Pathology - Research and Practice 270 (2025) 155960

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Prognostic impact of combined loss of RB1, p53 and p21 in muscle-invasive bladder cancer

Benedikt Ebner [a,*], Lennert Eismann [a], Julian Hermans [a], Marc Kidess [a], Nikolaos Pyrgidis [a], Marie Semmler [a], Yannic Volz [a], Alexander Buchner [a], Michael Chaloupka [a], Marie-Lisa Eich [b], Philipp Weinhold [a], Christian G. Stief [a], David Horst [c], Gerald B. Schulz [a], Simon Schallenberg [b,c]

[a] Department of Urology, University Hospital, Ludwig-Maximilians-University Munich, Munich, Germany
[b] Institute of Pathology, University Hospital Cologne, Cologne, Germany
[c] Institute of Pathology, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany

ARTICLE INFO

Keywords:
Urothelial carcinoma
Muscle-invasive bladder cancer
MIBC
RB1
P53
P21
Prognostic markers

ABSTRACT

Introduction: Muscle-invasive bladder cancer (MIBC) represents a genetically heterogeneous disease with limited prognostic markers. This study aimed to validate the prognostic relevance of combined alterations in cell cycle regulators RB1, p53, and p21 in a broad cohort of MIBC patients undergoing radical cystectomy (RC).
Material and Methods: We analyzed formalin-fixed paraffin-embedded material from MIBC patients who underwent RC at the Department of Urology, University Hospital, Ludwig-Maximilians-University Munich. Tissue microarrays (TMAs) from 251 MIBC patients (pT2-pT4) were constructed, incorporating triplicate cores from tumor center and front. Immunohistochemical expression of RB1, p53, and p21 was assessed using a four-grade scoring system. Prognostic associations with overall survival (OS) and cancer-specific survival (CSS) were evaluated using multivariable Cox regression, Kaplan-Meier curves, and log-rank tests.
Results: We assessed 4518 stainings from 251 patients. Single marker analysis revealed no significant association between the loss of RB1, p53, or p21 and OS or CSS. However, the loss of two or three markers was significantly associated with worse OS (HR 3.49, 95 % CI 1.28–9.50; $p = 0.01$) and CSS (HR 11.2, 95 % CI 1.46–86.04; $p = 0.02$).
Conclusions: RB1, p53, and p21 are insufficient as single prognostic markers in MIBC but demonstrate significant prognostic relevance when analyzed in combination. These findings underscore the need for multi-marker approaches in prognostic modeling and personalized treatment strategies for MIBC.

1. Introduction

MIBC encompasses a genetically diverse group of tumors [1], characterized by substantial intratumoral heterogeneity of molecular subtypes in almost every fourth patient [2]. These tumors demonstrate varying degrees of chemosensitivity [3–5]. Despite advances in molecular subtyping and immunohistochemical analysis, prognostic stratification for MIBC still relies predominantly on TNM staging [6]. The utilization of immunohistochemical markers has been demonstrated to be both cost-effective and capable of significantly impacting the field of MIBC biology and the development of personalized treatment concepts. Many marker studies in bladder cancer patients often do not consider co-expression of other markers, have small sample sizes or lack external validation [7]. Consequently, the European Association of Urology concludes in their current guidelines on MIBC that there is insufficient evidence to use tumor mutational burden, molecular variants, immune- or other gene expression signatures for the management of patients with urothelial cancer [6].

In a recent large multicenter study, Wang et al. evaluated tumor samples from 576 patients with pT3 bladder cancer undergoing RC using immunohistochemical tissue microarray (TMA) analysis [8]. They analyzed 10 markers including three cell cycle regulators (retinoblastoma protein (RB1), tumor suppressor protein (p53) and cyclin-dependent kinase inhibitor 1 (p21)). The study concluded that there was no association between the status of a single marker and recurrence risk or overall survival (OS) [8]. However, it was reported

* Correspondence to: Department of Urology, University Hospital Munich, LMU, Marchioninistr. 15, Munich 81377, Germany.
E-mail address: Benedikt.Ebner@med.uni-muenchen.de (B. Ebner).

https://doi.org/10.1016/j.prp.2025.155960
Received 1 February 2025; Accepted 8 April 2025
Available online 8 April 2025

that the number of altered cell cycle regulators (RB1, p53 and p21) was associated with increased risk of cancer recurrence [8].

RB1, a tumor suppressor gene, is frequently inactivated in various malignancies, contributing to tumorigenesis [9]. The tumor suppressor protein p53 is widely regarded as the "guardian of the genome" and is frequently mutated and inactivated in malignant tumors [10]. One such mediator is p21, a tumor suppressor inhibiting cell cycle progression [11]. Irreversible defects or dysregulation of RB1, p53 or p21 tumor suppressor functions can therefore all lead to tumorigenesis or tumor progression in a variety of cancers [9,12,13].

A notable limitation of the study by Wang et al. was its exclusive focus on pT3 bladder cancer patients [8]. The present study aimed to validate and extend these findings across all MIBC stages (pT2-pT4), evaluating the prognostic significance of these markers for overall survival (OS) and cancer-specific survival (CSS).

2. Material and methods

2.1. Study design and patient cohort

We analyzed formalin-fixed, paraffin embedded material from 251 patients with muscle-invasive bladder cancer (MIBC) who underwent RC at the Department of Urology, Ludwig-Maximilians-University Munich between 2004 and 2014. The inclusion criteria were muscle-invasive disease, urothelial BC as dominant histology, sufficient histological material for six tissue cores, adequate tissue quality, and available clinical follow-up data. Patient follow-up was conducted by postal mails at 3 and 12 months after RC, then annually. The study was performed according to the ethical principles for medical research of the Declaration of Helsinki. Approval was obtained from the institutional ethics committee of the Medical Faculty of the Ludwig Maximilians-University Munich (20–179).

2.2. Tissue-microarrays and immunohistochemistry

Formalin-fixed and paraffin-embedded MIBC tissue was collected from the archives of the Institute of Pathology. The tumors were reviewed once again regarding tumor type, stage and grade by a pathologist with extensive experience in genitourinary pathology (DH). The same cohort was used before for several other analyses [2,14,15]. For tissue microarray analysis (TMA), triplicates of one millimeter tissue cores of the tumor front and tumor center from each tumor were punched out and embedded in empty recipient paraffin blocks. Lymphoid tissue served as an internal control. In a first step, the TMA blocks were cut into 4µm sections. Subsequently the sections were incubated in CC1 mild buffer (Ventana Medical Systems, Tucson, AZ) for 30 min at 100 °C or in protease 1 for 8 min. Afterwards, the sections were stained with anti-p21 antibody (SX118, Dako, 1:25), anti-p53 antibody (DO-7, Dako, 1:50), anti-RB1 antibody (84-B3–1, Novocastra, 1:100) for 60 min at room temperature, and visualized using the avidin-biotin complex method and DAB. For the immunohistochemical stainings the BenchMark XT immunostainer (Ventana Medical Systems, Tucson, AZ) was used. A detailed list of the antibodies can be found in the Supplementary Table 1. The counterstaining of the cell nuclei took place by incubating for 12 min with hematoxylin and bluing reagent (Ventana Medical Systems, Tucson, AZ). The stainings were analyzed using an Olympus BX50 microscope and Olympus BX46 (Olympus Europe). Histological images were required with the digital slide scanner PANNORAMIC 1000 (3DHISTECH).

2.3. Immunohistochemistry scoring

One pathologist (S.S.) evaluated all cases of immunohistochemical staining. In accordance to Wang et al. [8], each antigen was analyzed as intensity of the expression, using a 0–3 scoring system (0 = absent staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining), and distribution of expression in increments of 10 % (0–9). Markers with a continuous scale of staining were assessed by rating the intensity. Markers where tumor cells are either positive or negative, such as RB1, were evaluated based on the percentage of positive cells. In the case of a mixed staining behavior, the combination of both methods was used. For antigens, which were analyzed by intensity or distribution, these values represent the final tumor cell scores, whereby the distribution of the values 0–9 was converted to 0–0.9. For antigens, which were evaluated as both intensity score (0–3) and distribution score (0–9), the two scores were multiplied and subsequently divided by 10. A detailed description of the tumor cell scoring system can be found in Supplementary Table 2. In total, six samples were evaluated per patient (three cores each from tumor center and tumor front). Patients were excluded from further analysis if less than two samples contained tumor cells or if the number of negative and positive samples was identic. The remaining patients were classified as marker-negative or marker-positive depending on whether the majority of samples tested negative or positive, respectively. In order to assess intratumoral heterogeneity, the concordance of marker expression between tumor front and tumor center was evaluated. Patients were excluded from this analysis if less than two tissue cores of tumor front or tumor center contained tumor cells. The remaining patients were classified as concordant if at least two tissue cores from the tumor front and tumor center showed the same results; otherwise, they were classified a non-concordant.

2.4. Outcomes and statistical analysis

The primary outcome of the present study was the impact of RB1, p53 and p21 marker status on OS of MIBC patients undergoing RC. The secondary outcomes included (i) the impact of marker status on CSS and (ii) the association of marker status and clinicopathological characteristics.

Continuous variables were shown as median with interquartile range (IQR) and categorical variables were shown as proportions. A two tailed *t*-test for independent samples was performed for comparisons between continuous variables. Before every *t*-test, a Levene test was performed to assess equality of variance. A Fisher's exact test was used for comparisons between categorical variables. Kaplan-Meier curves and log-rank tests were used to compare OS and CSS of different marker statuses. The effect of the tumor marker status on OS and CSS was evaluated through univariate and multivariable Cox regression analysis adjusted for age, gender, T-stage, pathological lymph node status as well as perineural-, vascular- and lymphovascular invasion. Included independent variables were chosen based on clinical relevance. For all survival outcomes, we estimated hazard ratios (HR) with 95 % confidence intervals (CI), and a two-sided p-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinicopathological characteristics

The clinicopathological characteristics are shown in Table 1. In total, 251 MIBC patients undergoing RC were included. The median age was 68 years and 73 % of patients were male. Histopathologic analysis revealed that 21 % of the tumors were organ confined, while 79 % were not. Specifically, 21 % were classified as T2, 55 % as T3, and 24 % as T4 stage. Lymph node metastases were detected in 47 % of patients who underwent lymph node dissection. Perineural invasion, vascular invasion, and lymphovascular invasion were observed in 24 %, 16 %, and 35 % of cases, respectively.

3.2. Immunohistochemical RB1, p53 and p21 status

In total, 4518 tissue cores were evaluated (Fig. 1 and Fig. 2). The results for the evaluation of the marker status of RB1, p53 and p21 are

Table 1
Clinicopathological characteristics of the included muscle-invasive bladder cancer patients undergoing radical cystectomy ($n = 251$). Values are presented as median and interquartile range, or as n (%).

Characteristic	$n = 251$
Age (years)	68 (± 11)
Gender	
Male	182 (73 %)
Female	69 (27 %)
T-stage	
T2	52 (21 %)
T3	138 (55 %)
T4	61 (24 %)
Lymph node status	
N0	119 (53 %)
N1	107 (47 %)
Perineural invasion	
PNI 0	189 (76 %)
PNI 1	61 (24 %)
Vascular invasion	
V 0	212 (84 %)
V 1	39 (16 %)
Lymphovascular invasion	
LV 0	162 (65 %)
LVI 1	89 (35 %)

shown in Fig. 3. For RB1, 21 out of 239 patients (8.8 %) were scored as negative, while 218 (91 %) were evaluated as positive. For p53, 109 out of 238 patients (46 %) were assessed as negative, and 129 (54 %) were scored as positive. The majority of patients (99 %) were negative for p21, while only 2 patients (0.8 %) expressed the marker.

3.3. Intratumoral heterogeneity of RB1, p53 and p21

The intratumoral heterogeneity analysis demonstrated a high concordance of marker expression between tumor front and tumor center (Supplementary Table 3). Concordant expression of RB1 was observed in 185 of 190 cases (97 %), while heterogeneous expression was detected in five cases (3 %). With regard to p53 expression, 186 patients showed homogeneous expression in the tumor front and tumor center (96 %), while only eight patients (4 %) demonstrated heterogeneous expression. The analysis of p21 expression revealed that homogeneous expression was present in 174 cases (98 %), whereas heterogeneous expression was detected in 3 cases (2 %).

3.4. Association of marker expression and clinicopathological characteristics

The association of marker expression and clinicopathological characteristics is shown in Supplementary Table 4. For RB1, there was no significant difference between RB1 negative and positive patients concerning age, gender, T-stage, lymph node status, or perineural-,

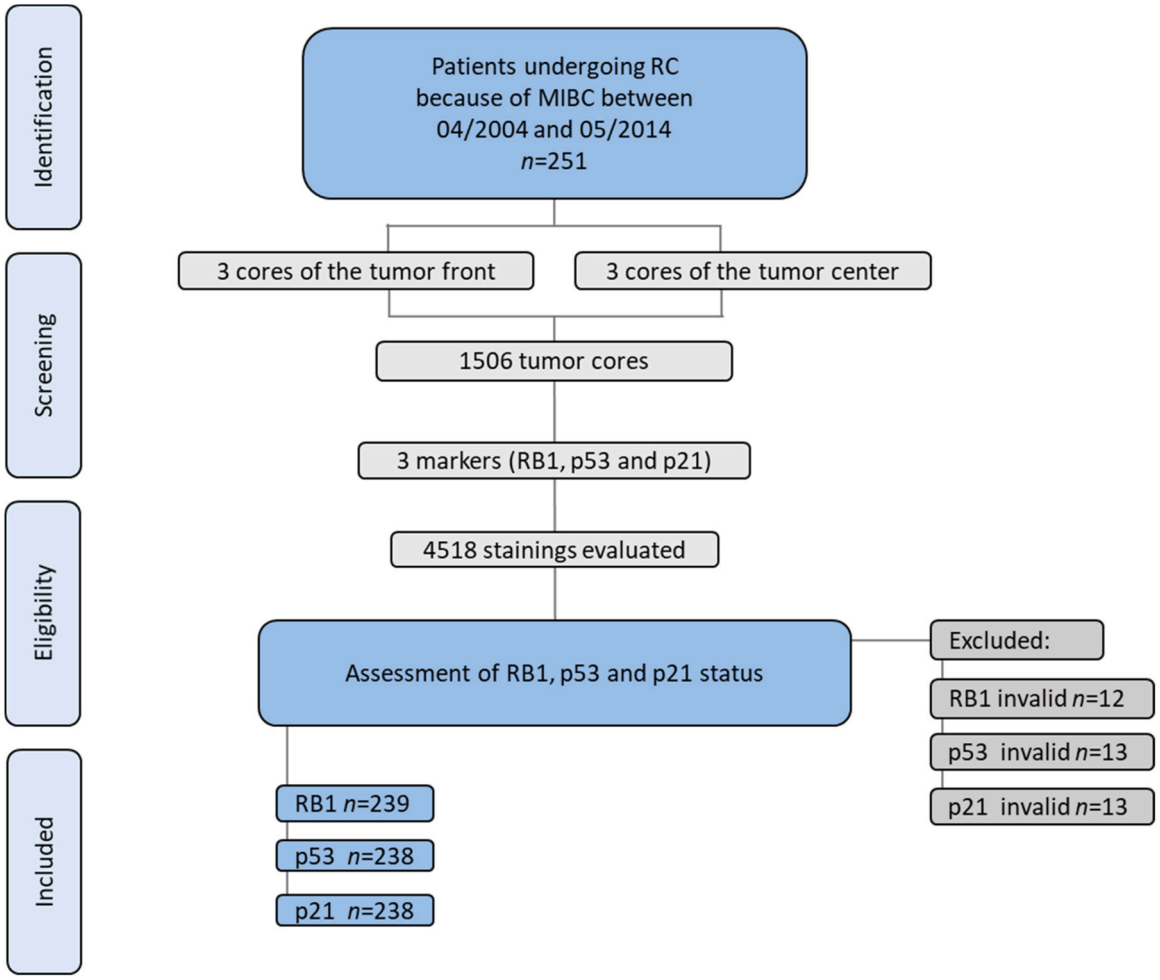

Fig. 1. Study flowchart of the patient selection process. RC: radical cystectomy; MIBC: muscle-invasive bladder cancer, RB1: retinoblastoma protein 1, p53: tumor suppressor protein 53, p21: cyclin-dependent kinase inhibitor 1. Patients were excluded, if tumor was found in less than 2 samples in the tissue microarray analysis (TMA), or if the number of negative and positive TMA samples was identic.

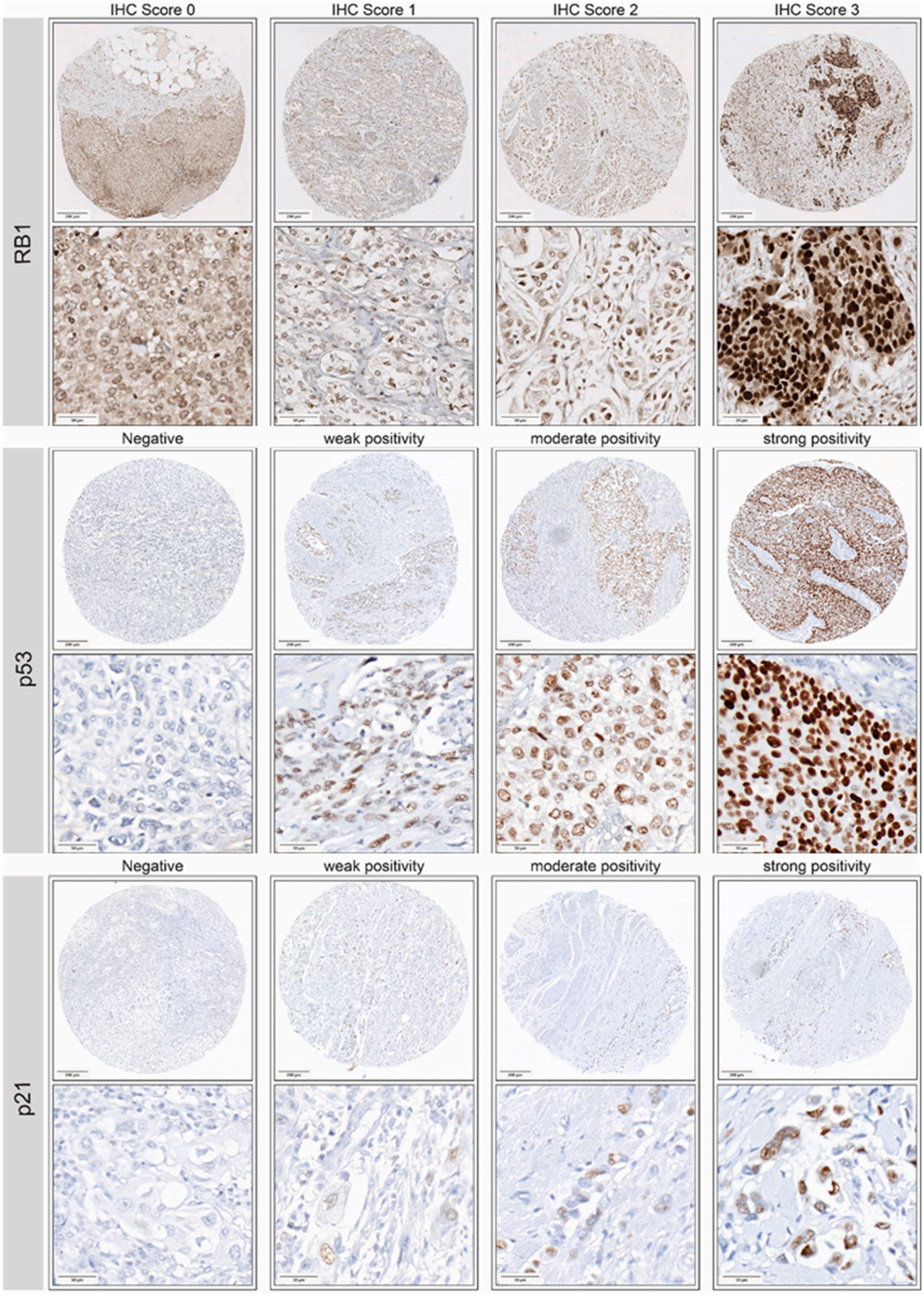

Fig. 2. Rating of retinoblastoma protein 1 (RB1), tumor suppressor protein 53 (p53) and cyclin-dependent kinase inhibitor 1 (p21) marker positivity based on immunohistochemical analysis of tissue microarrays. IHC Score 0: absent staining, IHC Score 1: weak staining, IHC Score 2: moderate staining, IHC Score 3: strong staining. Scale bars: 200 μm (overview) and 50 μm (details).

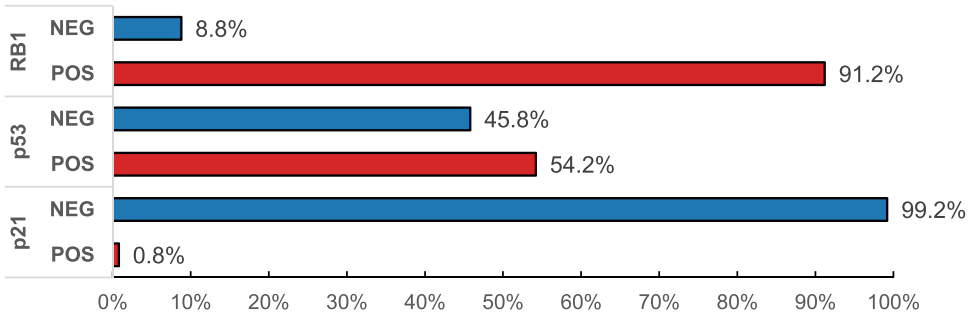

Fig. 3. Marker status of retinoblastoma protein 1 (RB1), tumor suppressor protein 53 (p53) and cyclin-dependent kinase inhibitor 1 (p21) after assessment of 6 tumor samples per patient (3 of the tumor front and 3 of the tumor center). The number given represents the percentage of patients with negative or positive marker status.

vascular- or lymphovascular invasion. A higher proportion of patients with pathologically confirmed lymph node metastases was observed in p53 negative patients compared to p53 positive patients (55 % vs. 40 %, $p = 0.03$). However, no statistically significant differences were observed in any of the other investigated parameters and p53 status. Given that only two of 238 patients (0.8 %) were rated as p21 positive, a statistical comparison of clinicopathologic characteristics between positive and negative patients was not feasible.

3.5. Association of marker expression and survival

We performed univariate and multivariable Cox regression analyses, adjusting for RB1, p53, and p21 expression status, as well as age, gender, local tumor stage, lymph node status, perineural invasion, vascular invasion, and lymphovascular invasion, to evaluate overall survival (OS) and cancer-specific survival (CSS). The results for OS are shown in Table 2 and for CSS in Table 3. In multivariable analysis, age over 68 years, T4 local tumor stage and vascular invasion were associated with worse OS. T4 local tumor stage and vascular invasion were also associated with worse CSS. However, in a single marker analysis, the expression of RB1, p53, or p21 did not correlate with OS or CSS.

RB1, p53, and p21 all possess tumor suppressive properties. Following the analysis of each marker individually, we evaluated whether the loss of two or three markers correlates with worse OS or CSS. Among the 233 patients for whom an analysis of all three markers was possible, 48 % (112 patients) were negative for zero or one marker, while 52 % (121 patients) were negative for two or three markers. Patients with a loss of two or three markers had a significantly worse OS (HR 3.49, 95 % CI 1.28–9.50, $p = 0.01$) and CSS (HR 11.2, 95 % CI 1.46–86.04, $p = 0.02$) in multivariable analysis compared to patients with a loss of zero or one marker.

The Kaplan-Meier curve with log-rank test displayed in Fig. 4A also revealed a statistically worse OS for patients with a loss of two or three

Table 3
Univariate and multivariable Cox regression analysis for **cancer specific survival** of muscle-invasive bladder cancer patients undergoing radical cystectomy. RB1: retinoblastoma protein 1, p53: tumor suppressor protein 53, p21: cyclin-dependent kinase inhibitor 1.

	Univariate			Multivariable		
Characteristic	**HR**	**95 % CI**	***p*=**	**HR**	**95 % CI**	***p*=**
RB1 negative	1.11	0.62–1.96	0.73	0.18	0.02, 1.27	0.09
p53 negative	1.05	0.74–1.49	0.77	0.11	0.01, 0.80	0.03
p21 negative	1.04	0.51–2.12	0.92	0.59	0.28, 1.26	0.17
2 or 3 markers negative	1.34	0.95–1.88	0.10	11.2	1.46, 86.04	**0.02**
Age > 68 years	1.10	0.78–1.55	0.58	1.14	0.80, 1.61	0.47
Gender female	1.06	0.72–1.56	0.77	1.27	0.84, 1.90	0.25
T-stage						
T3	1.92	1.15–3.19	**0.01**	1.60	0.94, 2.72	0.08
T4	3.14	1.82–5.44	**< 0.001**	2.63	1.50, 4.60	**0.001**
Positive lymph nodes	1.43	1.02–2.02	**0.04**	1.36	0.94, 1.98	0.10
Perineural invasion	1.59	1.09–2.32	**0.02**	1.37	0.92, 2.03	0.12
Vascular invasion	1.65	1.05–2.60	**0.03**	1.65	1.03, 2.66	**0.04**
Lymphovascular invasion	1.24	0.86–1.78	0.24	1.00	0.66, 1.50	0.99

Table 2
Univariate and multivariable Cox regression analysis for **overall survival** of muscle-invasive bladder cancer patients undergoing radical cystectomy. RB1: retinoblastoma protein 1, p53: tumor suppressor protein 53, p21: cyclin-dependent kinase inhibitor 1.

	Univariate			Multivariable		
Characteristic	**HR**	**95 % CI**	***p*=**	**HR**	**95 % CI**	***p*=**
RB1 negative	1.03	0.62–1.69	0.92	0.52	0.21–1.27	0.15
p53 negative	1.23	0.92–1.65	0.16	0.39	0.15–1.01	0.05
p21 negative	1.04	0.57–1.92	0.90	0.60	0.31–1.14	0.12
2 or 3 markers negative	1.42	1.06–1.90	**0.02**	3.49	1.28–9.50	**0.01**
Age > 68 years	1.38	1.03–1.84	**0.03**	1.43	1.06–1.92	**0.02**
Gender female	1.14	0.82–1.57	0.44	1.25	0.89–1.75	0.20
T-stage						
T3	1.63	1.09–2.44	**0.02**	1.41	0.93–2.15	0.11
T4	2.44	1.56–3.81	**< 0.001**	2.15	1.36–3.40	**0.001**
Positive lymph nodes	1.44	1.07–1.92	**0.02**	1.27	0.93–1.75	0.13
Perineural invasion	1.37	0.98–1.90	0.06	1.17	0.83–1.66	0.37
Vascular invasion	1.60	1.09–2.36	**0.02**	1.55	1.03–2.34	**0.04**
Lymphovascular invasion	1.40	1.03–1.89	**0.03**	1.13	0.80–1.58	0.50

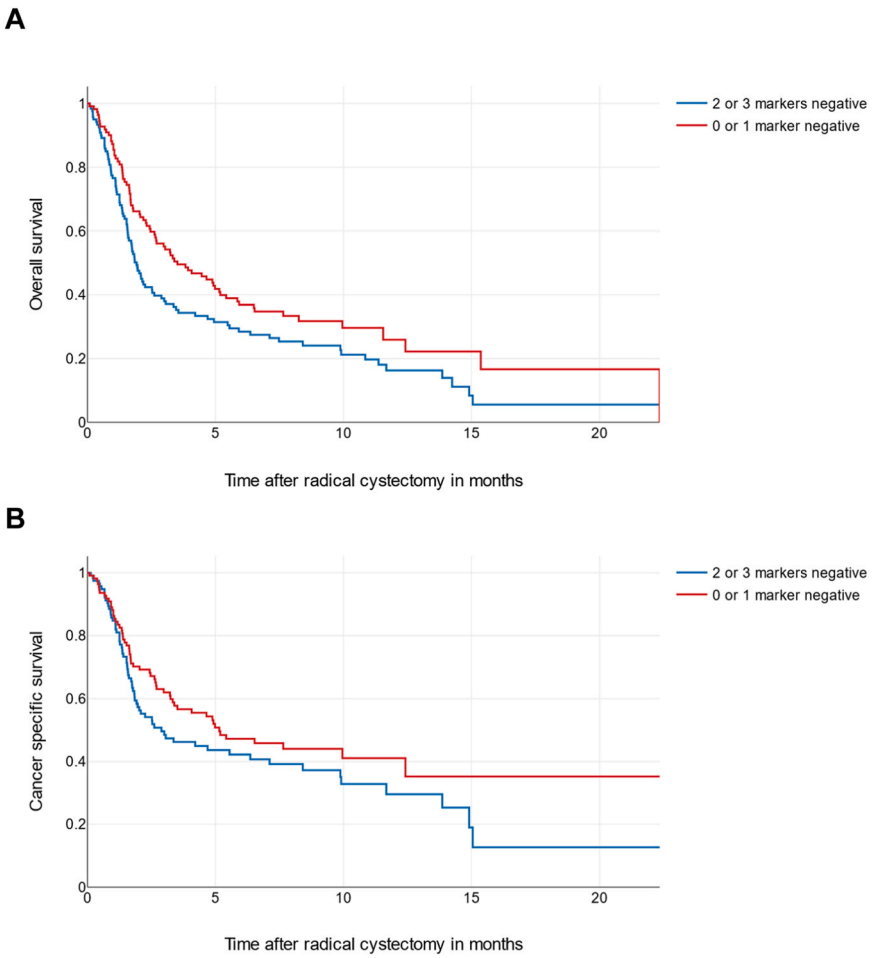

Fig. 4. A+B: Prognostic impact of marker status in muscle-invasive bladder cancer patients undergoing radical cystectomy: Kaplan-Meier curve for (A) overall survival and (B) cancer specific survival in patients with a loss of 0 or 1 marker vs. patients with a loss of 2 or 3 markers. Log-rank test (A) overall survival: p= 0.02, (B) cancer specific survival: p = 0.12.

markers (p = 0.02). The results for CSS (Fig. 4B) did not reach statistical significance (p = 0.12). The Kaplan-Meier curves with log-rank tests for single marker analysis of RB1 and p53 (Supplementary Figure 1 and 2) did not reveal any statistically significant differences between patients with negative and positive markers. The analysis of p21 was severely constrained by the limited number of positive patients (2 out of 238, 0.8 %). Consequently, the Kaplan-Meier curves and log-rank tests for p21 were essentially uninformative.

4. Discussion

This study validates and extends prior findings on the prognostic impact of RB1, p53, and p21 loss in MIBC. While single markers lacked predictive power, their combined analysis significantly correlated with poor survival outcomes, emphasizing the value of multimarker approaches.

Immunohistochemical markers such as RB1, p53 and p21 have significant potential to enhance our understanding of MIBC biology and to provide personalized treatment strategies. While several studies have investigated these markers, few have reached firm conclusions due to a focus on single-marker analyses, small sample sizes and a lack of long-term oncological follow-up data. Furthermore, the validation of these studies in external cohorts remains limited. The present study aimed to provide external validation of the findings reported by Wang et al., who observed an elevated risk of cancer recurrence in patients with pT3 bladder cancer who experienced a loss of two or more cell cycle regulators [8]. Building upon their analysis, we expanded our study to encompass all MIBC stages (pT2-pT4) and conducted a comprehensive analysis of the impact on both OS and CSS.

RB1, p53 and p21 are central tumor suppressors and are often found inactivated in various tumor types, including bladder cancer [16–18]. A key finding of our study is that none of the markers provides sufficient prognostic information when analyzed alone. However, a combined loss of two or more markers significantly correlates with worse survival outcomes, emphasizing the importance of multimarker analysis in the routine diagnostic. RB1 mutations are common in bladder cancer and are associated with high-grade tumors and drug resistance [8]. Our findings suggest that, while RB1 alone is not a strong predictor, it becomes critical when assessed alongside p53 and p21, significantly worsening patient outcomes. The role of p53 in bladder cancer prognosis has been debated, with some studies suggesting it predicts poor outcomes [8]. Our findings, however, indicate that p53 alone is insufficient to predict survival but becomes highly relevant when combined with RB1 and p21 loss.

The intratumoral heterogeneity analysis demonstrated a high concordance of RB1, p53, and p21 expression between the tumor front and center. These findings indicate that, for diagnostic purposes, the specific region from which samples are obtained may not be critical, as MIBC appears to show relatively homogeneous expression of these markers. This finding indicates that the diagnostic accuracy of evaluating RB1, p53, and p21 expression in MIBC can be sustained with a reduced number of samples. This has the potential to enhance efficiency and reduce associated costs.

Our study underscores the need for a multi-marker approach in

predicting outcomes for MIBC patients. Individual markers such as RB1, p53, and p21 may not provide reliable prognostic information alone, but their combined analysis significantly improves prognostic accuracy. Future treatment strategies should integrate multi-marker panels to identify high-risk patients who may benefit from more intensive therapies.

4.1. Limitations

This study has two main limitations. First, its retrospective and single-center design limits the generalizability of the results. The patient cohort, while well-characterized, may not fully represent the broader population of MIBC patients.

Second, immunohistochemical analysis only assesses protein expression but does not necessarily reflect functional loss. Future studies, incorporating functional assays and next-generation sequencing approaches, and employing prospective, multi-center designs could complement these analyses to provide a more comprehensive understanding of the biological mechanisms underlying marker expression patterns.

5. Conclusion

Our comprehensive immunohistochemical analysis of tissue microarrays demonstrates that RB1, p53, and p21 are unsuitable as single prognostic markers for MIBC patients undergoing radical cystectomy. However, the combined loss of two or three markers was significantly associated with worse overall and cancer-specific survival in our study. These findings confirm previous research and extend the results to a broader cohort (pT2–pT4). Future prognostic models should incorporate multi-marker analyses to identify high-risk patients more effectively and to personalize therapy accordingly.

Ethics statement

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and approved by the university ethics committee (project number: 20–179).

Funding

This research received no external funding.

CRediT authorship contribution statement

Schulz Gerald B.: Conceptualization, Data curation, Supervision, Writing – review & editing. **Schallenberg Simon:** Conceptualization, Data curation, Project administration, Writing – original draft. **Eich Marie-Lisa:** Writing – review & editing. **Weinhold Philipp:** Writing – review & editing. **Stief Christian G.:** Writing – review & editing. **Horst David:** Writing – review & editing. **Semmler Marie:** Writing – review & editing. **Volz Yannic:** Writing – review & editing. **Buchner Alexander:** Writing – review & editing. **Chaloupka Michael:** Writing – review & editing. **Eismann Lennert:** Writing – review & editing. **Hermans Julian:** Writing – review & editing. **Kidess Marc:** Writing – review & editing. **Pyrgidis Nikolaos:** Writing – review & editing. **Ebner Benedikt:** Data curation, Writing – original draft.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Microsoft Copilot and DeepL in order to enhance the fluency of the text and to perform grammar checks. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prp.2025.155960.

References

[1] W. Choi, et al., Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy, Cancer Cell 25 (2) (2014) 152–165.
[2] S. Schallenberg, et al., Intratumoral heterogeneity of molecular subtypes in muscle-invasive bladder cancer-an extensive multiregional immunohistochemical analysis, Eur. Urol. Focus 9 (5) (2023) 788–798.
[3] G. Iyer, et al., Multicenter prospective phase II trial of neoadjuvant dose-dense gemcitabine plus cisplatin in patients with muscle-invasive bladder cancer, J. Clin. Oncol. 36 (19) (2018) 1949–1956.
[4] M.Y. Teo, et al., Alterations in DNA damage response and repair genes as potential marker of clinical benefit from PD-1/PD-L1 blockade in advanced urothelial cancers, J. Clin. Oncol. 36 (17) (2018) 1685–1694.
[5] D.S. Helal, et al., Immunohistochemical based molecular subtypes of muscle-invasive bladder cancer: association with HER2 and EGFR alterations, neoadjuvant chemotherapy response and survival, Diagn. Pathol. 18 (1) (2023) 11.
[6] J. Alfred Witjes, et al., European association of urology guidelines on muscle-invasive and metastatic bladder cancer: summary of the 2023 guidelines, Eur. Urol. 85 (1) (2024) 17–31.
[7] J.H. Ku, et al., Diagnostic and prognostic markers in bladder cancer, Dis. Markers 2016 (1) (2016) 2425091.
[8] G. Wang, et al., Prognostic markers in pT3 bladder cancer: a study from the international bladder cancer tissue microarray project, Urol. Oncol. 39 (5) (2021) 301.e17–301.e28.
[9] P. Linn, et al., Targeting RB1 loss in cancers, Cancers (Basel) 13 (15) (2021).
[10] H. Wang, et al., Targeting p53 pathways: mechanisms, structures, and advances in therapy, Signal Transduct. Target. Ther. 8 (1) (2023) 92.
[11] T. Abbas, A. Dutta, p21 in cancer: intricate networks and multiple activities, Nat. Rev. Cancer 9 (6) (2009) 400–414.
[12] B. Shamloo, S. Usluer, p21 in cancer research, Cancers (Basel) 11 (8) (2019).
[13] H.E. Marei, et al., p53 signaling in cancer progression and therapy, Cancer Cell Int. 21 (1) (2021) 703.
[14] G.B. Schulz, et al., Therapeutic and prognostic implications of NOTCH and MAPK signaling in bladder cancer, Cancer Sci. 112 (5) (2021) 1987–1996.
[15] G.B. Schulz, et al., Prognostic value and association with epithelial-mesenchymal transition and molecular subtypes of the proteoglycan biglycan in advanced bladder cancer, Urol. Oncol. 37 (8) (2019) 530.e9–530.e18.
[16] K. Engeland, Cell cycle regulation: p53-p21-RB signaling, Cell Death Differ. 29 (5) (2022) 946–960.
[17] J.W. Slaton, W.F. Benedict, C.P.N. Dinney, p53 in bladder cancer: mechanism of action, prognostic value, and target for therapy, Urology 57 (5) (2001) 852–859.
[18] K. Tang, et al., Clinicopathologic and prognostic significance of p21 (Cip1/Waf1) expression in bladder cancer, Int J. Clin. Exp. Pathol. 8 (5) (2015) 4999–5007.