136)		Available DNA (N = 183)*		
	N	%	N	%	P
Mean age, y (range)	56 (32-65)		56 (29-65)		.28
Male sex	104	76	137	75	.74
BM involvement†					.95
Yes	108	79	144	79	
No	28	21	38	21	
MIPI					.57
Low	67	50	89	49	
Intermediate	34	26	56	31	
High	32	24	38	21	
MIPI-c					.62
Low	35	34	55	35	
Low-intermediate	30	29	46	29	
High-intermediate	27	26	34	22	
High	10	10	23	15	
Morphology					.41
Nonblastoid	108	79	152	84	
Blastoid	28	21	31	17	
Ki67 index					.34
<30%	66	63	90	57	
≥30%	39	37	68	43	
Clinical endpoints					
Median OS, y	11.7		12.8		.10
Median PFS, y	7.7		8.5		.49
Median CIR, y	11.8		9.9		.75

*Included in genetic analyses.
†Bone marrow involvement by morphologic assessment.

cohort in terms of baseline characteristics (Table 1). However, a significantly higher proportion of patients with available DNA achieved a complete remission after induction therapy ($P = .004$), and there was a trend toward superior OS, but no differences in PFS and CIR.

Deletions. On the basis of the findings in a recent study, we investigated the presence of *TP53* and *CDKN2A* deletions, which had both shown independent, prognostic impact.[22] Deletions of *CDKN2A* were detected in 35 (20%) of 177 patients, and deletions of *TP53* in 29 (16%) of 176, and both deletions were detected in 12 (7%) of 176 patients. In total, deletion of either gene was detected in 52 patients (30%) (Figure 1A). The applied method did not discriminate between homo- and heterozygous deletions.

Mutations. By targeted sequencing of 8 genes recurrently mutated in MCL, we detected a total of 154 mutations in the 176 patient samples examined, with *ATM* (27%), *KMT2D* (14%), *TP53* (11%), and *CCND1* (9%) being the most frequently mutated genes. Ninety (51%) patients carried at least 1 mutated gene, and 33 (19%) carried more than 1 mutated gene (Figure 1B).

Bone marrow involvement

By the original morphologic assessment, 144 (79%) patients had BM involvement at diagnosis. In the mutational analyses, morphological BM involvement was significantly associated with the presence of mutations in any of the genes tested ($P < .0001$); however, mutations were detected in 8 patients with no morphological BM involvement (Figure 1B; supplemental Table 2). In addition, we identified *TP53* and/or *CDKN2A* deletions in 45 (33%) of 138 (33%) patients with morphologic BM involvement compared with 7 (18%) of 38 patients with no morphologic BM involvement ($P = .09$; Figure 1A).

Prognostic relevance of genetic aberrations

In univariate log-rank analyses, *TP53* deletions, *CDKN2A* deletions, *TP53* mutations, and *NOTCH1* mutations were each significantly associated with poorer outcome, and *WHSC1* mutations were associated with shorter OS, but were not significant for PFS or CIR (Figure 2; supplemental Figure 2; supplemental Table 4). Likewise, the combined *TP53* aberrations (23%) (mutations and deletions) and the combined *NOTCH1* and *NOTCH2* mutations (7%) were associated with poorer outcome (Figure 3A-C; supplemental Table 4).

The presence of *TP53* mutations was significantly associated with *NOTCH1* mutations (5 of 7 *NOTCH1* mutations; $P = .0002$), deletions of *CDKN2A* (11 of 35 *CDKN2A* deletions; $P = .0002$), and *TP53* deletions (9 of 29 *TP53* deletions; $P = .001$). Of 169 patients, 39 (23%) carried *TP53* mutations and/or deletions, and of these, 9 (5%) carried both aberrations (Figures 1A and 3).

In the multivariate Cox regression analyses (n = 147), only *TP53* mutations (HR, 6.2; $P < .0001$) showed independent prognostic effect for OS, whereas for PFS and CIR, both *TP53* mutations (PFS: HR, 6.8 [$P < .0001$]; CIR: HR, 6.9 [$P < .0001$]) and MIPI-c high-risk (PFS: HR, 2.2 [$P = .01$]; CIR: HR, 2.6 [$P < .003$]) had significant prognostic impact (Table 2). The MIPI-c index was included as a bimodal variable

Figure 1. Deletions and point mutations in MCL2 and MCL3 patients. (A) Deletions of *CDKN2A* and *TP53* (blue) and mutations of *TP53* for comparison (red), according to BM involvement of MCL by morphologic review (yellow). Gray boxes indicate samples not tested. (B) Mutation frequency of 8 selected genes. *BM involvement by morphologic assessment.
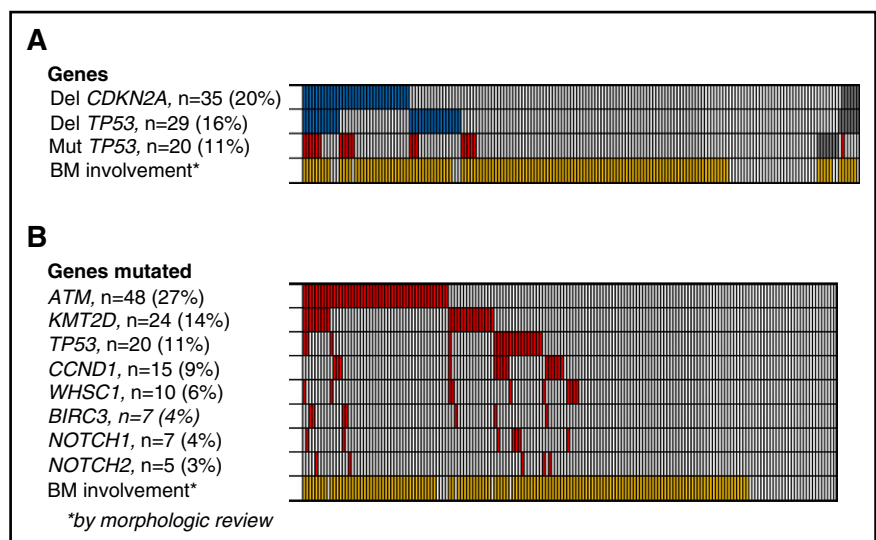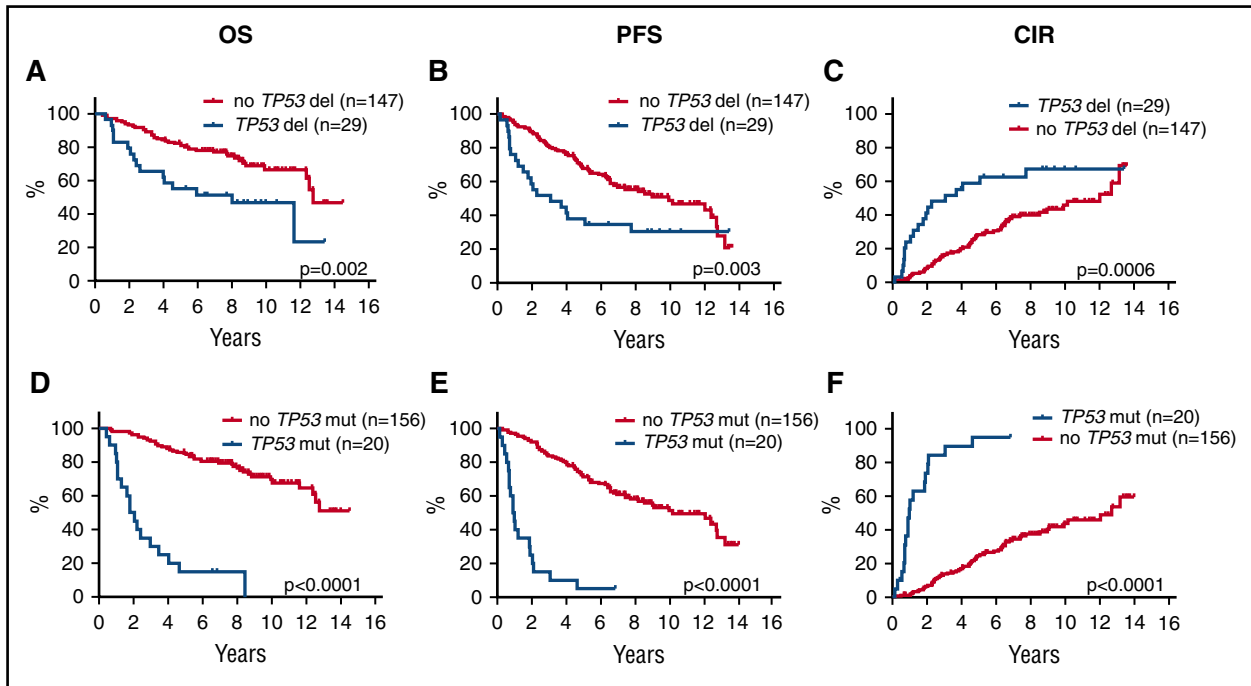
Figure 2. Prognostic impact of *TP53* deletions and mutations on OS, PFS, and CIR. Kaplan-Meier estimates of OS, PFS, and CIR stratified by the presence or absence of *TP53* deletions (A-C) and *TP53* mutations (D-F).

(high-risk or not) to obtain the highest effect (MIPI-c included as categorical variable did not show significant individual effect; data not shown). Surprisingly, MIPI high-risk and Ki67 $\geq$ 30% included as separate values did not show independent prognostic value (data not shown). All factors with $P < .05$ in univariate analyses were included in

the multivariate analyses: *TP53* and *NOTCH1* mutations, *TP53* and *CDKN2A* deletions, blastoid morphology, and MIPI-c high-risk. *WHSC1* was only included for OS, as it had no significant effect for PFS and CIR in the univariate log-rank analyses (Table 2; supplemental Table 4).
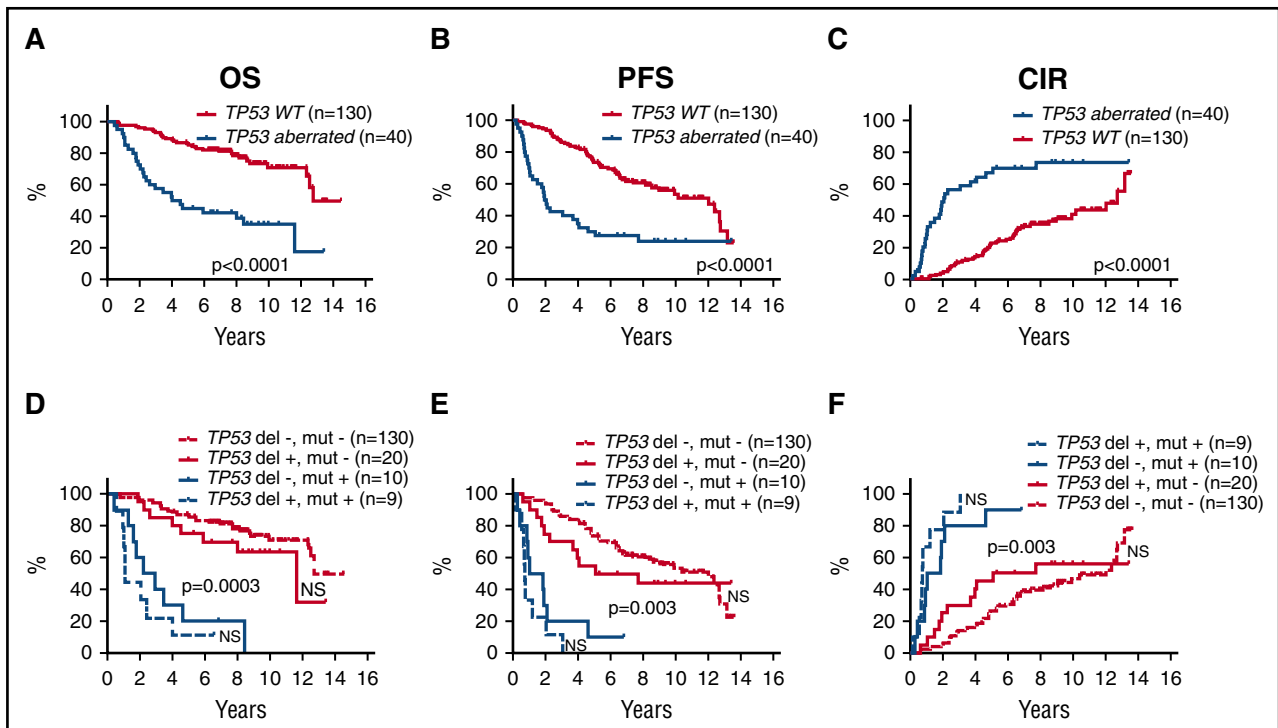

Figure 3. Prognostic impact of *TP53* aberrations. Kaplan-Meier estimates of OS, PFS, and CIR according to the presence of the either *TP53* aberrations (deletions and/or mutations) compared with WT *TP53* (A-C), and stratified into 4 groups by the presence or absence of *TP53* deletions and mutations, respectively (D-F). *P* values indicate log-rank tests of adjacent curves. NS, not significant.

Table 2. Multivariate Cox regression analyses (n = 147)

Variables	OS HR	95% CI	P	PFS HR	95% CI	P	CIR HR	95% CI	P
mut TP53	6.2	(2.6-14.9)	<.0001	6.8	(3.4-13.8)	<.0001	6.9	(3.3-14.5)	<.0001
mut NOTCH1	2.7	(0.9-8.6)	.09	2.3	(0.9-6.3)	.10	2.2	(0.7-6.5)	.17
del TP53	1.4	(0.7-2.8)	.37	1.5	(0.9-2.7)	.15	1.7	(0.9-3.0)	.10
del CDKN2A	1.3	(0.6-2.7)	.55	1.3	(0.7-2.4)	.40	1.3	(0.7-2.5)	.43
Blastoid	1.3	(0.6-2.5)	.53	0.8	(0.4-1.6)	.62	0.9	(0.4-1.7)	.65
MIPI-c high-risk*	1.8	(0.9-3.9)	.11	2.2	(1.2-4.0)	.01	2.6	(1.4-4.9)	.003
mut WHSC1†	0.8	(0.3-1.9)	.58	—	—	—	—	—	—

*MIPI-c index included as a bimodal variable of MIPI-c high-risk or not.
†WHSC1 mutations only included for OS, as they did not show significant prognostic effect for PFS and CIR in univariate analyses.

Combining TP53 mutations and deletions in the multivariate analyses also showed significant prognostic value (OS: HR, 3.1 [P = .0004]; PFS: HR, 2.8 [P < .0001]; CIR: HR, 3.1 [P < .0001]). The different influence on outcome between TP53 mutations and deletions is illustrated in Figure 3D-F. Inclusion of the combined NOTCH1 and NOTCH2 mutations did not change the multivariate results (data not shown).

Characteristics of patients with MCL with TP53 mutations

The above multivariate analyses clearly demonstrated that cases with TP53 mutations represent a unique subset of MCL. Stratification of all 176 patients according to TP53 mutational status revealed 2 cohorts of very different outcomes. The median OS for the TP53-mutated cases was 1.8 years, median PFS was 0.9 years, and median time to relapse was 1.0 years compared with a median OS not reached, median PFS of 10.2 years, and median time to relapse of 12.3 years for the TP53-unmutated cases (Figure 2D-F; supplemental Table 4). Comparably, the median OS, PFS, and time to relapse were 8.0, 3.1, and 3.1 years for the TP53-deleted cases, respectively (Figures 2A-C and 3D-F). To gain further insight into the specific characteristics of patients with and without TP53 mutations, we analyzed these cohorts separately.

Nineteen patients carried a single TP53 mutation, and 1 patient carried 2. Of the 21 detected mutations, 16 were missense mutations in the DNA binding domain (supplemental Table 2).

TP53-mutated cases displayed highly aggressive baseline characteristics and were highly associated with blastoid morphology, Ki67 ≥ 30%, MIPI high-risk, and MIPI-c high-risk, and significantly fewer TP53-mutated patients achieved CR after induction chemotherapy and ASCT, respectively (Table 3). Furthermore, MRD evaluation showed a significant association between PCR positivity and TP53 mutations post-ASCT and pre-ASCT (Table 3).

Comparing TP53 mutations with already available data on microRNA expression from the same cohort,[25] we found a significant correlation of TP53 mutations and higher expression of miR-18b and miR-378d, but not miR-92a-3p (P = .001, P = .043, and P = .20, respectively) (supplemental Table 6).

We found no significant relation of TP53 mutations to IGHV mutational data (available for 93 patients, including 14 with TP53 mutations; data not shown).

One patient (no. 3098; supplemental Table 2) carried a TP53 mutation, p.Pro191Arg (c.572C>G), with variant allele frequency 48.9% despite no morphologic BM involvement. This mutation had not previously been reported in relation to hematological cancer in COSMIC, or as a rare SNP. Sanger sequencing of normal DNA from the patient confirmed its germ line origin. The patient was still in first remission after 7 years of follow-up and had never experienced any other cancer. This case was referred to the local department of clinical

genetics, who interpreted the mutation as a rare variant of unknown significance, class 3. Hence, the mutation was excluded from our analyses.

All these features advocate that MCL with TP53 mutations represents a molecular and clinical distinct disease entity.

Patients with unmutated TP53

With TP53 mutations confidently assigning 11% of patients to a dismal outcome, we investigated the possibility of stratifying the remaining 89% of TP53-unmutated cases. Interestingly, among the 156 TP53-unmutated cases, no other biomarker showed any prognostic value (mutations in the remaining 7 genes, TP53 and CDKN2A deletions, MIPI, blastoid morphology, Ki67 > 30%, and MIPI-c; Figure 4). However, MIPI-c high-risk cases showed a significantly shorter time to relapse compared with MIPI-c non-high-risk cases (5.9 vs 12.0 years; P = .035), but did not have significant prognostic value for OS (not reached vs 12.8 years; P = .18) or PFS (5.9 years vs 10.2 years; P = .11).

Discussion

Here, we report the prognostic value of 8 recurrently mutated and 2 recurrently deleted genes on outcome for younger patients with MCL treated in the Nordic MCL2 and MCL3 trials, representing current standard-of-care regimens. Despite the high efficacy of these regimens, cases with TP53 mutations were resistant. Thus, our data show that TP53 mutations identify a unique MCL subtype associated with

Table 3. Characterization of TP53-mutated MCLs

|	N	TP53 unmutated (N = 156) N	%	TP53 mutated (N = 20) N	%	P
Baseline characteristics						
MIPI high risk	176	24	15	14	70	<.0001
MIPI-c high risk	152	12	9	11	58	<.0001
Blastoid morphology	176	19	12	12	60	<.0001
KI67 >30%	152	50	38	15	79	.001
Treatment response						
CR pre-ASCT*	176	111	71	5	25	.0002
CR post-ASCT*	176	141	90	9	45	<.0001
No ASCT	176	8	5	5	25	.001
MRD assessment						
MRD positivity, pre-ASCT	99	32	36	7	70	.037
MRD positivity, post-ASCT	135	10	8	6	50	<.0001

*CR or CRu.
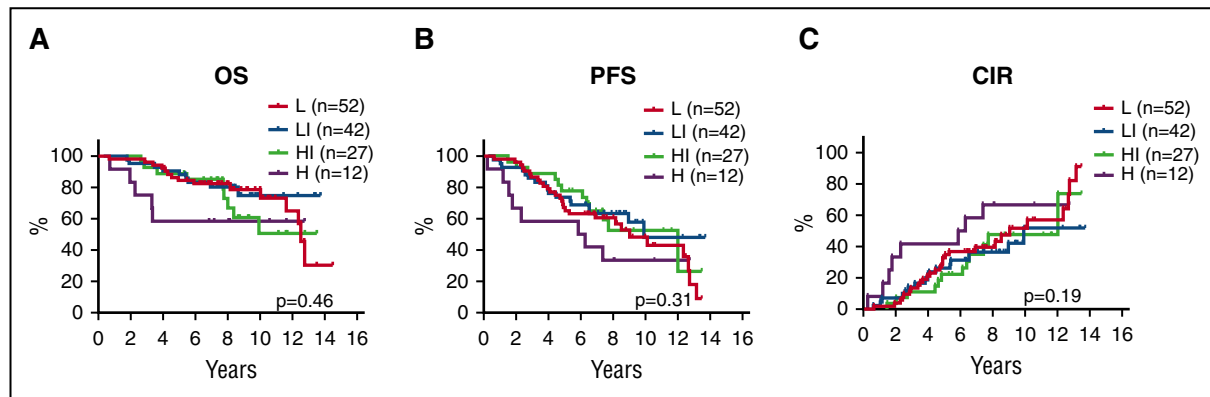
Figure 4. Prognostic impact of MIPI-c index in *TP53*-unmutated cases. Kaplan-Meier estimates of OS (A), PFS (B), and CIR (C) stratified by the MIPI-c index into low (L), low-intermediate (LI), high-intermediate (HI), and high (H) risk groups.

high-risk baseline characteristics, dismal response to standard treatment, and poor clinical outcome.

TP53 mutations have repeatedly been associated with a poor prognosis in previous MCL studies; however, these cohorts were heterogeneously treated and included patients of all ages.[14,17,18] Interestingly, both Greiner et al[18] and Halldórsdóttir et al[17] reported a median OS of 1.1 years for the *TP53*-mutated cases (16 and 17 patients, respectively). Here, we demonstrate for the first time that *TP53* mutations are associated with poor prognosis in a homogeneously treated cohort of younger, fit patients with MCL. The addition of rituximab and high-dose cytarabine to high-dose chemotherapy has improved responses significantly for younger patients with MCL in general (median OS, 12.7 years), but *TP53*-mutated MCL cells seem to escape eradication by these drugs, and patients with *TP53*-mutated MCL still have a dismal outcome with a median survival of 1.8 years.

Deletions of *CDKN2A* and *TP53* have been thoroughly investigated in MCL. Although *TP53* deletions have shown diverse results, *CDKN2A* deletions have consistently been associated with inferior clinical outcome.[12,17,19-21] Importantly, 1 recent study reported CNVs in patients from the MCL Younger trial,[22] of whom 72 of 134 had been treated with a rituximab- and cytarabine-containing induction regimen followed by ASCT; that is, a regimen similar to the cohort in our study. Using a panel of 8 gene regions, the authors showed that only deletions of *TP53* (22%) and *CDKN2A* (25%) retained individual prognostic impact; hence, only these 2 genomic regions were analyzed for deletions in our studies. We detected slightly lower frequencies of *TP53* and *CDKN2A* deletions (16% and 20%, respectively), which can possibly be explained by the unsorted selection of BM samples in our studies, whereas Delfau-Larue et al[22] selected samples with higher tumor content. Importantly, we confirmed the prognostic effect of both deletions in the univariate setting; however, both were highly associated with *TP53* mutations and, hence, lost effect in the multivariate analyses.

Our data show a clear difference in impact on all outcomes of *TP53* deletions vs mutations (Figure 3D-E). This pattern has previously been reported in a more heterogeneous cohort of 119 Swedish patients with MCL, which showed prognostic significance of *TP53* mutations, but not of *TP53* deletions.[17] Similar results have been reported in diffuse large B-cell lymphomas.[26] The explanation for this difference may relate to the dominant negative effect of some *TP53* mutations: although some mutated forms of p53 disrupt the function of the entire p53 protein-tetramer, deletions only reduce the amount of transcribed p53,

and thus may affects the protein function to a lesser degree.[27] Furthermore, an evaluation of the effect of homo- versus heterozygous deletion is needed.

In this report, we confirm the prognostic impact of blastoid morphology, Ki67 ≥ 30%, MIPI, and MIPI-c in the largest cohort of younger, optimally treated patients with MCL reported to date. Interestingly, all these factors were strongly associated with *TP53* mutations in our study cohort, and only MIPI-c high-risk maintained independent prognostic impact in the multivariate analyses (Table 3). Moreover, in the cohort of *TP53*-unmutated patients (n = 156), none of these widely validated biomarkers showed any prognostic value; still, this remains to be validated in an independent cohort of patients stratified according to *TP53* mutations. Indeed, for MIPI and MIPI-c, it needs to be stressed that these indices were trained for an unselected cohort of patients, rather than a *TP53*-stratified cohort.[6,7]

Mutations of *NOTCH1* and *NOTCH2* have previously been reported to have prognostic impact.[14,15] In our study, *NOTCH1* mutations (n = 7) were associated with a poor outcome in univariate analyses, but lost prognostic value in multivariate analyses, as they were strongly associated with *TP53* mutations (5 of 7). We did not find any prognostic impact of *NOTCH2* mutations.

For mutation detection, we used a targeted next-generation sequencing panel based on the most commonly mutated MCL genes according to previous whole-genome/exome sequencing studies.[10,14] We found a similar distribution of mutations,[10,14,28,29] but the frequencies observed in our study were generally lower. This difference most likely reflects differences in study cohorts, sample origin, processing of material, and method of sequencing. Our patient cohort consisted of fit younger, untreated patients with MCL, whereas other studies included both diagnostic and relapse samples from patients of various age. We consistently investigated BM samples, whereas other studies investigated DNA from a mixture of blood, lymph nodes, BM, and other sources. Because of the high coverage (>2900×) of our targeted approach, we decided to include patients regardless of known BM involvement from morphological assessment; however, we still detected significantly fewer mutations in patients with no morphological BM involvement, suggesting we miss identification of some mutated cases in patients with no or very little BM involvement of MCL.

TP53 aberrations have been widely examined in hematologic cancer and showed negative prognostic value in most.[30] In chronic lymphocytic leukemia, *TP53* aberrations (mutations and deletions) have consistently been shown to confer poor prognosis and treatment resistance, and recently, the novel small molecule inhibitors, ibrutinib,

venetoclax, and idelalisib, have been approved for first-line treatment in *TP53*-disrupted chronic lymphocytic leukemia, as they seem to partly overcome this deleterious effect.[31-33] Interestingly, ibrutinib and venetoclax have shown high response rates in relapsed MCLs,[34,35] and hence, it is tempting to speculate whether a similar approach could be applied in MCL. At this time, novel trials, including these targeted drugs, are appearing in both the frontline and relapse setting, and it will be interesting to investigate the responses in light of the *TP53* status.[36] Our preliminary results from the Nordic relapse trial, MCL6 Philemon (ibrutinib, lenalidomide, and rituximab), indicate that the presence of a *TP53* mutations may have less effect on outcome with this nonchemotherapeutic regimen; however, longer follow-up is needed to substantiate this finding.[37] Interestingly, from our MCL2 and MCL3 cohort, 2 patients with *TP53* mutations seemed to respond to salvage therapy. Both progressed during induction treatment, and both received bortezomib- and cytarabine-containing regimens for their relapses, the combination of which has previously been shown to have a synergistic effect.[38] One patient remains alive in second remission after 6.5 years, and the other went on to allogeneic stem cell transplantation but died of complications after 4.5 years, still being in MCL remission.

In conclusion, we show that *TP53*-mutated MCL represents a phenotypically distinct and highly aggressive disease entity with poor or no response to the high-dose regimens including cytarabine, rituximab, and ASCT. Thus, we suggest stratification of patients with MCL according to *TP53* mutational status, and inclusion in separate clinical trials exploring novel targeted agents.

Acknowledgments

Authorship

Contribution: C.W.E., C.D., J.W.H., M.W., C.F., S.E., M.K.A., P.G., M.J., and K.G. conceived and designed the experiments; C.W.E., C.D., J.W.H., L.B.P., C.P.M.-A., S.H., and A.P. performed the experiments; C.W.E., C.D., J.W.H., P.B., P.G., and K.G. interpreted the data; A.K., R.R., C.N., C.H.G., and M.J. conducted the trials, handled patient material, and collected clinical data; C.W.E., C.D., M.J., and K.G. wrote the manuscript; and all authors critically reviewed the final version of the manuscript.

Conflict-of-interest disclosure: K.G. is on Celgene and Janssen advisory boards. M.J. received research funding from Janssen, Celgene, AbbVie, and Gilead. C.H.G. is a member of the advisory boards for Janssen, Celgene, and Novartis. A.K. has received research funding from Merck and Roche and is a member of the scientific advisory board for Nordic Nanovector. C.N. received research support from AbbVie; has been involved with consultancy with Roche, Gilead, AbbVie, and Jannsen; and has received travel grants from Roche, Novartis, Gilead, and AbbVie. The remaining authors declare no competing financial interests.

Correspondence: Kirsten Grønbæk, Department of Hematology/ The Epi-/Genome Laboratory, Rigshospitalet Department 3733, Bartholin Institute, Copenhagen Biocenter, Building 2, 3rd Floor, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark; e-mail: kirstengroenbaek@regionh.dk.

References

1. Herrmann A, Hoster E, Zwingers T, et al. Improvement of overall survival in advanced stage mantle cell lymphoma. *J Clin Oncol.* 2009;27(4): 511-518.

2. Abrahamsson A, Albertsson-Lindblad A, Brown PN, et al. Real world data on primary treatment for mantle cell lymphoma: a Nordic Lymphoma Group observational study. *Blood.* 2014;124(8): 1288-1295.

3. Geisler CH, Kolstad A, Laurell A, et al; Nordic Lymphoma Group. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group. *Blood.* 2008;112(7):2687-2693.

4. Hermine O, Hoster E, Walewski J, et al; European Mantle Cell Lymphoma Network. Addition of high-dose cytarabine to immunochemotherapy before autologous stem-cell transplantation in patients aged 65 years or younger with mantle cell lymphoma (MCL Younger): a randomised, open-label, phase 3 trial of the European Mantle Cell Lymphoma Network. *Lancet.* 2016;388(10044): 565-575.

5. Eskelund CW, Kolstad A, Jerkeman M, et al. 15-year follow-up of the Second Nordic Mantle Cell Lymphoma trial (MCL2): prolonged remissions without survival plateau. *Br J Haematol.* 2016; 175(3):410-418.

6. Hoster E, Klapper W, Hermine O, et al. Confirmation of the mantle-cell lymphoma International Prognostic Index in randomized trials of the European Mantle-Cell Lymphoma Network. *J Clin Oncol.* 2014;32(13):1338-1346.

7. Hoster E, Rosenwald A, Berger F, et al. Prognostic value of Ki-67 index, cytology, and growth pattern in mantle-cell lymphoma: results from randomized trials of the European Mantle Cell Lymphoma Network. *J Clin Oncol.* 2016; 34(12):1386-1394.

8. Kolstad A, Pedersen LB, Eskelund CW, et al; Nordic Lymphoma Group. Molecular monitoring after autologous stem cell transplantation and preemptive rituximab treatment of molecular relapse; results from the Nordic Mantle Cell Lymphoma Studies (MCL2 and MCL3) with median follow-up of 8.5 years. *Biol Blood Marrow Transplant.* 2017;23(3):428-435.

9. Beà S, Ribas M, Hernández JM, et al. Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants. *Blood.* 1999; 93(12):4365-4374.

10. Zhang J, Jima D, Moffitt AB, et al. The genomic landscape of mantle cell lymphoma is related to the epigenetically determined chromatin state of normal B cells. *Blood.* 2014;123(19):2988-2996.

11. Beà S, Salaverria I, Armengol L, et al. Uniparental disomies, homozygous deletions, amplifications, and target genes in mantle cell lymphoma revealed by integrative high-resolution whole-genome profiling. *Blood.* 2009;113(13): 3059-3069.

12. Rosenwald A, Wright G, Wiestner A, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. *Cancer Cell.* 2003;3(2):185-197.

13. Scott DW, Abrisqueta P, Wright GW, et al; Lymphoma/Leukemia Molecular Profiling Project. New molecular assay for the proliferation signature in mantle cell lymphoma applicable to formalin-fixed paraffin-embedded biopsies. *J Clin Oncol.* 2017;35(15):1668-1677.

14. Beà S, Valdés-Mas R, Navarro A, et al. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. *Proc Natl Acad Sci USA.* 2013;110(45):18250-18255.

15. Kridel R, Meissner B, Rogic S, et al. Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. *Blood.* 2012;119(9):1963-1971.

16. Nordström L, Sernbo S, Eden P, et al. SOX11 and TP53 add prognostic information to MIPI in a homogenously treated cohort of mantle cell lymphoma–a Nordic Lymphoma Group study. *Br J Haematol.* 2014;166(1):98-108.

17. Halldórsdóttir a M, Lundin a, Murray F, et al. Impact of TP53 mutation and 17p deletion in mantle cell lymphoma. *Leukemia*. 2011;25(12): 1904-1908.

18. Greiner TC, Dasgupta C, Ho VV, et al. Mutation and genomic deletion status of ataxia telangiectasia mutated (ATM) and p53 confer specific gene expression profiles in mantle cell lymphoma. *Proc Natl Acad Sci USA*. 2006;103(7): 2352-2357.

19. Fernàndez V, Salamero O, Espinet B, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. *Cancer Res*. 2010;70(4):1408-1418.

20. Rubio-Moscardo F, Climent J, Siebert R, et al. Mantle-cell lymphoma genotypes identified with CGH to BAC microarrays define a leukemic subgroup of disease and predict patient outcome. *Blood*. 2005;105(11):4445-4454.

21. Grønbaek K, de Nully Brown P, Møller MB, et al. Concurrent disruption of p16INK4a and the ARF-p53 pathway predicts poor prognosis in aggressive non-Hodgkin's lymphoma. *Leukemia*. 2000;14(10):1727-1735.

22. Delfau-Larue MH, Klapper W, Berger F, et al; European Mantle Cell Lymphoma Network. High-dose cytarabine does not overcome the adverse prognostic value of CDKN2A and TP53 deletions in mantle cell lymphoma. *Blood*. 2015;126(5): 604-611.

23. Kolstad A, Laurell A, Jerkeman M, et al; Nordic Lymphoma Group. Nordic MCL3 study: 90Y-ibritumomab-tiuxetan added to BEAM/C in non-CR patients before transplant in mantle cell lymphoma. *Blood*. 2014;123(19):2953-2959.

24. Andersen NS, Pedersen LB, Laurell A, et al. Pre-emptive treatment with rituximab of molecular relapse after autologous stem cell transplantation in mantle cell lymphoma. *J Clin Oncol*. 2009; 27(26):4365-4370.

25. Husby S, Ralfkiaer U, Garde C, et al. miR-18b overexpression identi fi es mantle cell lymphoma patients with poor outcome and improves the MIPI-B prognosticator. 2015;125(17):2669-2677.

26. Xu-Monette ZY, Wu L, Visco C, et al. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study. *Blood*. 2012;120(19):3986-3996.

27. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol*. 2010;2(1):a001008.

28. Meissner B, Kridel R, Lim RSEA, et al. The E3 ubiquitin ligase UBR5 is recurrently mutated in mantle cell lymphoma. *Blood*. 2013;121(16): 3161-3164.

29. Rahal R, Frick M, Romero R, et al. Pharmacological and genomic profiling identifies NF-κB-targeted treatment strategies for mantle cell lymphoma. *Nat Med*. 2014;20(1):87-92.

30. Tessoulin B, Eveillard M, Lok A, et al. p53 dysregulation in B-cell malignancies: More than a single gene in the pathway to hell. *Blood Rev*. 2017;31(4):251-259.

31. Woyach JA, Furman RR, Liu T-M, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med*. 2014; 370(24):2286-2294.

32. Byrd JC, Brown JR, O'Brien S, et al; RESONATE Investigators. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med*. 2014;371(3):213-223.

33. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med*. 2016;374(4):311-322.

34. Wang ML, Rule S, Martin P, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med*. 2013;369(6):507-516.

35. Davids MS, Roberts AW, Seymour JF, et al. Phase I first-in-human study of venetoclax in patients with relapsed or refractory non-hodgkin lymphoma. *J Clin Oncol*. 2017;35(8):826-833.

36. Dreyling M, Ferrero S; European Mantle Cell Lymphoma Network. The role of targeted treatment in mantle cell lymphoma: is transplant dead or alive? *Haematologica*. 2016;101(2): 104-114.

37. Jerkeman M, Hutchings M, Räty R, et al. Ibrutinib-lenalidomide-rituximab in patients with relapsed/refractory mantle cell lymphoma: first results from the nordic lymphoma group MCL6 (PHILEMON) phase II trial. [abstract] *Blood*. 2016;128(22). Abstract 148.

38. Weigert O, Weidmann E, Mueck R, et al. A novel regimen combining high dose cytarabine and bortezomib has activity in multiply relapsed and refractory mantle cell lymphoma – long-term results of a multicenter observation study. 2009;50(5):716-722.

TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy

Christian W. Eskelund, Christina Dahl, Jakob W. Hansen, Maj Westman, Arne Kolstad, Lone B. Pedersen, Carmen P. Montano-Almendras, Simon Husby, Catja Freiburghaus, Sara Ek, Anja Pedersen, Carsten Niemann, Riikka Räty, Peter Brown, Christian H. Geisler, Mette K. Andersen, Per Guldberg, Mats Jerkeman and Kirsten Grønbæk

Updated information and services can be found at:
http://www.bloodjournal.org/content/130/17/1903.full.html

Articles on similar topics can be found in the following Blood collections
Clinical Trials and Observations (4680 articles)
Free Research Articles (4806 articles)
Lymphoid Neoplasia (2679 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml