p53 is associated with high-risk and pinpoints *TP53* missense mutations in mantle cell lymphoma

Joana M. Rodrigues,[1] May Hassan,[1] Catja Freiburghaus,[1] Christian W. Eskelund,[2,3] Christian Geisler,[2] Riikka Räty,[4] Arne Kolstad,[5] Christer Sundström,[6] Ingrid Glimelius,[6] Kirsten Grønbæk,[2,3] Anna Kwiecinska,[7] Anna Porwit,[8] Mats Jerkeman[9] and Sara Ek[1]

[1]Department of Immunotechnology, Lund University, Lund, Sweden, [2]Department of Haematology, Rigshospitalet, [3]Biotech Research and Innovation Centre BRIC, and The Danish Stem Cell Center (Danstem) Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [4]Department of Haematology, Helsinki University Hospital, Helsinki, Finland, [5]Department of Oncology, Oslo University Hospital, Oslo, Norway, [6]Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, [7]Department of Pathology-Oncology, Karolinska Institute, Stockholm, [8]Department of Pathology, Lund University, and [9]Department of Oncology, Lund University, Lund, Sweden

Received 3 June 2020; accepted for publication 12 July 2020

Correspondence: Sara Ek, Department of Immunotechnology, Lund University, Medicon Village 406, Scheelevägen 8, 223 87 Lund, Sweden.
E-mail: sara.ek@immun.lth.se

Summary

Survival for patients diagnosed with mantle cell lymphoma (MCL) has improved drastically in recent years. However, patients carrying mutations in tumour protein p53 (*TP53*) do not benefit from modern chemotherapy-based treatments and have poor prognosis. Thus, there is a clinical need to identify missense mutations through routine analysis to enable patient stratification. Sequencing is not widely implemented in clinical practice for MCL, and immunohistochemistry (IHC) is a feasible alternative to identify high-risk patients. The aim of the present study was to investigate the accuracy of p53 as a tool to identify patients with *TP53* missense mutations and the prognostic impact of overexpression and mutations in a Swedish population-based cohort. In total, 317 cases were investigated using IHC and 255 cases were sequenced, enabling analysis of p53 and *TP53* status among 137 cases divided over the two-cohort investigated. The accuracy of predicting missense mutations from protein expression was 82%, with sensitivity at 82% and specificity at 100% in paired samples. We further show the impact of p53 expression and *TP53* mutations on survival (hazard ratio of 3·1 in univariate analysis for both), and the association to risk factors, such as high MCL International Prognostic Index, blastoid morphology and proliferation, in a population-based setting.

Keywords: immunohistochemistry, p53, *TP53*, mantle cell lymphoma, digital pathology, targeted sequencing.

Mantle cell lymphoma (MCL) is a rare and aggressive mature B-cell lymphoma with a historically median overall survival (OS) of 3–5 years.[1] Sub-groups of young and fit patients have benefitted from modern treatment such as the Nordic MCL2/3 protocol, which includes cytarabine, rituximab and consolidation with high-dose therapy and autologous stem-cell transplantation, and can achieve longer survival times.[2]

In contrast, high-risk patients identified by the MCL International Prognostic Index (MIPI), blastoid morphology or tumour protein p53 (*TP53*) mutational status still have a short survival. Patients with MCL with *TP53* mutated cells have a median OS of 1·8 compared to 12·7 years in patients with *TP53* wild-type (WT) MCL tumours.[3] Patients with mutated *TP53* tumours also have a shorter progression-free survival and higher incidence of relapse, with hazard ratios (HRs) of 6·8 and 6·9, respectively, in a multivariate analysis including MIPI and blastoid morphology.[3] Further, multiple studies have reported higher frequency of *TP53* mutations in

First published online 4 August 2020
doi: 10.1111/bjh.17023

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd. *British Journal of Haematology*, 2020, **191**, 796–805
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

groups of patients with blastoid morphology and highly proliferative tumours.[4–7] Mutations are also more frequent among relapsed (22%) compared to diagnostic (10%) patients, as shown in the PHILEMON clinical trial (ClinicalTrials.gov Identifier: NCT02460276).[8] This was also reinforced by the AIM clinical trial (ClinicalTrials.gov Identifier: NCT02471391), where half of the patients had *TP53* aberrations.[9]

It is known that deletions of *TP53* in MCL are less significant than mutations (HR 1·4 vs. 6·2, respectively),[3] and they lose significance in multivariate analysis, emphasising the relevance of combining *TP53* mutations with MIPI for risk stratification, rather than deletions.[10]

Despite association to other high-risk characteristics, *TP53* mutated MCLs cannot be identified based on morphology or proliferation,[8] supporting that assessment of *TP53* status needs to be used for improved risk stratification. Targeted sequencing is not available in most clinics and alternative methods are warranted. It is known that missense mutations may lead to accumulation of the protein in the cell,[11,12] which can be identified through routine immunohistochemistry (IHC).[13] Currently, p53 expression is being widely accepted as a marker for *TP53* missense mutation in other malignancies.[14–16]

Information on *TP53* status may guide treatment selection, as chemotherapy-free treatment, which is independent on functional DNA repair pathways, could provide an alternative for patients with *TP53* mutated malignancies. This was supported by a study where relapsed MCL patients treated with ibrutinib, lenalidomide and rituximab, responded equally to the treatment independent of their *TP53* mutational status.[8]

In the present study, we aimed to investigate the accuracy of p53 as a tool to identify patients with *TP53* missense mutations and investigate the prognostic impact of overexpression and mutations in a Swedish population-based cohort of patients. We show that p53 identifies MCL tumours with *TP53* missense mutations with high accuracy, and that sub-clones of p53-positive tumour cells can be identified when whole tissue sections are used. We further report on the prognostic impact of both *TP53* mutations and p53 overexpression, emphasising the importance in identifying these high-risk patients. We propose that risk stratification using routine IHC staining of p53 should be used to stratify patients with MCL to alternative treatment options.

Patients and methods

Patient characteristics, IHC protocols and quantification

In the present study, material from diagnostic MCL patients, part of the population-based cohorts BLISS (Biobank of Lymphomas in Southern Sweden) and F1 (Finnish MCL patients) and the Nordic MCL2 and MCL3 (N-MCL2/3) clinical trials,[17,18] were used (Fig 1 and Data S1; Patient characteristics).

Tissue microarrays (TMAs) were constructed as previously described.[19] Briefly, representative 1-mm tumour areas were transferred to a recipient block using an automated device (ATA-27; Beecher Instruments, Sun Prairie, WI, USA). The Benchmark Ultra Ventana platform (Roche, Basel, Switzerland) was used to stain TMAs with anti-p53, anti-cyclin D1, anti-SRY-box transcription factor (SOX11) and anti-Ki-67 (Table SI). Scanning (×40) was performed using a NanoZoomer 60 (Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan).

Two methods were used to quantify p53 expression: manual scoring based on routine clinical pathology procedures and digital software HALO® (Indica Labs, Albuquerque, NM, USA). Manual pathology review was performed by a trained pathologist and staining was considered when nuclei were dark brown, as this has been shown to correlate to the presence of mutations.[20] Faint staining was not considered. Cores with >30% positive nuclei were classified as p53-overexpression cases and the others as low p53-expressing cores. The cut-off was selected based on clinical routine at Lund University Hospital, and consistent with previous publications from the Nordic lymphoma group.[10]

For digital pathology scoring, the Cytonuclear module of HALO was used and the threshold for a positive cell was defined based on the criteria used during manual pathology review (Figure S1). Digital measurement allowed quantification using both continuous measurements of the percentage of positive cells, and dichotomisation at defined cut-offs (1% and 20%). The 20% cut-off was selected prior to performing survival analyses to identify similar number of cases as the manual pathology review. The 1% cut-off was selected to be a qualitative measurement of p53 expression in contrast to no expression.

For SOX11 and Cyclin D1 staining, digital scoring using the software HALO with the Cytonuclear module was performed.

The number of Ki-67-positive cells was manually accessed in areas with most cells positive for CD20. Ki-67 was analysed both as a continuous variable and dichotomised using a 30% positive cells cut-off, according to the clinical standard.[21]

Sequencing of TP53 *gene*

The DNA of formalin-fixed paraffin-embedded (FFPE) samples was extracted with High Pure FFPET DNA isolation kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer instructions. DNA quality was assessed by quantitative polymerase chain reaction and samples with less than six delta quantification cycles were sequenced.

The tumours from the BLISS cohorts ($n = 72$) were sequenced on a TWIST cancer panel covering the exon parts

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

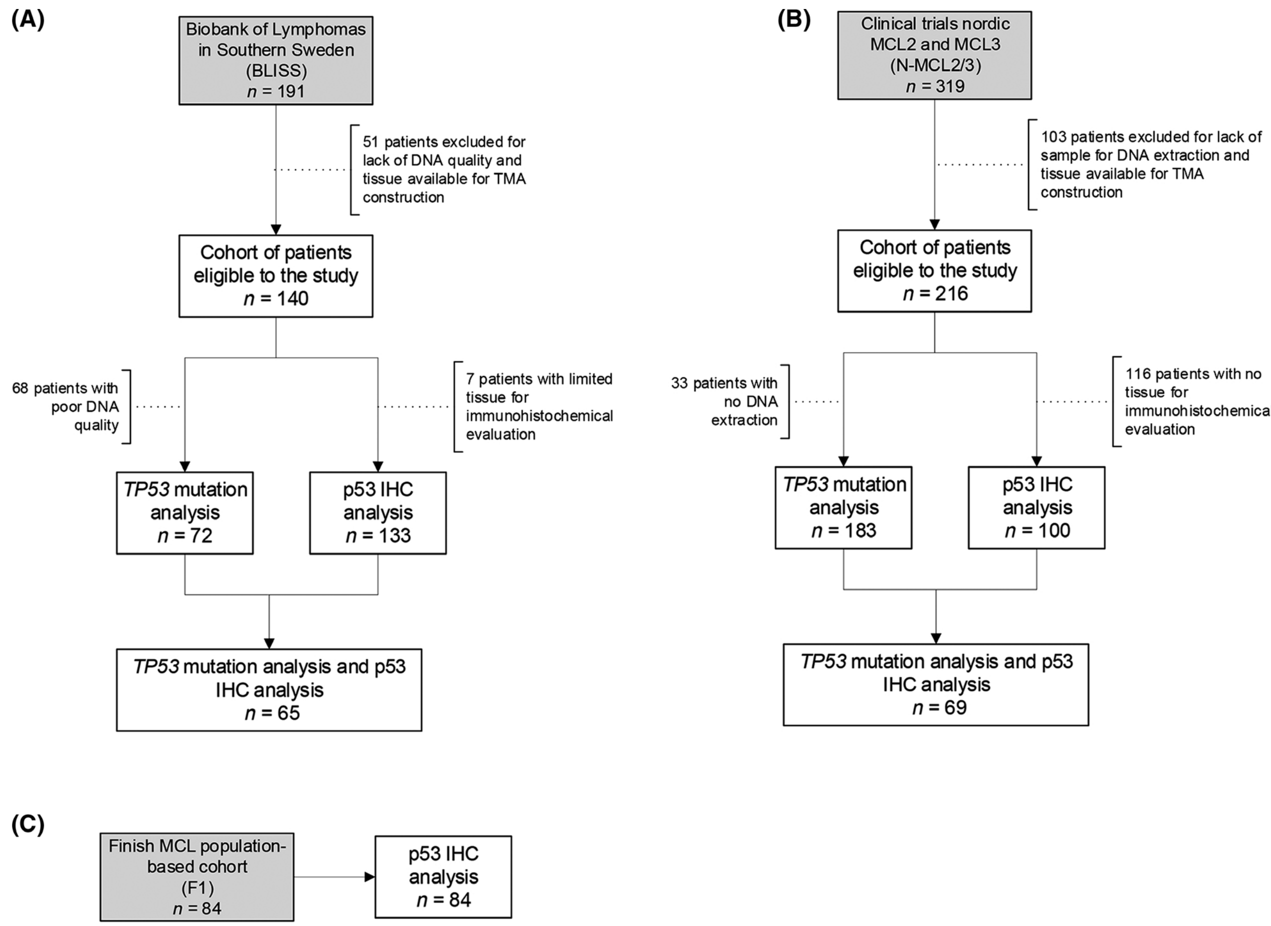

Fig 1. Overview of the clinical mantle cell lymphoma (MCL) material used in the present study. Three cohorts were used including (A) the population-based BLISS (Southern Sweden), (B) the Nordic MCL 2/3 trial cohort, and (C) F1 (Finnish cohort). IHC, immunohistochemistry, *n*, number; TMA, tissue microarray.

of ~200 genes with capture probes. Detailed information is found in Supplementary Information (Data S1; Sequencing of the *TP53* gene in a population-based material). *TP53* mutations were validated using the Integrative Genomic Viewer (IGV) software.[22]

In the present study, ion Torrent Next-Generation Sequencing Technology was carried out according to previous protocol to assess *TP53* mutations in eight additional samples to complement data from the N-MCL2/3 clinical trials.[3]

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS®) version 25.0 for Windows (IBM Corp., Armonk, NY, USA) and R version 3.6 (R Foundation for Statistical Computing, Vienna, Austria). Differences were considered statistically significant at $P < 0{\cdot}05$. For a detailed description, see Supplementary Information (Data S1; Statistical analysis).

Results

Patient characteristics and survival in the study material

Clinical information from the BLISS cohort was available for 140 patients and is shown in Table SII. Patients had a median (range) age at diagnosis of 71 (45–94) years, and a median OS of 4·1 years. The follow-up time using reversed censoring (Kaplan–Meier estimator) was 5·4 years.

Male patients comprised 76% of the cohort. In all, 14% of all tumours had Ki-67 ≥30% and 8% had blastoid/pleomorphic morphology. The Ki-67 ≥30% (HR 3·1, 95% CI 1·7–5·5; $P < 0{\cdot}001$) and blastoid/pleomorphic variants (HR 4·2, 95% CI 1·9–9; $P < 0{\cdot}001$) were associated with a worse outcome in univariate Cox analysis. The follow-up time using reversed censoring (Kaplan–Meier estimator) was 8·8 years.

Clinical information from the N-MCL2/3 cohort was available for 216 patients and is shown in Table SIII. Patients had a median (range) age at diagnosis of 57 (28–65) years

 © 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

and a median OS and time to progression (TTP) of 12·8 and 9·9 years, respectively. In all, 73% were male, 22% were MIPI high, 37% were high proliferative tumours, and 14% had blastoid/pleomorphic morphology. As reported by Eskelund *et al.*,[3] blastoid morphology (HR 2·3, 95% CI 1·4–3·9; $P = 0{\cdot}001$), high MIPI (HR 1·9, 95% CI 1·4–2·5; $P < 0{\cdot}001$) and Ki-67 ≥30% (HR 1·8, 95% CI 1·1–2·8; $P = 0{\cdot}02$) were associated with poorer outcomes in univariate Cox analysis. No clinical information was available from the Finnish MCL cohort, which was only used to assess the frequency of p53-positive cells.

Correlation between TP53 *mutational status and p53 overexpression by pathology review*

Among the 11 identified missense mutations in the BLISS cohort, nine were classified as p53 positive based on manual pathology review (Fig 2A). Thus, an accuracy of 82% was achieved with sensitivity at 82% (nine of 11) and specificity at 100% (61/61). In addition to the analyses on TMA, p53 staining was performed on whole tissue sections for five mutated cases with moderate-to-low p53 expression in the population-based cohort. A representative case where a positive subclone for p53 expression is shown can be seen in Figure S2.

To access the applicability in a different cohort, we used the previously published sequencing data from the N-MCL2/3 cohorts ($n = 183$). However, the same tissue was not available for IHC, and it was performed on separate patient material (tissue for IHC vs. mainly bone marrow for sequencing). Thus, we identified three cases with high p53 expression that lacked missense mutations. In this data set, an accuracy of 71% was achieved with sensitivity at 75% (six of eight) and specificity at 95% (58/61) using manual pathology review (Fig 2B). Of note, full tissue sections were not available from the N-MCL2/3 material and thus extended evaluation was not possible to perform.

Comparison of manual and digital scoring of p53 overexpression IHC as a tool to determine TP53 *missense mutations*

Correlation between digital pathology scoring (continuous parameter) and manual pathology review was strong ($R^2 = 0{\cdot}86$), independent of the cohorts. The individual values are presented in Table SIV. The regression coefficient was similar between cohorts (BLISS cohort: 0·029, N-MCL2/3 cohort: 0·023), allowing for comparison between scoring systems. Receiver operating characteristic (ROC) analysis based on p53 as a continuous variable from digital pathology scoring, showed an area under the curve (AUC) value of 0·96 for the BLISS cohort (Fig 3).

Four cases with *TP53* mutations that scored negative for p53 expression (two in N-MCL2/3 and two in BLISS) had <3% p53-positive cells. Three of them would be possible to identify if a cut-off of 1% of positive cells would be selected based on digital pathology scoring (see Table SVI). This cut-off would adjust the specificity to 79% and sensitivity to 100% for missense mutations.

TP53 *mutation frequency and correlation with survival*

Mutational analysis showed that 21% (15/72) of patients with MCL collected within the population-based cohort BLISS had a mutated *TP53* allele in the malignant cells (Table SII). Detailed information about the mutations and the clinical information of patients with mutated tumours can be found in Supplementary Information (Tables SV and SVI). Most aberrations 73% (11/15) were missense mutations (Table SII). Consistent with previous reports, most of the

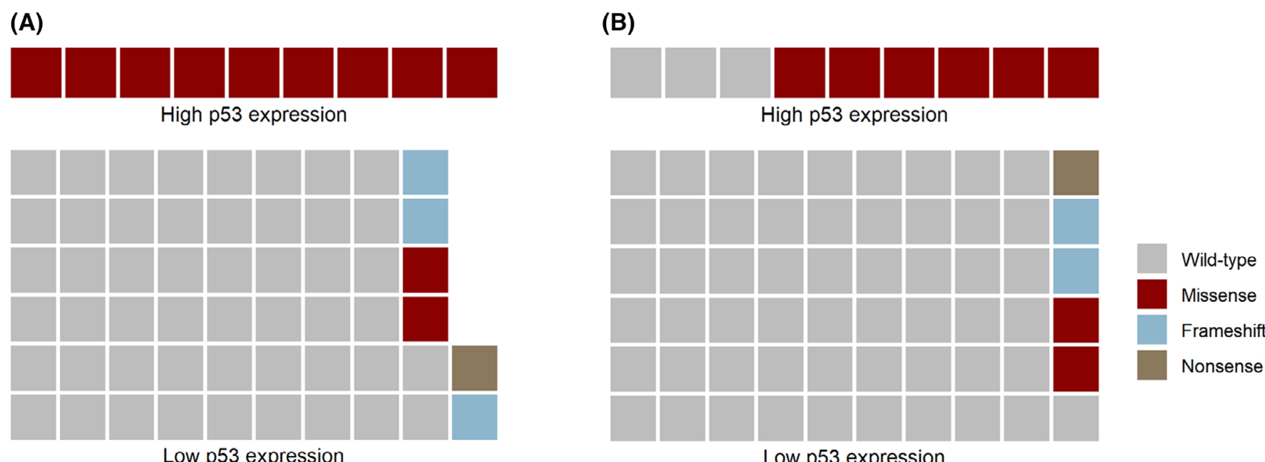

Fig 2. *TP53* mutational status and manual scoring of p53 expression. Each square represents a tumour for which both immunohistochemistry and sequencing data was available. Grey squares are identified as wild-type *TP53* cases, Red squares are missense mutations, blue squares match frameshift mutations and brown are classified as nonsense mutations. (A) BLISS cohort; (B) N-MCL2/3 clinical trials. [Colour figure can be viewed at wileyonlinelibrary.com]

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

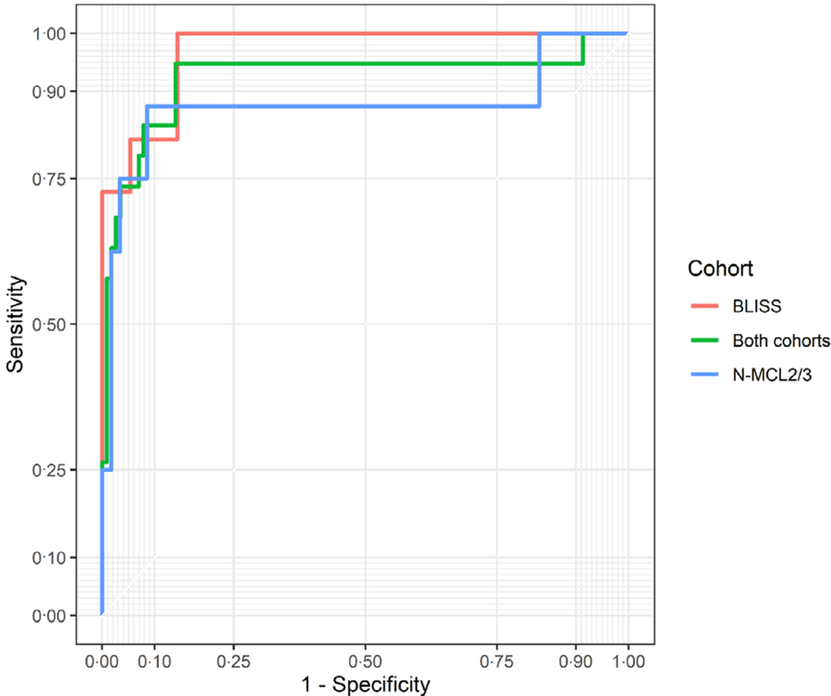

Fig 3. Receiver operating characteristic (ROC) analysis for N-MCL2/3 and/or BLISS cohorts. Area under the curve (AUC) was estimated using the digital pathology scoring as a continuous measurement of the percentage of positive cells in the different cohorts. AUC value for BLISS was 0·96; AUC value for N-MCL2/3 was 0·88, whereas for both cohorts combined, the AUC value was 0·92. [Colour figure can be viewed at wileyonlinelibrary.com]

mutations occurred in the DNA-binding domain of the gene.[23] R175H was the most frequent mutation, with three patients reported to have the specific alteration (Figure S3). The OS for patients with *TP53*-mutated MCL (n = 15) was 1·4 years (HR 3·1, 95% CI 1·5–6·4; $P < 0{\cdot}001$) compared to 6·2 years for unmutated MCL cases. Thus, 80% of the patients harbouring *TP53* mutations died in the first 5 years after diagnosis, as visualised using Kaplan–Meier curve and with log-rank statistics (Fig 4A).

Cases with missense mutations (n = 11) had no difference in outcome compared to cases with other truncations and frameshifts (n = 4), at 1·4 vs. 1·3 years (Table SII]). Due to the few non-missense mutations, no further analysis on this subgroup was conducted.

The increased number of samples sequenced for *TP53* in the N-MCL2/3 lead to similar results to those previously published.[3] The OS for patients with *TP53* WT MCL was 14 years, compared to 2·4 years (HR 8·8, 95% CI 5–15·3; $P < 0{\cdot}001$) for mutated MCL. Patients carrying *TP53*-mutated tumours also relapsed faster (HR 11·7, 95% CI 6·8–20·3; $P < 0{\cdot}001$) than patients with WT MCL tumours (Table SIII).

p53 overexpression and correlation to survival

The overall frequency of p53 protein overexpression was 13%, consistent with previous reports.[10,24] The variation among the individual cohorts was minor with 14% (18/133) in BLISS, 11% (11/100) among N-MCL2/3, and 15·5% (13/84) among Finnish MCL patients (F1). Clinical information for the BLISS patients with tumour p53 overexpression can be found in the Supplementary Information (Table SVI).

Patients in the population-based cohort (BLISS) with no/low p53 expression in the tumour cells had a median survival of 4·5 years (Table SII), whereas patients with high p53 expression based on manual pathology review had a median overall survival of 0·9 years (HR 3·1, 95% CI 1·7–5·7; $P < 0{\cdot}001$). The difference in survival between patients with high and low p53 expression is visualised in Fig 4B together with the log-rank statistics. The same difference was seen for the N-MCL2/3 cohort (Fig 5; Table SIII), although the median survival of p53 low cases is longer due to the difference in treatment and age and fitness of patients at diagnosis.

p53 as a continuous variable in all cohorts, assessed through digital pathology, was significantly associated with poor OS (HR 1·02, 95% CI 1·01–1·03, $P < 0{\cdot}001$ for OS in BLISS; HR 1·02, 95% CI 1·003–1·03, $P < 0{\cdot}05$ for OS in N-MCL2/3; and HR 1·02, 95% CI 1·01–1·03, $P < 0{\cdot}001$ for TTP in N-MCL2/3).

Correlation of TP53 *mutations and p53 expression with high-risk parameters*

It has previously been proposed that SOX11-negative cases may represent indolent MCL that gain *TP53* mutations and

 © 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

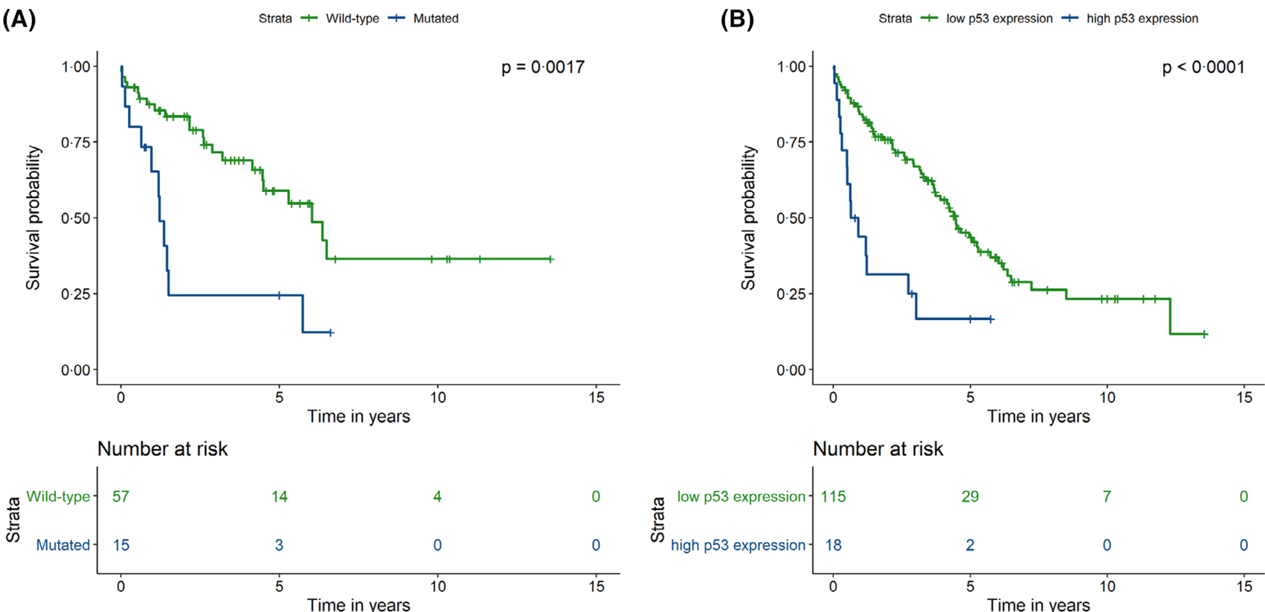

Fig 4. Prognostic impact (overall survival) of *TP53* mutations (n = 72) and p53 overexpression (n = 133) in BLISS. Samples were scored based on the targeted sequencing analysis and the Kaplan–Meier estimates were calculated and plotted for (A) mutations and (B) p53 overexpression based on manual pathology review. Log-rank statistics were used to assess the prognostic significance. [Colour figure can be viewed at wileyonlinelibrary.com]

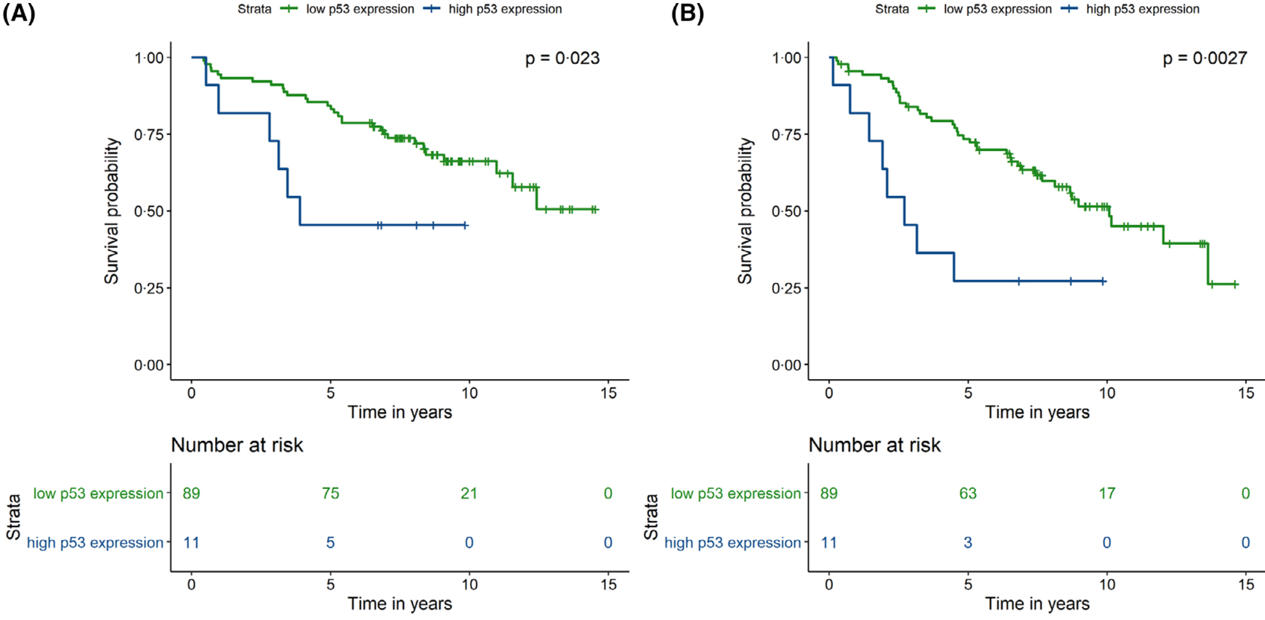

Fig 5. Prognostic impact of p53 overexpression (n = 100) in N-MCL2/3. Samples were scored based on manual pathology review and the Kaplan–Meier estimates were calculated and plotted for (A) overall survival and (B) time to progression. Log-rank statistics were used to assess the prognostic significance. [Colour figure can be viewed at wileyonlinelibrary.com]

undergo blastoid transformation.[25] Although these cohorts represent nodal, symptomatic MCL, we evaluated the associations between SOX11 and p53/*TP53* aberrations but found no significance (data not shown). Also, the association between age and p53/*TP53* aberrations was investigated but showed no significance (Figure S4).

Although non-classical morphology, Ki-67 and p53 were all associated with worse survival (data not shown), in multivariate analysis only p53 expression remained significant (HR 2·1, 95% CI 1·001–4·3; $P < 0·05$) in the BLISS cohort.

p53 overexpression was strongly associated with more aggressive variants of MCL, with higher p53 expression

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

observed in cases with high Ki-67 and non-classic variants ($P < 0{\cdot}05$) (Fig 6; Tables SII and SIII). Albeit the strong correlation, patients with high-risk variants do not exclusively display p53 abnormalities. Patient-specific information on treatment (when available), proliferation, mutational status, p53 expression and outcome is shown for the population-based cohort BLISS in Table SVI.

Discussion

The frequency of *TP53* aberrations in the population-based cohort was 21%, consistent with previous studies, where 7–31% of patients had aberrations in *TP53*.[3,26,27] *TP53* mutations conferred a threefold increased risk of death in the population-based material analysed. Most mutations were missense and occurred in the "hot spot" regions of the gene binding domain. Updated data from the N-MCL2/3 cohort showed a 8·8-times increased risk of death with *TP53* aberrations, similar to the original report.[3]

Although the impact of *TP53* mutations in MCL is well known,[3,28] p53 overexpression associated with missense mutations is less well studied, and little is known on the impact on outcome in MCL. In the present study, where material from both population-based and clinical trials were used (n = 317 patients), 13% of MCL expressed p53 at diagnosis, similar to previous studies.[10,24] It is widely accepted, although poorly understood, that mutations in *TP53* will confer a high stability to the p53 mutated protein, as opposed to WT p53, leading to accumulation of the protein in the cells.[11,29,30] In our present study, assessment of p53 expression correctly identified the majority of the missense mutations, with an AUC value of 0·96 for the population-based material.

The correlation between manual and digital scoring methods was high and the overall value was $R^2 = 0{\cdot}86$ (n = 317). In clinical routine, p53 is considered positive when >30% of the nuclei are strongly stained for p53. However, in some cases a focal expression of p53 can be found while the overall frequency is <30%. These cases may be considered negative by the pathologists, but a subclone that expresses p53 is reported. Subsequently, the predictive power of both techniques to assess missense mutations in *TP53* was high. Pathology review provided an accuracy at 82%, with a 100% specificity, and 82% sensitivity when whole tissue section could be used to further evaluate mutated cases that had <30% p53-positive cells based on TMA analysis. This emphasises the need to use full tissue sections for clinical evaluation and risk scoring. For the clinical trial cohort (N-MCL2/3), where different tissue was used for IHC and sequencing, accuracy reached 71%.

Digital pathology scoring provides the possibility to analyse p53 expression as a continuous parameter and allow qualitative assessment of p53 with a cut-off at 1% positive cells. With this methodology, 100% sensitivity was reached, but with an overall lower accuracy of 79%.

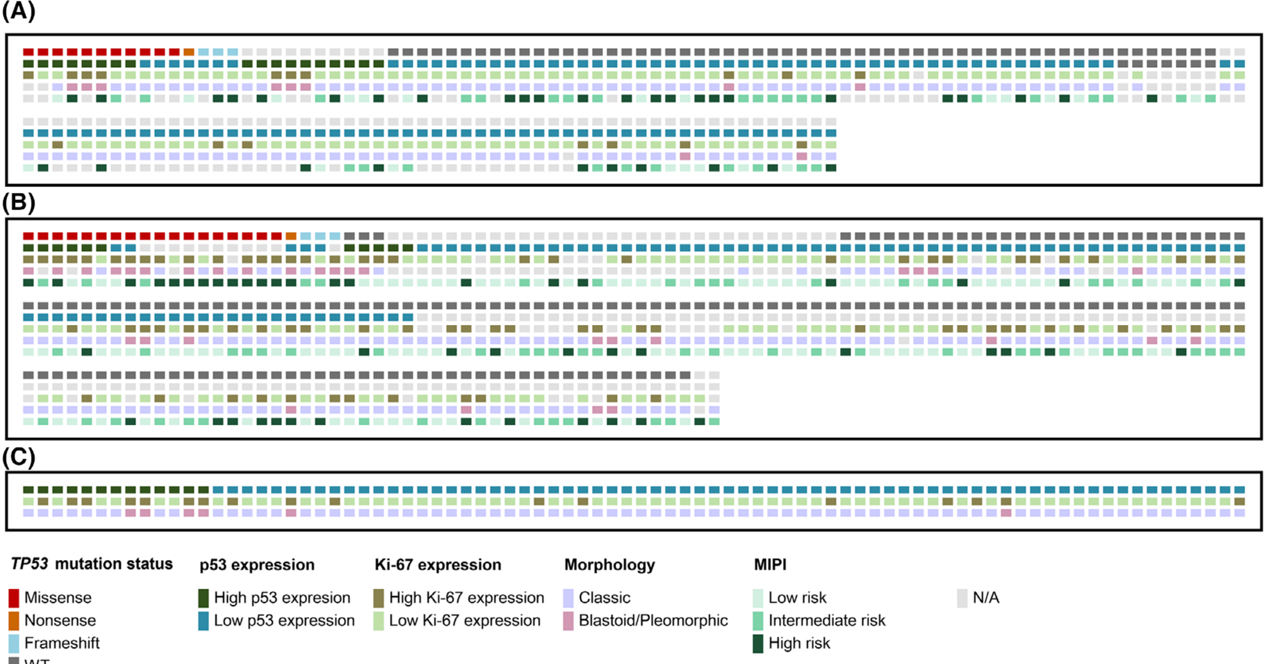

Fig 6. Correlation between p53 scoring, *TP53* mutation, morphology variant, Ki-67 and MIPI. (A) BLISS; (B) N-MCL2/3; (C) F1 cohort. Each column corresponds to one patient. High-risk variants are enriched in mutated and/or overexpressing cases, although, they are not exclusive of patients carrying mantle cell lymphoma tumours with aberrations on p53. [Colour figure can be viewed at wileyonlinelibrary.com]

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

It is known that patients with MCL are molecularly diverse,[31] with *TP53* aberrations being one of the strongest risk factors.[3] Thus, IHC analysis of p53 can serve multiple purposes. Stand-alone, it will identify patients with a shorter OS. It can also be used as a screening method to select patients for further genomic *TP53* analysis. Targeted sequencing in routine clinical practice is not yet implemented, and selection of patients constitutes an approach to reduce the practical and financial burden of a patient-wide screening using next-generation sequencing. Using p53 as a surrogate marker has already been suggested for other malignancies with a similar accuracy of ~90%.[14,32,33] IHC brings the advantage that cases with a mutated subclone can be visualised. Such mutated cases may be challenging to identify through sequencing due to low allele frequency. This makes IHC a relevant technique to screen larger areas and shape genomic analysis for more accurate study of mutations in cases with clearly heterogeneity and clones arising within the tumour.

Our present study does not come without limitations, including the low absolute number of p53-positive cases available for analysis and the fact that only missense mutations can be identified using p53 overexpression. By using this technique, we failed to identify two out of 11 missense mutations. Technical factors such as age of the paraffin block, fragmentation of DNA could possibly account for the difference in results, but also unknown biological factors that influence the degradation of the p53 protein.

p53 expression has previously been associated to blastoid morphology and Ki-67.[5,6,34] However, in the present study we use IHC information from a large cohort of 233 patients to associate p53 expression also to outcome. We show that there was a significant number of MCLs with *TP53* mutations and/or p53 expression among MCL with non-classical morphology ($P < 0{\cdot}05$) and high Ki-67 expression ($P < 0{\cdot}05$). In total, 52 patients had MCL with either *TP53* mutations or p53 expression, of these, 22 were non-classic and 27 highly proliferative. Thus, p53 is independently associated with survival, and only half of the patients carrying mutations shown other high-risk clinicopathological factors.[4,5,10]

TP53 mutations are a key independent prognostic factor for patients with MCL, with an OS related HR of 3 in our present population-based material. Due to the current lack of routine target sequencing for *TP53* mutations in the clinic, fast and reliable IHC alternatives are in high demand for improved patient stratification. Our present results show that p53 IHC analysis can be used as a surrogate marker for the detection of missense mutations. IHC analysis of p53 can benefit clinical workflows where sequencing capability is limited or not available and optimise treatment decision-making, as it identifies high-risk patients independent of treatment regimen, age and aggressive morphology variants to allow stratification of primary MCL patients using a combined MIPI according to previous suggestion.[35]

To date, although it is known that rituximab + high dose cytarabine is not sufficient for these patients, it remains to be determined what these patients should be offered. The recently published AIM trial[9] showed promising results for treating *TP53*-mutated patients, which included ibrutinib in combination with venetoclax. Allogenic stem-cell transplantation could, additionally, be an option for these patients with adverse outcomes.[36] Ongoing clinical trials with different combinations including ibrutinib and/or venetoclax will, hopefully, present more options for this challenging subgroup of patients.

Acknowledgements

We would like to thank Kristina Lövgren for her expert practical help with IHC. We would also like to acknowledge the collaboration with the Nordic lymphoma group, and specifically the Nordic MCL network.

Conflict of interest

None of the authors declare any conflict of interest.

Author contributions

Joana M. Rodrigues performed data and statistical analysis and wrote the manuscript. May Hassan performed part of the digital pathology scoring using the HALO software; Catja Freiburghaus was involved in the planning of the study and organisation of clinical information; Christian W. Eskelund performed the sequencing analysis of the MCL2/3 cohort; Christian Geisler, Riikka Räty and Arne Kolstad were involved in the collection of clinical information and nationally responsible for the MCL2 and MCL3 trials; Christer Sundström was involved in the pathology review; Ingrid Glimelius was involved in the collection of clinical information for the MCL2/3 follow-up; Kirsten Grønbæk was responsible for the sequencing of TP53 in the MCL2/3 cohort; Anna Kwiecinska and Anna Porwit performed the pathology review of the BLISS cohort; Mats Jerkeman was nationally responsible for the MCL2 and MCL3 trials, involved in the planning of the study and main responsible for the collection of the BLISS material; and Sara Ek was responsible for the planning of the study, analysis of the data and drafting the manuscript. All authors approved the final version of the manuscript.

Funding

The research leading to these results has received funding from the European Community's Horizon 2020 Framework Programme for Research and Innovation (EU-H2020-MSCA-COFUND-2016-754299), Cancerfonden 2016/465 and 2019/0309 and Fru Berta Kamprad FBKS-2018-7-(149).

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Material and methods.

Figure S1. p53 overexpression analysis in HALO®.

Figure S2. Whole tissue section for patient BLISS-4.

Figure S3. *TP53* mutations detected.

Figure S4. Frequency of *TP53* and p53 expression in relation to age.

Table SI. Specification of antibodies used.

Table SII. Patient characteristics for the BLISS population-based cohort.

Table SIII. Patient characteristics for the N-MCL2/3 clinical trial cohort.

Table SIV. p53 manual and digital scoring for N-MCL2/3, BLISS and F1, with *TP53* status information.

Table SV. Description of the identified *TP53* mutations in the BLISS cohort.

Table SVI. Selected clinicopathological data for p53 high and *TP53* mutated cases.

References

1. Abrahamsson A, Albertsson-Lindblad A, Brown PN, Baumgartner-Wennerholm S, Pedersen LM, D'Amore F, et al. Real world data on primary treatment for mantle cell lymphoma: a Nordic Lymphoma Group observational study. *Blood*. 2014;**124**:1288–95.
2. Eskelund CW, Kolstad A, Jerkeman M, Räty R, Laurell A, Eloranta S, 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**:410–8.
3. Eskelund CW, Dahl C, Hansen JW, Westman M, Kolstad A, Pedersen LB, et al. TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. *Blood*. 2017;**130**:1903–10.
4. Aukema SM, Hoster E, Rosenwald A, Canoni D, Delfau-Larue MH, Rymkiewicz G, et al. Expression of TP53 is associated with the outcome of MCL independent of MIPI and Ki-67 in trials of the European MCL Network. *Blood*. 2018;**131**:417–20.
5. Slotta-Huspenina J, Koch I, de Leval L, Keller G, Klier M, Bink K, et al. The impact of cyclin D1 mRNA isoforms, morphology and p53 in mantle cell lymphoma: P53 alterations and blastoid morphology are strong predictors of a high proliferation index. *Haematologica*. 2012;**97**:1422–30.
6. Solenthaler M, Matutes E, Brito-Babapulle V, Morilla R, Catovsky D. p53 and mdm2 in mantle cell lymphoma in leukemic phase. *Haematologica*. 2002;**87**:1141–50.
7. Stefancikova L, Moulis M, Fabian P, Ravcukova B, Vasova I, Muzik J, et al. Loss of the p53 tumor suppressor activity is associated with negative prognosis of mantle cell lymphoma. *Int J Oncol*. 2010;**36**:699–706.
8. Jerkeman M, Eskelund CW, Hutchings M, Räty R, Wader KF, Laurell A, et al. Ibrutinib, lenalidomide, and rituximab in relapsed or refractory mantle cell lymphoma (PHILEMON): a multicentre, open-label, single-arm, phase 2 trial. *Lancet Haematol*. 2018;**5**:e109–16.
9. Tam CS, Anderson MA, Pott C, Agarwal R, Handunnetti S, Hicks RJ, et al. Ibrutinib plus venetoclax for the treatment of mantle-cell lymphoma. *N Engl J Med*. 2018;**378**:1211–23.
10. Nordström L, Sernbo S, Eden P, Grønbæk K, Kolstad A, Räty R, 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**:98–108.
11. Blandino G, Di Agostino S. New therapeutic strategies to treat human cancers expressing mutant p53 proteins. *J Exp Clin Cancer Res*. 2018;**37** (1):1–13.
12. Hientz K, Mohr A, Bhakta-Guha D, Efferth T. The role of p53 in cancer drug resistance and targeted chemotherapy. *Oncotarget*. 2017;**8**:8921–46.
13. Oren M, Rotter V. Mutant p53 gain-of-function in cancer. *Cold Spring Harbor Perspect Biol*. 2010;**2**:a001107.
14. Cole AJ, Dwight T, Gill AJ, Dickson KA, Zhu Y, Clarkson A, et al. Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. *Sci Rep*. 2016;**18**:6.
15. Zenz T, Kreuz M, Fuge M, Klapper W, Horn H, Staiger AM, et al. *TP53* mutation and survival in aggressive B cell lymphoma. *Int J Cancer*. 2017;**141**:1381–8.
16. Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih IM, et al. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. *Mod Pathol*. 2011;**24**:1248–53.
17. Geisler CH, Kolstad A, Laurell A, Andersen NS, Pedersen LB, Jerkeman M, et al. 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**:2687–93.
18. Kolstad A, Laurell A, Jerkeman M, Grønbæk K, Elonen E, Räty R, et al. Nordic MCL3 study: 90Y-ibritumomab-tiuxetan added to BEAM/C in non-CR patients before transplant in mantle cell lymphoma. *Blood*. 2014;**123**:2953–9.
19. Kononen J, Bubendorf L, Kallionimeni A, Bärlund M, Schraml P, Leighton S, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med*. 1998;**4**:844–7.
20. Saft L, Karimi M, Ghaderi M, Matolcsy A, Mufti GJ, Kulasekararaj A, et al. p53 protein expression independently predicts outcome in patients with lower-risk myelodysplastic syndromes with del(5q). *Haematologica*. 2014;**99**:1041–9.
21. Croci GA, Hoster E, Beà S, Clot G, Enjuanes A, Scott DW, et al. Reproducibility of histologic prognostic parameters for mantle cell lymphoma: cytology, Ki67, p53 and SOX11. *Virchows Arch*. 2020;**477**:259–67.
22. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. *Nat Biotechnol*. 2011;**29**:24–6.
23. Baugh EH, Ke H, Levine AJ, Bonneau RA, Chan CS. Why are there hotspot mutations in the TP53 gene in human cancers? *Cell Death Differ*. 2018;**25**:154–60.
24. Izban KF, Alkan S, Singleton TP, Hsi ED. Multiparametric immunohistochemical analysis of the cell cycle proteins cyclin D1, Ki-67, p21(WAF1), p27(KIP1), and p53 in mantle cell lymphoma. *Arch Pathol Lab Med*. 2000;**124**:1457–62.
25. Royo C, Navarro A, Clot G, Salaverria I, Giné E, Jares P, et al. Non-nodal type of mantle cell lymphoma is a specific biological and clinical subgroup of the disease. *Leukemia*. 2012;**26**:1895–8.
26. Ahmed M, Zhang L, Nomie K, Lam L, Wang M. Gene mutations and actionable genetic lesions in mantle cell lymphoma. *Oncotarget*. 2016;**7**:58638–48.
27. Bea S, Valdes-Mas R, Navarro A, Salaverria I, Martin-Garcia D, Jares P, et al. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. *Proc Natl Acad Sci USA*. 2013;**110**:18250–5.
28. Ferrero S, Rossi D, Rinaldi A, Bruscaggin A, Spina V, Eskelund CW, et al. KMT2D mutations and TP53 disruptions are poor prognostic biomarkers in mantle cell lymphoma receiving high-dose therapy: a FIL study. *Haematologica*. 2019;**105**:1604–12.
29. Muller PAJ, Vousden KH. Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell*. 2014;**25**:304–17.
30. Mantovani F, Collavin L, Del Sal G. Mutant p53 as a guardian of the cancer cell. *Cell Death Differ*. 2019;**26**:199–212.
31. Mareckova A, Malcikova J, Tom N, Pal K, Radova L, Salek D, et al. ATM and TP53 mutations show mutual exclusivity but distinct clinical impact in mantle cell lymphoma patients. *Leuk Lymphoma*. 2019;**60**:1420–8.

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.

32. Guedes LB, Almutairi F, Haffner MC, Rajoria G, Liu Z, Klimek S, et al. Analytic, preanalytic, and clinical validation of p53 IHC for detection of TP53 missense mutation in prostate cancer. *Clin Cancer Res*. 2017;**23**:4693–703.
33. Köbel M, Piskorz AM, Lee S, Lui S, LePage C, Marass F, et al. Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma. *J Pathol Clin Res*. 2016;**2**:247–58.
34. Kimura Y, Sato K, Arakawa F, Karube K, Nomura Y, Shimizu K, et al. Mantle cell lymphoma shows three morphological evolutions of classical, intermediate, and aggressive forms, which occur in parallel with increased labeling index of cyclin D1 and Ki-67. *Cancer Sci*. 2010;**101**:806–14.
35. Liebers N, Dreger P, Dreyling M, Dietrich S. Risk stratification of mantle cell lymphoma (MCL). *Ann Lymphoma*. 2018;**2**:10.
36. Lin RJ, Ho C, Hilden PD, Barker JN, Giralt SA, Hamlin PA, et al. Allogeneic haematopoietic cell transplantation impacts on outcomes of mantle cell lymphoma with TP53 alterations. *Br J Haematol*. 2018;**184**: 1006–10.

© 2020 The Authors. British Journal of Haematology published by British Society for Haematology and John Wiley & Sons Ltd.