SOX11 and TP53 add prognostic information to MIPI in a homogenously treated cohort of mantle cell lymphoma – a Nordic Lymphoma Group study

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Summary

Mantle cell lymphoma (MCL) is an aggressive B cell lymphoma, where survival has been remarkably improved by use of protocols including high dose cytarabine, rituximab and autologous stem cell transplantation, such as the Nordic MCL2/3 protocols. In 2008, a MCL international prognostic index (MIPI) was created to enable stratification of the clinical diverse MCL patients into three risk groups. So far, use of the MIPI in clinical routine has been limited, as it has been shown that it inadequately separates low and intermediate risk group patients. To improve outcome and minimize treatment-related morbidity, additional parameters need to be evaluated to enable risk-adapted treatment selection. We have investigated the individual prognostic role of the MIPI and molecular markers including SOX11, TP53 (p53), MKI67 (Ki-67) and CCND1 (cyclin D1). Furthermore, we explored the possibility of creating an improved prognostic tool by combining the MIPI with information on molecular markers. SOX11 was shown to significantly add prognostic information to the MIPI, but in multivariate analysis TP53 was the only significant independent molecular marker. Based on these findings, we propose that TP53 and SOX11 should routinely be assessed and that a combined TP53/MIPI score may be used to guide treatment decisions.

Keywords: lymphoid malignancies, molecular diagnostics, prognostic factors.

Mantle cell lymphoma (MCL) is an aggressive B cell lymphoma defined by cyclin D1 (CCND1) overexpression (Oka et al, 1994) and, until recently, a short median survival of 3–5 years (Weisenburger & Armitage, 1996; Herrmann et al, 2009). However, more recent treatment protocols, including high dose cytarabine, rituximab and autologous stem cell transplantation (ASCT), have achieved long-term remission in subgroups of patients (Geisler et al, 2008; Romaguera et al, 2010). A follow-up of the Nordic MCL2 trial showed a median survival of more than 10 years, a significant improvement in survival compared to previously used regimens (Geisler et al, 2012). However, to further improve

survival, prognostic markers are needed to identify patients with poorer outcome, to enable the delivery of alternative treatments for these patients.

The Mantle Cell Lymphoma International Prognostic Index (MIPI) has recently been designed to stratify MCL patients into risk groups (low, intermediate or high risk) based on clinical prognostic factors, such as age, performance, leucocyte count and lactate dehydrogenase (LDH) level (Hoster et al, 2008). MKI67 (Ki-67) expression may also be included in the MIPI to account for proliferation; this combined score is referred to as the biological MIPI (MIPI-B). The MIPI index is based on similar parameters to those used in the International Prognostic Index (IPI) and the follicular lymphoma IPI (FLIPI) (Hoster et al, 2008). However, MIPI predicted survival significantly better than the IPI, as shown in the Nordic MCL2 study of 158 patients treated with intensive immunochemotherapy followed by high-dose chemotherapy and ASCT (Geisler et al, 2010). The MIPI could clearly identify high-risk patients and separate those from intermediate and low risk patients, however patients with low and intermediate MIPI scores were poorly segregated (Geisler et al, 2010). In addition to MIPI, molecular markers, such as TP53 mutational status has been shown to have prognostic value (Louie et al, 1995; Bernard et al, 2001; Stefancikova et al, 2010; Nygren et al, 2012; Slotta-Huspenina et al, 2012). However, despite the correlation of TP53 mutational status and strong TP53 (p53) staining in immunohistochemistry (IHC) (Stefancikova et al, 2010), enabling routine analysis, TP53 status is today not used in clinical routine.

In addition to CCND1, we defined SOX11 as a diagnostic antigen in MCL (Ek et al, 2008), which was widely confirmed by others (Wang et al, 2008; Mozos et al, 2009; Fernandez et al, 2010; Royo et al, 2012; Salaverria et al, 2013), and recent studies emphasized the importance of SOX11 in identifying CCND1-negative MCLs and preventing suboptimal treatment (Salaverria et al, 2013). However, previous studies investigating the prognostic role of SOX11 have shown conflicting results (Wang et al, 2008; Fernandez et al, 2010; Navarro et al, 2012; Nygren et al, 2012). This might be explained by the lack of (i) international guidelines to separate MCL into treatment groups depending on clinical behavior (indolent versus classical MCL), (ii) use of heterogeneously-treated patients as a basis of SOX11 prognostic analysis and (iii) potential cross reactivity of the polyclonal reagents used (Nordstrom et al, 2012).

In this study, we used a novel, well characterized monoclonal SOX11 antibody (Nordstrom et al, 2012) to assess the clinical significance of SOX11 in the combined Nordic MCL2/3 cohort of patients. SOX11 was expressed in most patients (95%) at different levels and could be categorized into a dichotomized variable, where SOX11^{high} correlated to improved overall survival (OS) and event-free survival (EFS), in agreement with our previous experimental in vitro data (Gustavsson et al, 2010; Conrotto et al, 2011). With the aim

to use information on molecular markers, such as SOX11, TP53, MKI67 and CCND1 that routinely can be assessed using IHC, we optimized a combined molecular/MIPI score. SOX11 could be used to improve the MIPI but, in multivariate analysis, TP53 was the only independent molecular marker that was able to improve the prognostic value of MIPI. We thus propose that SOX11 and TP53 can be used to guide treatment decisions, preferably in combination with the well-established MIPI.

Methods

Patients, cohorts and treatment protocols

Material from patients included in the Nordic Lymphoma Group MCL2 and MCL3 trials, at hospitals in Sweden, Denmark, Norway or Finland was collected. In the Nordic MCL2 trial protocol, patients were treated with first-line intensive immunochemotherapy, followed by high-dose chemotherapy and ASCT (Geisler et al, 2010). The Nordic MCL3 protocol was identical, except for the addition of ibritumumab tiuxetan to patients in partial remission or complete remission unconfirmed (CRu) after induction chemotherapy. The inclusion criteria were (i) MCL stage II-IV, (ii) previously untreated, (iii) CCND1 positivity or presence of t(11;14) and (iv) age 18–65 years. Enrolled patients had a median age at diagnosis of 57 years (range 37–65 years). All tumours had been classified regarding histological subtype prior to tissue microarray (TMA) construction. The separate clinicopathological characteristics for the MCL2 and MCL3 cohorts have been described elsewhere (Geisler et al, 2008; Kolstad et al, 2012), and relevant data for the available patients from the combined cohort, hereafter referred to as the Nordic MCL2/3 cohort, is presented in Table I. Data for the full MCL2/3 cohort is shown in Table SI. The study was approved by the ethic committees in Sweden, Denmark, Norway and Finland.

Table I. Patient characteristics of the MCL2/3 cohort.

Parameter	n (%)
Male	83 (74)
Stage IV	95 (85)
MIPI^{low}	60 (54)
MIPI^{intermediate}	28 (25)
MIPI^{high}	23 (21)
Blastoid variant of MCL	26 (23)
Common variant of MCL	86 (77)
TP53^{weak}	78 (70)
TP53^{intermediate}	5 (4)
TP53^{strong}	10 (9)
TP53$^{not\ available}$	19 (17)
MKI67 (0–9%)	12 (11)
MKI67 (10–29%)	47 (42)
MKI67 (30–100%)	38 (34)
MKI67 (not available)	15 (13)

In total, 127 cases were available from the combined MCL2 ($n = 58$) and MCL3 ($n = 69$) trials.

TMA construction

TMAs were constructed according to a method previously described (Kononen *et al*, 1998). Representative tumour areas were chosen from haemotoxylin and eosin-stained sections from paraffin blocks and duplicate cores with a diameter of 1 mm tissue were transferred to a recipient block using an automated device (ATA-27; Beecher Instruments, Sun Prairie, WI, USA).

IHC and scoring

Immunohistochemistry was performed on 2-μm sections that were dried, deparaffinized, rehydrated and microwave-treated as previously described (Nordstrom *et al*, 2012). The sections were stained for SOX11 (SOX11-C1 developed in-house as previously described (Nordstrom *et al*, 2012), CCND1 (ab M3635; Dako, Glostrup, Denmark) or TP53 (sc-126; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). All samples were routinely processed and embedded in paraffin for tissue conservation. Antigen retrieval was performed using the PT-LINK system (Dako) at pH 9. All sections were analysed using a Nikon ECLIPSE 80i microscope (Nikon Instrument Inc., Melville, NY, USA) at a magnification of 20× (Plan Fluor 20× DIC M N2, Nikon) with a numerical aperture of 0·5. Images were captured using a Nikon DS-U2/L2 USB (Nikon) camera and NIS Elements BR 3·10 (Nikon) as acquisition software. For each antigen (CCND1, TP53 and SOX11), the fraction of positive nuclei was scored and samples divided into groups as follows; (i) negative, (ii) weak, (iii) intermediate (strong nuclear staining in <30% of cells) and (iv) strong (nuclear staining in ≥30% of cells) staining. For SOX11, negative, weak and intermediate cases are referred to as SOX11^{low}, while strong cases are referred to as SOX11^{high} when the dichotomized variable is used. Among the collected 127 cases, tissues from 120 patients were evaluable for SOX11 and CCND1 staining, while 93 cases were evaluable for TP53 staining. MKI67 scoring was available since previously (Geisler *et al*, 2010).

Statistical analysis

Among the collected 127 cases, clinicopathological information was available for 112 cases. OS was defined as time from study entry to death or last follow-up. EFS was defined as time from study entry to the date of last follow-up or failure resulting from any of the following events: death from any cause, nonresponse to induction treatment, lymphoma relapse or progression, any toxic event that prohibited treatment according to protocol, failure to harvest stem cells from peripheral blood or bone marrow, failure to engraft, or patient refusal to undergo ASCT, as previously described (Geisler *et al*, 2008). Both OS and EFS were estimated according to the Kaplan-Meier method and the log-rank test was used to compare survival between different groups. To compare clinicopathological and biological differences between SOX11^{low} and SOX11^{high} cases, χ^2-test and linear-by-linear association were used. Cox regression using both uni and multivariate models were used to investigate the significance of SOX11, TP53, MKI67, CCND1, morphology and MIPI in relation to OS and EFS. The statistical analysis was performed using either IBM SPSS STATISTICS Version 20 (IBM, Armonk, NY, USA) or MATLAB (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, MA, USA).

SOX11/MIPI and TP53/MIPI

Multivariate Cox regression on all of the MIPI components (age, white blood cell [WBC] count, LDH and Eastern Cooperative Oncology Group performance score [ECOG]) gave slightly different coefficients compared to original MIPI (see Table SII). However, to be able to compare with previous studies, MIPI was kept constant in further analyses.

The original MIPI was calculated for each patient (Hoster *et al*, 2008), in brief: MIPI = $[0{\cdot}03535 \times \text{age (years)}] + 0{\cdot}6978$ (if ECOG > 1) + $[1{\cdot}367 \times \log10(\text{LDH/ULN})] + [0{\cdot}9393 \times \log10(\text{WBC count})]$, where ULN is defined as the upper limit of mormal. The high, intermediate and low subgroups were defined according to standard guidelines (Hoster *et al*, 2008). A score <5·7 indicates low-risk, 5·7–6·2 indicates intermediate risk, and a score >6·2 indicates high risk disease. Similarly, the biological MIPI was calculated as: MIPI-B = $[0{\cdot}03535 \times \text{age (years)}] + 0{\cdot}6978$ (if ECOG > 1) + $[1{\cdot}367 \times \log10(\text{LDH/ULN})] + [0{\cdot}9393 \times \log10(\text{WBC count})] + [0{\cdot}02142 \times \text{MKI67}(\%)]$. The high, intermediate and low subgroups were defined according to standard guidelines (Hoster *et al*, 2008). A score <5·7 indicates low-risk, 5·7–6·5 indicates intermediate risk, and a score >6·5 indicates high risk disease. Using Cox regression (Cox, 1972), the statistical value of adding information on molecular markers, SOX11, TP53, MKI67 and CCND1, to the MIPI was assessed. The optimal regression coefficient (maximizing likelihood of observed events) for each significant molecular marker was also calculated. The combined indices were calculated using Cox-regression coefficients. Expressed in terms of hazard ratios (HRs), each parameter (X) was scaled against the MIPI, log HR(X)/log HR (MIPI).

Results

In this study, we have investigated SOX11 in relation to prognostic use and clinicopathological and biological parameters in the Nordic MCL2/3 cohort. We further explored the prognostic use of MIPI and the well-known molecular mark-
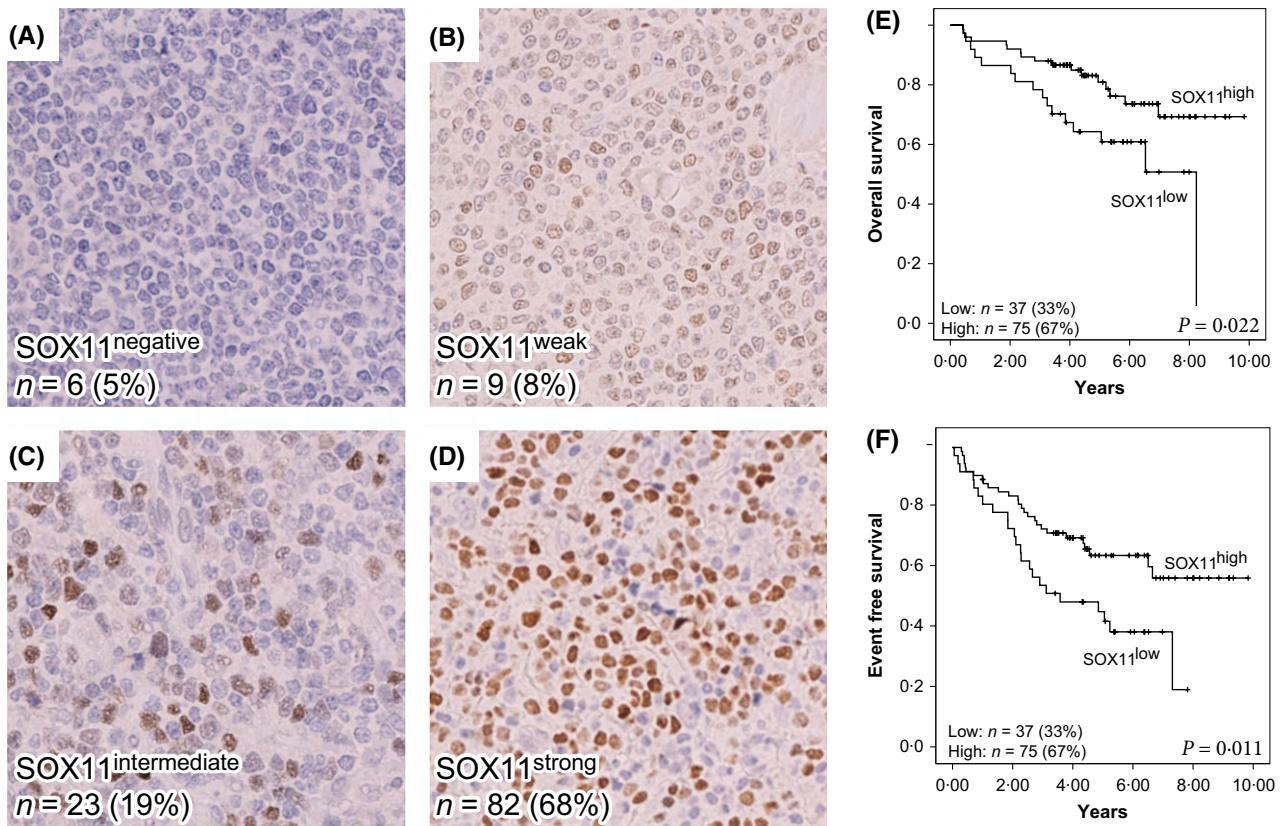
Fig 1. Representative immunostaining of SOX11 and correlation to overall survival and event-free survival. The immunohistochemistry panel shows representative figures of (A) negative SOX11 staining, detected in 5% of cases, (B) weak SOX11 staining, detected in 8% of cases, (C) intermediate SOX11 staining, detected in 19% of cases and (D) strong SOX11staining, detected in 68% of cases. Cases were grouped into a dichotomized variable depending on SOX11 expression. Strong cases are referred to as SOX11^{high} and negative, weak or intermediate cases are referred to as SOX11^{low}. Survival analysis using Kaplan-Meier's method comparing SOX11^{high} (D) *versus* SOX11^{low} (A-C) shows a positive correlation between SOX11 and (E) overall survival and (F) event-free survival with *P*-values of 0·022 and 0·001, respectively, as determined by the log rank test.

ers TP53, MKI67 and CCND1. The potential of SOX11, TP53, MKI67 and CCND1 to add prognostic value to the MIPI score was assessed and optimized with the aim of establishing a combined molecular marker/MIPI score as a tool for treatment decisions.

IHC analysis of SOX11, CCND1 and TP53

The expression of SOX11, CCND1 and TP53 was evaluated using IHC. Representative stainings and information on the frequency and number of cases for each marker are shown in Figs 1 and 2.

Among the evaluable 120 cases, 68% ($n = 82$) showed strong SOX11 staining in a large fraction of cells while 27% ($n = 32$) showed a weak or intermediate staining (refers to Groups 2 and 3). Only 5% ($n = 6$) of cases were SOX11-negative. Thus overall, 95% of cases were SOX11-positive, in accordance with previous studies (Ek *et al*, 2008; Wang *et al*, 2008; Mozos *et al*, 2009; Nygren *et al*, 2012). In all subsequent correlation analyses, a dichotomized variable combining SOX11 nuclear fraction and intensity was used. These two groups are referred to as SOX11^{high} (includes SOX11[strong] cases) and SOX11^{low} (includes SOX11[negative/weak/intermediate] cases). This optimal grouping of the SOX11 cases in relation to survival was assessed by Cox univariate analysis (see Table SIII).

In accordance with the Nordic MCL2/3 inclusion criteria, all cases were CCND1-positive. Among these, 60% and 34% of patients showed strong or intermediate CCND1 staining, respectively, and only 6% showed weak staining (Fig 2A–C). In non-selected cohorts, 6–15% of MCL were negative for CCND1 (Yatabe *et al*, 2000; Rosenwald *et al*, 2003). Out of the 127 cases in the cohort, 93 were evaluable for TP53 expression, of which 15% showed strong or intermediate TP53 staining (Fig 2E, F). Of major interest, all strong TP53 cases were found among the SOX11^{low} subgroup (Fig 1A–C, Table II), indicating that these may harbour an increased frequency of *TP53* mutations and potentially other genetic aberrations, as previously suggested (Stefancikova *et al*, 2010; Navarro *et al*, 2012).
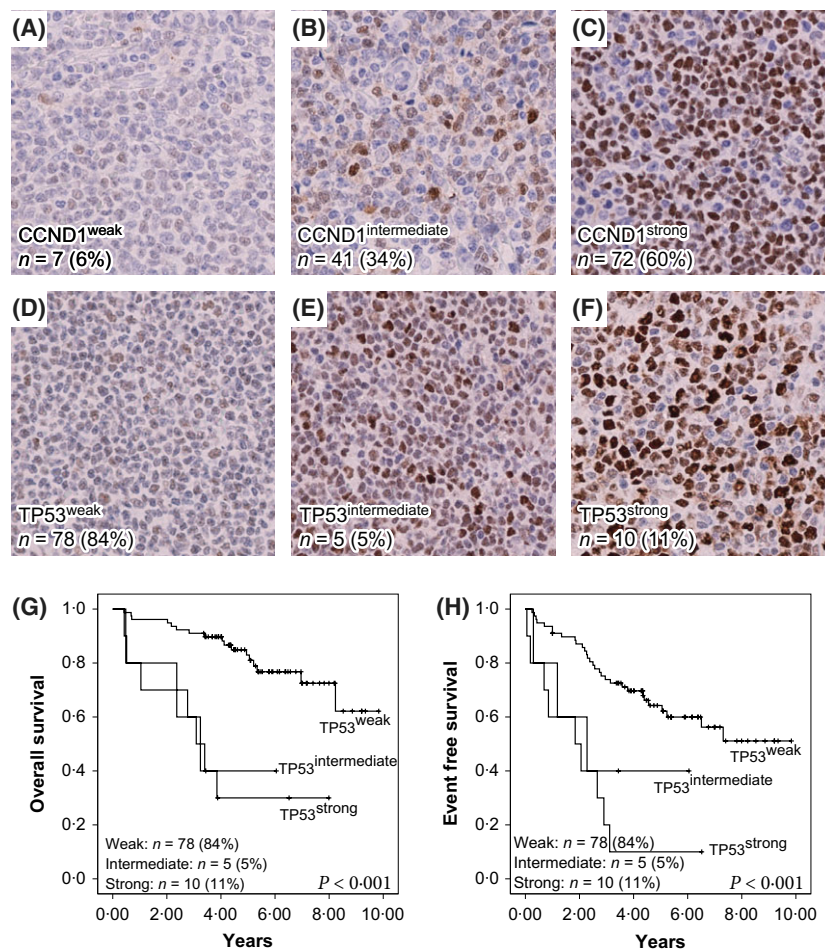
Fig 2. Representative immunostainings of CCND1 and TP53 and correlation of TP53 to overall survival and event-free survival. (A) Weak CCND1 staining was detected in 6% of cases, (B) intermediate CCND1 staining was detected in 34% of cases, and (C) strong CCND1 staining was detected in 60% of cases. (D) Weak TP53 staining was detected in 84% of cases, (E) intermediate TP53 staining was detected in 5% of cases and (F) strong TP53 was detected in 11% of cases. Survival analysis using Kaplan-Meier's method comparing p53^{weak} (F), p53^{intermediate} (E) and p53^{strong} (F) shows a negative correlation between TP53 and (G) overall survival and (H) event-free survival with *P*-values <0·001 in both cases, as determined by the log rank test.

Survival in relation to SOX11 and TP53 expression

The median OS and EFS for the cases used and available from the combined Nordic MCL2/3 cohort were 9·0 and 7·0 years, respectively. SOX11^{high} identified a large subgroup of patients (67%) with both favourable 5- and 10-year OS (81%, 69%) and EFS (64%, 56%). Similarly, TP53^{weak} identified a subgroup of patients (84%) with favourable 5- and 10-year OS (83%, 62%) and EFS (64%, 51%; Fig 2G, H).

Correlation between SOX11 and established clinicopathological and biological parameters

The dichotomized variable for SOX11 was also used to investigate the correlation between SOX11 and established clinicopathological and biological parameters (Table II). A positive correlation between SOX11 and CCND1 ($P = 0·006$) was seen, in contrast to previous data, where SOX11 expression was found to be independent of the t(11;14) translocation (Chen *et al*, 2010). Blastoid morphology ($P < 0·002$), TP53 ($P < 0·001$) and MKI67 ($P = 0·006$) showed a negative correlation to SOX11, indicating that SOX11^{high} may identify patients with lower proliferation, non-blastoid morphology and functional TP53.

Prognostic significance of SOX11, TP53, MKI67, CCND1, blastoid morphology and MIPI

To determine the prognostic significance of relevant molecular and clinicopathological parameters, Cox univariate analyses were performed. SOX11 expression positively correlated to OS and EFS ($P = 0·025$ and $0·013$; Table III). MIPI and TP53 correlated negatively to both OS (<0·001) and EFS

Table II. Clinical, pathological and biological features of the SOX11^{low} compared to SOX11^{high} subgroups in the MCL2/3 cohort.

Clinical and pathologic features	SOX11[low] n = 37 (32%)	SOX11[high] n = 77 (68%)	P-value*
Median age (years)	54 (41–65)	58 (37–65)	0·108
Age >60 years	10/37 (27%)	21/75 (28%)	0·914
Male sex	27/37 (73%)	56/75 (75%)	0·848
Blastoid morphology	15/37 (41%)	11/75 (15%)	**0·002**
CCND1[weak]	4/37 (11%)	3/75 (4%)	
CCND1[intermediate]	18/37 (49%)	21/75 (28%)	**0·006**
CCND1[strong]	15/37 (41%)	51/75 (68%)	
TP53[strong]	10/35 (29%)	0/58 (0%)	**≤0·001**
MIPI[low]	18/37 (49%)	42/74 (57%)	
MIPI[intermediate]	9/37 (24%)	19/74 (26%)	0·276
MIPI[high]	10/37 (27 %)	13/74 (17%)	
MKI67 (30–100%)	20/35 (57%)	18/62 (29%)	**0·006**

*Statistical significant P-values are shown in bold.

(<0·001), while histology and MKI67 showed negative correlation to OS (P = 0·001 and P = 0·02) but showed no significant correlation to EFS. CCND1 showed no significant correlation to either OS or EFS.

TP53 adds independent prognostic significance to the MIPI

As previously discussed, patients with low and intermediate MIPI are poorly separated based on survival in the Nordic MCL2/3 cohort (Fig 3A, B). It has previously been suggested that MKI67 may add prognostic value to MIPI (Hoster et al, 2008) but the MIPI-B also failed to separate the low and intermediate risk groups in this combined cohort (Fig 3C, D). The multimodality of the cohort was investigated by Gaussian Mixture Model analysis, optimizing maximum likelihood and evaluating with the Akaike Information Criterion (Akaike, 1974). This analysis confirmed that the cohort is bimodal in MIPI, with a transition point at 5·92 (where both risk groups are equally probable). However, to be able to compare the novel proposed indices with previous studies of MIPI, the low, intermediate and high risk groups, and the sizes thereof, were kept constant when visualizing the indices using Kaplan Meier.

To assess the ability of SOX11 to add information to MIPI, these were analysed together in a multivariate analysis where MIPI was used as a continuous variable. It was shown that SOX11 significantly improved the MIPI and that an optimal score should be calculated as MIPI-0·72[if SOX11^{high}] for OS and MIPI-0·92[if SOX11^{high}] for EFS respectively (see Table IV for HR values). When divided into the standard low, intermediate and high risk groups for visualization using Kaplan Meier, the adjusted scores were 3·94–5·23 for the low risk group, 5·27–5·77 for the intermediate risk group and 5·8–7·77 for the high risk group using the combined SOX11/MIPI, optimized for OS. Similarly, when optimized for EFS,

Table III. Cox univariate analysis of SOX11, TP53, MKI67, CCND1, blastoid morphology and MIPI in relation to overall and event-free survival in the MCL2/3 cohort.

	Patients (n)	HR	95% CI	P-value*
Overall survival				
Common morphology	86	1·0		
Blastoid morphology	26	3·2	1·6–6·5	**0·001**
CCND1[weak]	7	1·0		
CCND1[intermediate]	39	3·3	0·4–25·3	0·248
CCND1[strong]	66	2·5	0·3–18·8	0·373
MKI67 (continuous)	97	1·0	1·0–1·0	**0·02**
MIPI (continuous)	111	3·1	2·0–4·7	**<0·001**
TP53[weak]	78	1·0		
TP53[intermediate]	5	5·1	1·5–17·9	**0·010**
TP53[strong]	10	5·7	2·3–14·1	**<0·001**
SOX11[high]	75	1·0		
SOX11[low]	37	2·2	1·1–4·3	**0·025**
Event-free survival				
Common morphology	86	1·0		
Blastoid morphology	26	1·6	0·9–3·0	0·118
CCND1[weak]	7	1·0		
CCND1[intermediate]	39	2·0	0·4–8·4	0·370
CCND1[strong]	66	2·1	0·5–8·8	0·312
MKI67	97	1·0	1·0–1·0	0·086
MIPI (continuous)	111	2·3	1·5–3·5	**<0·001**
P53[weak]	78	1·0		
P53[intermediate]	5	2·6	0·8–8·4	0·123
P53[strong]	10	4·8	2·2–10·3	**<0·001**
SOX11[high]	75	1·0		
SOX11[low]	37	2·0	1·2–3·6	**0·013**

HR, hazard ratio; 95% CI, 95% confidence interval; MIPI, Mantle cell lymphoma International Prognostic Index.
*Statistical significant P-values are shown in bold.

the adjusted scores for the three risk groups were 3·73–5·13 for the low risk group, 5·17–5·75 for the intermediate risk group and 5·77–7·77 for the high risk group. Using the combined SOX11/MIPI, survival analysis showed the improved separation of low, intermediate and high risk groups for OS and EFS, although the separation of low and intermediate risk groups was still not statistically significant (see Table IV and Fig 3E, F). Cox regression was used to further evaluate the potential of SOX11 to add prognostic value to MIPI in relation to known molecular markers including TP53, MKI67 and/or CCND1 (see Methods). When using all these parameters in a multivariate analysis, only TP53 was able to independently add prognostic information to MIPI (see Table SIV). The optimal scaled factors were 1·47 and 1·65 for OS and EFS, respectively (see Table SIV and Table V). Thus, the TP53-adjusted MIPI was calculated as: MIPI + 1·47 [if p53[strong]] for OS and MIPI + 1·65 [if p53[strong]] for EFS. TP53 was also assessed together with the individual MIPI parameters, which slightly changed the indices for OS and EFS (see Table SV). The adjusted scores for the different risk groups were 4·66–5·71 for the low risk group, 5·73–6·67 for the intermediate risk group and 6·76–8·84 for the high risk
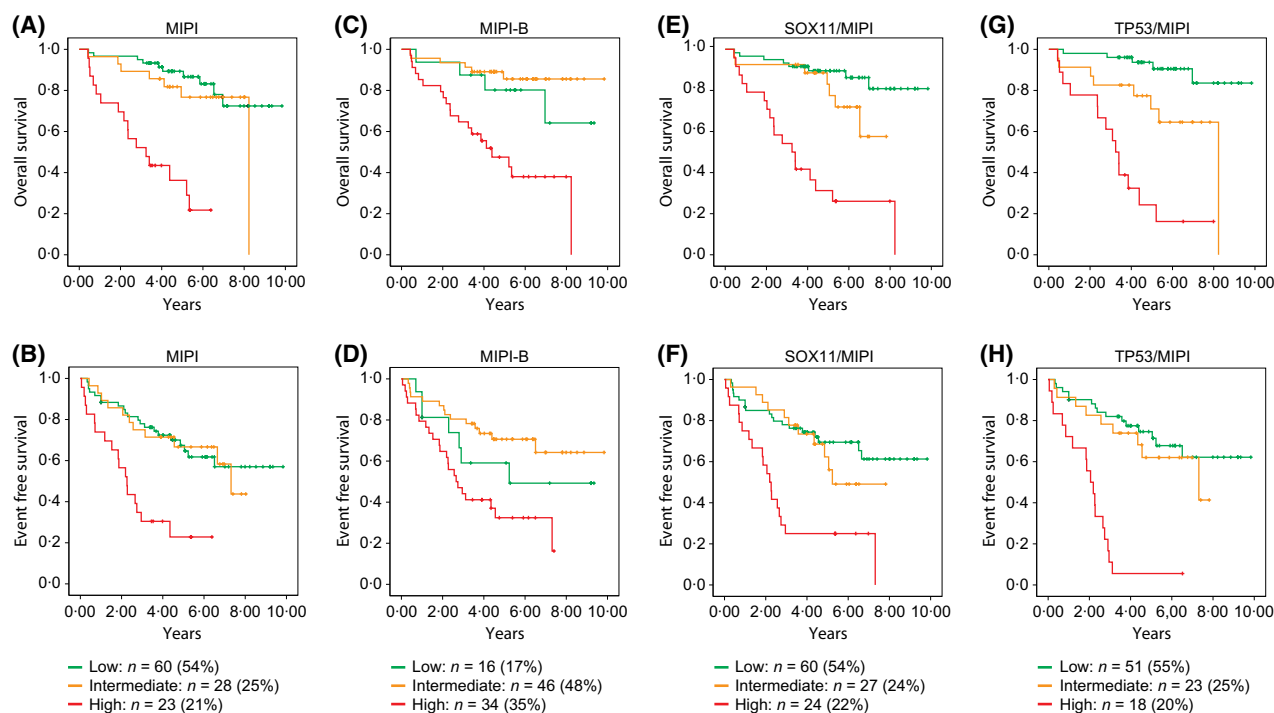
Fig 3. Kaplan-Meier's estimate of overall survival and event-free survival in relation to MIPI, MIPI-B, SOX11/MIPI and TP53/MIPI. (A) OS and (B) EFS analysis using the Kaplan-Meier method reveal a poor separation between MIPI^low and MIPI^intermediate groups. When applying the proposed biological MIPI (MIPI-B) both (C) OS and (D) EFS analysis show inverted low and intermediate survival curves. The combined SOX11/MIPI improves separation between low and intermediate risk groups (although, $P > 0.05$) in relation to (E) OS and (F) EFS. The combined TP53/MIPI show significant ($P = 0.006$) separation between low and intermediate risk groups in relation to (G) OS and identifies a high risk group with decreased (H) EFS. OS, overall survival; EFS, event-free survival; MIPI, Mantle cell lymphoma International Prognostic Index; MIPI-B, biological MIPI.

Table IV. Cox multivariate analysis of SOX11 and MIPI in relation to overall and event free survival in the MCL2/3 cohort.

	Patients (*n*)	HR	95% CI	*P*-value*
Overall survival				
SOX11^high	74	1·0		
SOX11^low	37	2·3	1·2–4·6	**0·017**
MIPI (continuous)	111	3·2	2·1–4·9	**<0·001**
Event-free survival				
SOX11^high	74	1·0		
SOX11^low	37	2·2	1·3–4·0	**0·005**
MIPI (continuous)	111	2·4	1·6–3·6	**<0·001**

HR, hazard ratio; 95% CI, 95% confidence interval; MIPI, Mantle cell lymphoma International Prognostic Index.
*Statistical significant *P*-values are shown in bold.

Table V. Cox multivariate analysis of TP53 and MIPI in relation to overall and event free survival in the MCL2/3 cohort.

	Patients (*n*)	HR	95% CI	*P*-value*
Overall survival				
TP53^weak/intermediate	82	1·0		
TP53^strong	10	6·4	2·6–16·1	**<0·001**
MIPI (continuous)	92	3·6	2·3–5·6	**<0·001**
Event-free survival				
TP53^weak/intermediate	82	1·0		
TP53^strong	10	6·1	2·8–13·4	**<0·001**
MIPI (continuous)	92	3·0	1·9–4·6	**<0·001**

HR, hazard ratio; 95% CI, 95% confidence interval; MIPI, Mantle cell lymphoma International Prognostic Index.
*Statistical significant *P*-values are shown in bold.

group when optimized in relation to OS. When optimized for EFS, the adjusted risk group scores were 4·66–5·71 for the low risk group, 5·73–6·67 for the intermediate risk group and 6·86–8·94 for the high risk group. Using the combined TP53/MIPI, survival analysis showed the improved separation of low and intermediate risk groups for OS ($P = 0.006$; see Table V and Fig 3G, H). Furthermore, the 5-year EFS for the combined TP53/MIPI identified a high risk group with lower EFS (6%) compared to MIPI (23%) and TP53 (10%)

as stand-alone biomarkers. Thus, by combining MIPI with information on TP53, improved prognostic information was achieved.

TP53 is not routinely assessed, and as TP53 data was missing for a number of cases, the multivariate analysis was also performed with only MIPI, SOX11, MKI67 and CCND1. In this analysis, SOX11 and MKI67 were the only molecular markers that independently added prognostic value to the MIPI in relation to EFS and OS, respectively (see Table SVI).

The optimal scaled factor was 0·012 (OS) for MKI67, which is similar to the previously established MIPI-B, calculated as MIPI + 0·021[MKI67%] (Hoster et al, 2008).

Discussion

The treatment of MCL is ever-changing and recent improvements of clinical protocols have had a pronounced effect on patient outcome (Geisler et al, 2008; Romaguera et al, 2010; Delarue et al, 2013). In younger patients (<65 years), the introduction of autologous stem cell transplantation, high dose cytarabine, and rituximab has clearly improved PFS and OS (Delarue et al, 2013). Even more recently, it was shown that an inhibitor of Bruton's tyrosine kinase (BTK), ibrutinib, induces a response rate of >70% in relapsed and refractory MCL as a single agent (Wang et al, 2013). Combinatory studies with ibrutinib are still lacking, but the initial results may indicate a shift from chemotherapy-based approach to therapies targeting the underlying biological mechanisms of disease in MCL.

Prognostic factors, such as the MIPI and proliferation rate, are part of the routine work-up for patients with MCL, but are still rarely used for treatment decisions, as recently discussed by the European MCL network (Dreyling et al, 2013). Even in recent studies of MCL, MIPI is not used to assess differences in response to treatment (Delarue et al, 2013). It is evident that molecular subtype, MIPI (Hoster et al, 2008) and other biological factors need to be tested as potential companion biomarkers to enable individualized treatment selection among the plethora of current treatment strategies. To be clinically useful, these markers need to be robust and easily scored in routine IHC analysis.

It is well established that aberrations of TP53 is associated with aggressive behavior (Louie et al, 1995), the blastoid subtype (Bernard et al, 2001) and high proliferation (Slotta-Huspenina et al, 2012). Recent studies have shown a prognostic value of mutational status of TP53 but also correlation to TP53 levels by IHC (Stefancikova et al, 2010), and thus able to be included in routine assessments. In a recent population-based series, this was used to show the prognostic value of TP53 IHC status (Nygren et al, 2012).

Another important biomarker in MCL is SOX11, which during recent years has been identified as a diagnostic (Ek et al, 2008; Wang et al, 2008; Mozos et al, 2009; Fernandez et al, 2010; Royo et al, 2012; Salaverria et al, 2013) and prognostic antigen (Wang et al, 2008; Fernandez et al, 2010; Navarro et al, 2012; Nygren et al, 2012). The prognostic significance of SOX11 has so far only been assessed in population-based cohorts, and thus the potential of using SOX11 as a tool for treatment selection has not been evaluable. In this study, we showed that SOX11 correlates with favourable survival among MCL patients treated according to the Nordic MCL2/3 protocols. However, the molecular mechanism of SOX11 in MCL is still not fully elucidated. It is very likely that SOX11 contributes to the tumour development of classical MCLs, as recently presented by Vegliante et al (2013) who showed that SOX11 regulated PAX5 expression and blocked terminal B-cell differentiation. However, in relation to treatment response among aggressive MCLs, a high level of SOX11 is beneficial as shown here, in agreement with our previous molecular studies (Gustavsson et al, 2010; Conrotto et al, 2011).

Previous studies correlating the absence or presence of nuclear SOX11 to survival in MCL have shown conflicting results. A positive correlation between SOX11 and improved survival was reported in two studies with cohorts of 53 and 186 MCL patients, respectively (Wang et al, 2008; Nygren et al, 2012). In contrast, absence of SOX11 correlated to better survival in two other series (Fernandez et al, 2010; Navarro et al, 2012). Of note, in the studies correlating SOX11 negativity to better survival, the SOX11-negative cases were classified as an indolent form of MCL characterized by more frequent non-nodal presentation, hypermutated IGHV and less genomic complexity, which represents a distinct clinical subgroup of MCL (Navarro et al, 2012; Royo et al, 2012). It can be argued that these cases might even be classified as a different disease, as their clinical course is very different from that of the classical, more aggressive MCL. No indolent cases were included in the present Nordic MCL2/3 cohort. In the study reported by Nygren et al (2012), in which a positive correlation between SOX11 staining and survival was seen, 69% of the SOX11-negative cases showed strong TP53 staining. It has been argued that these cases might harbour a TP53 mutation associated with a more rapid clinical evolution, compared to the SOX11-negative cases with wild type TP53 that has a stable disease and long survival (Navarro et al, 2012). Also in our study the TP53 strong cases were found within the SOX11^{low} subgroup, but future investigations of the TP53 mutational status need to be performed to verify this potential negative correlation to SOX11. We here show that TP53 status, as assessed by IHC, is associated with inferior survival. In addition to strong TP53 expression, an increased fraction of cases with blastoid morphology (Bernard et al, 2001) and high MKI67 was found within the SOX11^{low} subgroup, in agreement with a more aggressive clinical course for these patients.

It has previously been suggested that the proliferation index (fraction of MKI67-positive cells) may add prognostic value to MIPI, referred to as biological MIPI (MIPI-B) (Hoster et al, 2008). We here explored the potential of a range of molecular markers, including TP53, SOX11, MKI67 and/or CCND1, to add prognostic value to MIPI for patients treated with the Nordic MCL2/3 protocol. Although SOX11 significantly added prognostic value to MIPI, TP53 was the only molecular marker that remained significant in multivariate analysis. The combined TP53/MIPI was able to separate low and intermediate risk groups in relation to OS and identified a high risk group of patients, with poor EFS, in need of alternative treatment. When TP53 was omitted, SOX11

was the only independent molecular marker that could add prognostic value to MIPI in relation to EFS and may thus be used for patient stratification when data on TP53 is missing.

In summary, we have used the homogenously treated Nordic MCL2/3 cohort to demonstrate the prognostic significance of SOX11, using a novel monoclonal antibody. A quantitative assessment showed that OS and EFS were superior for SOX11^{high} compared to SOX11^{low} patients, and that a large group of patients (81%) with long-term response (5-year EFS) to the Nordic MCL2/3 protocol was identified. Of note, p53^{strong} cases were only found among the SOX11^{low} subgroup, indicating that these may constitute cases with more complex genetic aberrations, consistent with a shorter survival. In multivariate analysis, TP53 was the only molecular marker that significantly added prognostic value to the MIPI. Based on these findings, we propose that SOX11 and TP53 should routinely be assessed by IHC and that a combined TP53/MIPI score may be used for treatment decisions in MCL.

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Authorship and disclosures

LN developed the antibody, performed the statistical analyses and took active part in the writing of the manuscript. PE performed all Cox regression analysis in relation to the combined MIPI indexes. SS evaluated IHC stainings and wrote parts of the manuscript. KG, AK, RR, MLK, CG, ER, CS, AL, JD and ME were involved in the collection of material and clinicopathological parameters. MJ took active part in the design of the study, interpretation of the data and writing of the manuscript. SE was responsible for the design of the study, interpretation of data and writing of the manuscript. All authors read and approved the final manuscript. A patent has previously been filed on the diagnostic, prognostic and therapeutic use of SOX11 in B cell lymphomas.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table SI. Patient characteristics of the full MCL2/3 cohort compared to available and used cohort.

Table SII. Iterative Cox-regression of individual MIPI components where the least significant parameter is removed in each step until only statistical significant parameters remain.

Table SIII. Univariate analysis to determine most significant cut-off point for SOX11 in relation to survival.

Table SIV. Iterative Cox-regression including TP53, SOX11, MKI67 and CCND1 where the least significant parameter is removed in each step until only statistical significant parameters remain.

Table SV. Final parameters after Cox-regression of all individual MIPI components and TP53.

Table SVI. Iterative Cox-regression SOX11, MKI67 and CCND1 where the least significant parameter is removed in each step until only statistical significant parameters remain.

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