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ORIGINAL ARTICLE

Loss of CHEK2 Predicts Progression in Stage pT1 Non-Muscle-Invasive Bladder Cancer (NMIBC)

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Abstract

Downregulation of checkpoint protein kinase 2 (CHEK2), which is involved in DNA repair, is associated with poorer outcome in various tumors. Little is known about the role of CHEK2 in urothelial carcinoma of the bladder (UCB). In the present study, we investigated the prognostic impact of CHEK2 protein expression in stage pT1 UCB. This retrospective, single-center analysis was carried out in a cohort of patients initially diagnosed with a pT1 UCB between 2007 and 2015. Immunohistochemical (IHC) staining of CHEK2 was performed. CHEK2 expression was correlated with recurrence-free survival (RFS), progression-free survival (PFS), and cancer-specific survival (CSS) using Kaplan-Meier analysis and multivariable Cox regression analysis. The analysis included 126 patients (86% male, median age 71 years). Loss of immunohistochemical protein expression of CHEK2 (<10%) was associated with significantly worse PFS ($p = 0.041$). Likewise, CHEK2 loss identified a subgroup of patients with worse PFS in the high-risk groups with concomitant CIS ($p = 0.044$), multifocal tumors ($p < 0.001$) and tumor grading G3 according to WHO1973 ($p = 0.009$). Multivariable Cox regression analysis revealed both loss of CHEK2 expression (HR: 4.18, 95%-CI: 1.35–12.93; $p = 0.013$) and multifocal tumors (HR: 4.53, 95%-CI:1.29–15.92; $p = 0.018$) as the only predictive factors for progression. Loss of IHC expression of CHEK2 in pT1 UCB is an independent predictor for progression to muscle-invasive disease and is also associated with worse PFS. This could help to identify high-risk patients who would benefit from early cystectomy.

Keywords Non-muscle-invasive bladder cancer · NMIBC · CHEK2 · Smoking status · Progression

Introduction

Urothelial carcinoma of the bladder accounts for 549,393 new cases per year worldwide and displays the 12th most common malignancy [1]. About 75% are initially diagnosed as non-muscle-invasive bladder cancer (NMIBC) [2]. NMIBC is treated by transurethral resection of the bladder tumor (TUR-B) and instillation therapy and characterized by a high recurrence rate up to 70%, while up to 30% will progress to muscle-invasive bladder cancer (MIBC) [2]. To improve prediction of recurrence and progression, several clinical and pathological parameters have been evaluated. Of these, tumor stage, tumor grade according to the WHO1973 classification, focality, concomitant carcinoma in situ (Cis), tumor size and number of recurrences have been implemented by the European Organization for Research and Treatment of Cancer (EORTC) as a score (EORTC score) [3].

While less invasive stage (pTa tumors) is treated with a bladder sparing approach, NMIBC at stage pT1 often cannot be cured by TUR-B and instillation therapy. It has been shown that especially pT1G3 tumors benefit from an early cystectomy (CE), however up to date, the treatment decision is an individual decision based on the established clinical and pathological parameters [4]. Thus, further objective parameters are needed to support the clinician.

Bladder cancer is among the tumors with the highest mutational rate. Recently, Heeke et al. could also show that bladder cancer is the tumor with the 3rd most mutations of

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homologous recombination-related DNA damage repair genes like ATM, BRCA1/2 and CHEK2 [5]. Checkpoint-kinase 2 (CHEK2), located at chromosome 22, is a well-known tumor suppression gene and encodes for protein CHEK2, which regulates cell apoptosis and DNA repair [6]. CHEK2 interacts with BRCA1, PI3K and p53 to facilitate DNA-repair cell-cycle arrest and apoptosis following DNA-damage [6, 7]. Some germline mutations of CHEK2 are associated with a higher risk of colorectal [8], Hodgkin Lymphoma [9], Non-Hodgkin Lymphoma [10], prostate cancer [11] and bladder cancer [12]. Loss of CHEK2 protein expression in IHC has been correlated with adverse outcome in gastric cancer [13] or in ovarian cancer [14]. Furthermore, germline mutations of CHEK2 also seem to increase the recurrence risk of NMIBC [15].

To date, there is no study on the prognostic role of CHEK2 protein expression in IHC in stage pT1 NMIBC. In the present study, we investigated on the prognostic role of CHEK2 expression in this challenging entity.

Patients and Methods

Study Population

The total study cohort consisted of 126 patients with stage pT1 NMIBC at initial diagnosis who underwent transurethral resection of the bladder (TUR-B) in a single center between 2007 and 2015. All patients underwent re-resection 6 weeks after initial resection. Patients' histopathological and clinical data as well as follow-up was recorded retrospectively after approval of the local ethics committee (16–321-101). All cases were re-evaluated and classified according to the most recent TNM-classification (2017) and the WHO 1973 and 2016 grading classification of genitourinary tumors by experienced uropathologists.

Immunohistochemical Assessment and Analysis

4 μm sections were cut from formalin-fixed and paraffin-embedded tissue-blocks and mounted on poly-L-lysine-coated glass slides. Immunohistochemistry was carried out in a *BenchMark IHC Full System immunostainer (Roche Diagnostics, Mannheim, Germany)* using the avidin-biotin peroxidase method with diaminobenzidine as chromogen according to the manufacturer's instructions. As primary antibody *mouse monoclonal Chk2 antibody 1C12 (Cell Signaling Technology Inc., Danvers MA, USA, dilution 1:150)* was used.

Expression of CHEK2 was visualised with a *Primo Star microscope (Carl Zeiss Microimaging, Jena, Germany)* under 40- and 100-fold magnification. Evaluation of the immunostained slides was performed independently by two reviewers without knowledge of clinical and follow-up data. CHEK2 was assessed by reporting staining in steps of 10%, with loss of CHEK2 being <10% staining (Fig. 1).

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (*IBM Deutschland GmbH, Ehningen, Germany*). Recurrence-free survival (RFS) rates, progression-free survival (PFS) rates and cancer-specific survival (CSS) rates were calculated by Kaplan-Meier analysis and Log rank test. The Spearman product-moment correlation coefficient r was used as a measure of the strength and direction of the linear relationship between variables. Multivariable Cox regression analyses were used to assess the value of CHEK2 expression, clinical and histopathological parameters for RFS, PFS and CSS. p values <0.05 were considered statistically significant.

Results

Patient Population

Of the 126 patients that could be included in the final analysis 108 were male, the median age was 71 years (IQ range: 72–78 years). 82 patients were graded WHO1973 grade 3 (70.6%), 53 (42.1%) had a concomitant Cis (Table 1). The median follow-up was 32 months (IQ range: 20–50 months), 45 patients had a tumor recurrence (35.7%), 19 patients suffered from progression to muscle-invasive disease (15.1%) with 16 who died from the disease (12.7%) (Table 1).

Of the 126 patients 26 (20.6%) never smoked, with 35 current smokers (27.8%) and 43 former smokers (34.1%). Due to the retrospective nature of the study there was no information about the smoking status of 22 patients (17.5%). The complete clinical and histopathological demographics are displayed in Table 1.

Immunohistochemical Expression of CHEK2 and Correlation with Clinical and Histopathological Parameters

CHEK2 expression could be evaluated in all 126 samples and was found to be <10% in 11 samples (8.7%) (Fig. 1). Correlating CHEK2 expression with various clinical and histopathological features revealed a significant correlation between loss of CHEK2 expression and a concomitant Cis (p = 0.031, Table 2).

Loss of CHEK2 Expression Predicts Progression

Kaplan-Meier survival analysis revealed a statistically significant association between loss of CHEK2 expression (<10%) and worse PFS (p = 0.041, Fig. 2a). No correlation between

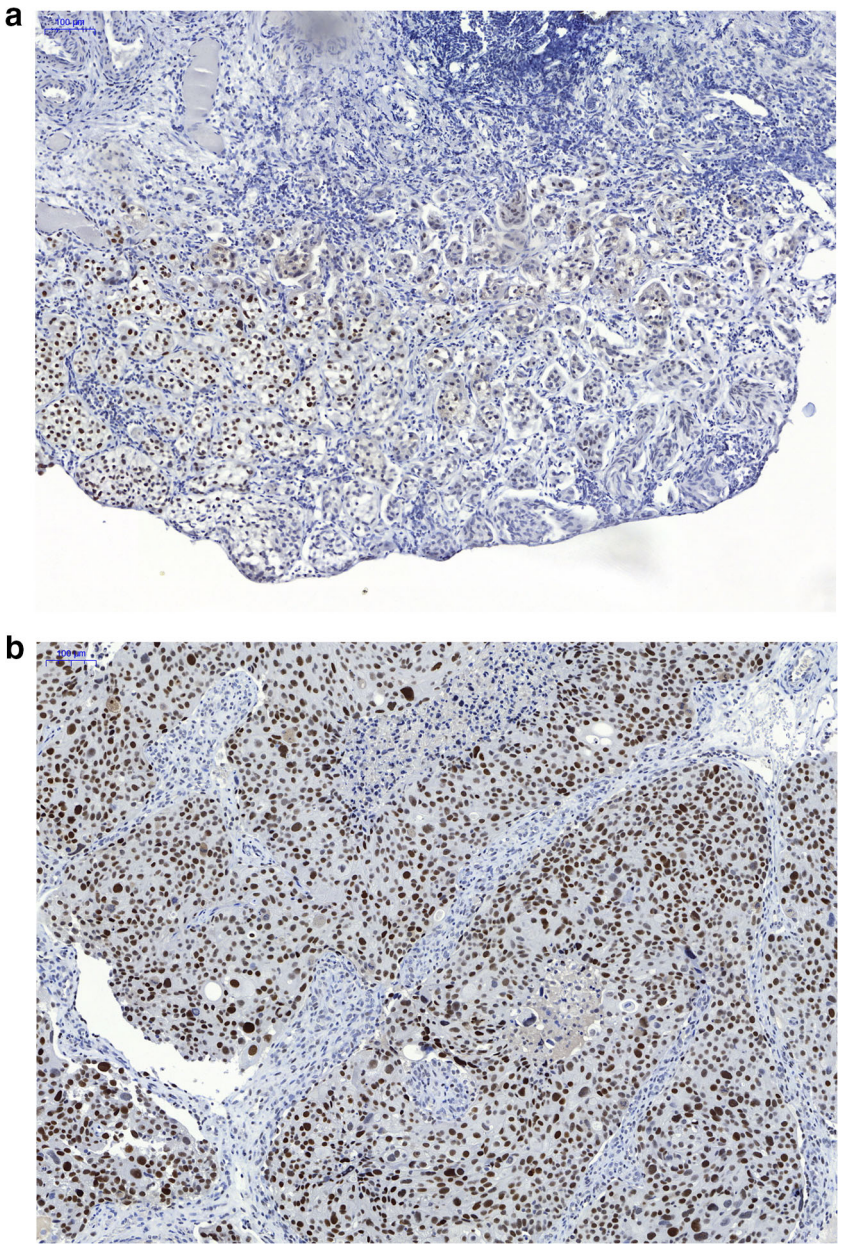

Fig. 1 Examples of weak (**a**) and strong (**b**) immunohistochemical staining of CHEK2 in stage pT1 non-muscle-invasive bladder cancer under 10-fold magnification

CHEK2 expression and RFS and CSS could be found. Of the clinical and pathological parameters, only multifocal tumors were associated with worse RFS ($p = 0.007$), PFS ($p = 0.025$) and CSS ($p = 0.022$). Multivariable cox regression analysis revealed both loss of CHEK2 expression (HR: 4.18, 95%-CI: 1.35–12.93; $p = 0.013$) and multifocal tumors (HR: 4.53, 95%-CI:1.29–15.92; $p = 0.018$) as the only predictive factors for progression (Table 3).

Loss of CHEK2 Expression is Associated with Worse PFS in High Risk Subgroups

Furthermore, loss of CHEK2 expression was associated with worse PFS in the high-risk subgroups of tumors with concomitant Cis ($p = 0.047$, 50% vs. 82% 5-years survival rate), Grading WHO1973 G3 tumors ($p = 0.009$, 52% vs. 88% 5-years survival rate) and multifocal tumors ($p < 0.001$, 20% vs. 82% 5-years survival rate) (Fig. 2 b-d).

Loss of CHEK2 is Associated with Worse PFS in Smokers

Correlating CHEK2 expression with PFS in smokers revealed a statistically significant association with worse PFS in active smokers ($p = 0.047$, 33% vs. 81% 5-years survival rate) and active and former smokers ($p < 0.001$, 22% vs. 83% 5-years survival rate) (Fig. 3).

Table 1 Patient characteristics in stage pT1 NMIBC study cohort

Parameter	n (%)
Patient data	
Total stage pT1 2007–2015	126 (100)
Female patients	18 (14.3)
Male patients	108 (85.7)
Median age (years)	71 [IQ range: 72–78]
Clinical and pathological parameters	
Grading WHO1973	
G1	0 (0)
G2	37 (29.4)
G3	89 (70.6)
Grading WHO2016	
low grade	8 (6.3)
high grade	110 (87.3)
n.a.	8 (6.3)
Tumor diameter	
<3 cm	60 (47.6)
≥3 cm	66 (52.4)
Concomitant Cis	
Yes	53 (42.1)
No	73 (57.9)
Focality	
Unifocal	50 (39.7)
Multifocal	76 (60.3)
Smoking status	
Never	26 (20.6)
Active	35 (27.8)
Former	43 (34.1)
n.a.	22 (17.5)
Treatment	
Instillation therapy	93 (73.8)
MMC	22 (17.5)
BCG	71 (56.3)
Early cystectomy	13 (10.3)
Deferred cystectomy	14 (11.1)
Follow-up information	
Median follow-up (months)	32 [IQ range: 20–50]
Maximum follow-up (months)	112
Recurrence ≤pT1	45 (35.7)
Progression	19 (15.1)
Death	32 (25.4)
Death of disease	16 (12.7)

IQ interquartile, *n.a.* not available

Discussion

NMIBC at stage pT1 remains one of the most challenging entities for the treating urologist. Up to one third of the patients with a pT1G3 tumor will suffer progression to MIBC and will need a radical cystectomy [16]. Furthermore, it has been shown that those patients benefit from an early cystectomy [4]. However, a majority of the patients will be overtreated by early cystectomy, an operation that resembles high morbidity and mortality [17].

The present study investigates on the prognostic role of CHEK2 protein expression by IHC in stage pT1 NMIBC. Loss of CHEK2 expression was associated with worse PFS and was the best independent predictor for progression to muscle-invasive disease in multivariable analysis. To date, this is the first study that proves a prognostic effect of CHEK2 expression in bladder cancer. Slojewski et al. could show a higher recurrence risk in NMIBC with germline mutations [15]. Others also suggested an increased risk for development of bladder cancer with existing CHEK2 germline mutations [12, 18]. However, Ge et al., could show that depending on the mutated variant CHEK2 mutations can be associated with increased risk of bladder cancer as well as with decreased risk of bladder cancer [18]. This could also be shown for breast cancer [19]. Specific mutations of CHEK2 like the c.1100delC variant are associated with a loss of CHEK2 IHC expression in breast cancer and correlate with higher tumor stage and grade, worse CSS but not with overall survival [20–23]. Furthermore, it could be shown that CHEK2 mutations correlate with an increased risk of gastric cancer [24] and loss of CHEK2 expression in IHC correlates with worse CSS in gastric cancer [13]. An adverse role for CHEK2 on OS could be shown in ovarian cancer [14]. As described above, due to the different functional outcomes of CHEK2 mutations it is necessary to look at protein expression of CHEK2 to get a functional and potential prognostic information.

Table 2 Correlation of CHEK2 expression with clinical and histopathological parameters ($n = 126$). Significant results indicated in bold

Parameter	CHEK2 expression <10% ($n = 11$)	CHEK2 expression ≥10% ($n = 115$)	p value
Age			
<75	6	77	0.407
≥75	5	38	
Gender			
Male	11	97	0.156
Female	0	18	
Smoking status			
Never	3	23	0.546
Ever	6	72	
Grading WHO1973			
G2	2	35	0.394
G3	9	80	
Concomitant Cis			
No	3	70	**0.031**
Yes	8	45	
Focality			
Unifocal	6	44	0.292
Multifocal	5	71	
Tumor diameter			
<3 cm	6	54	0.630
≥3 cm	5	61	

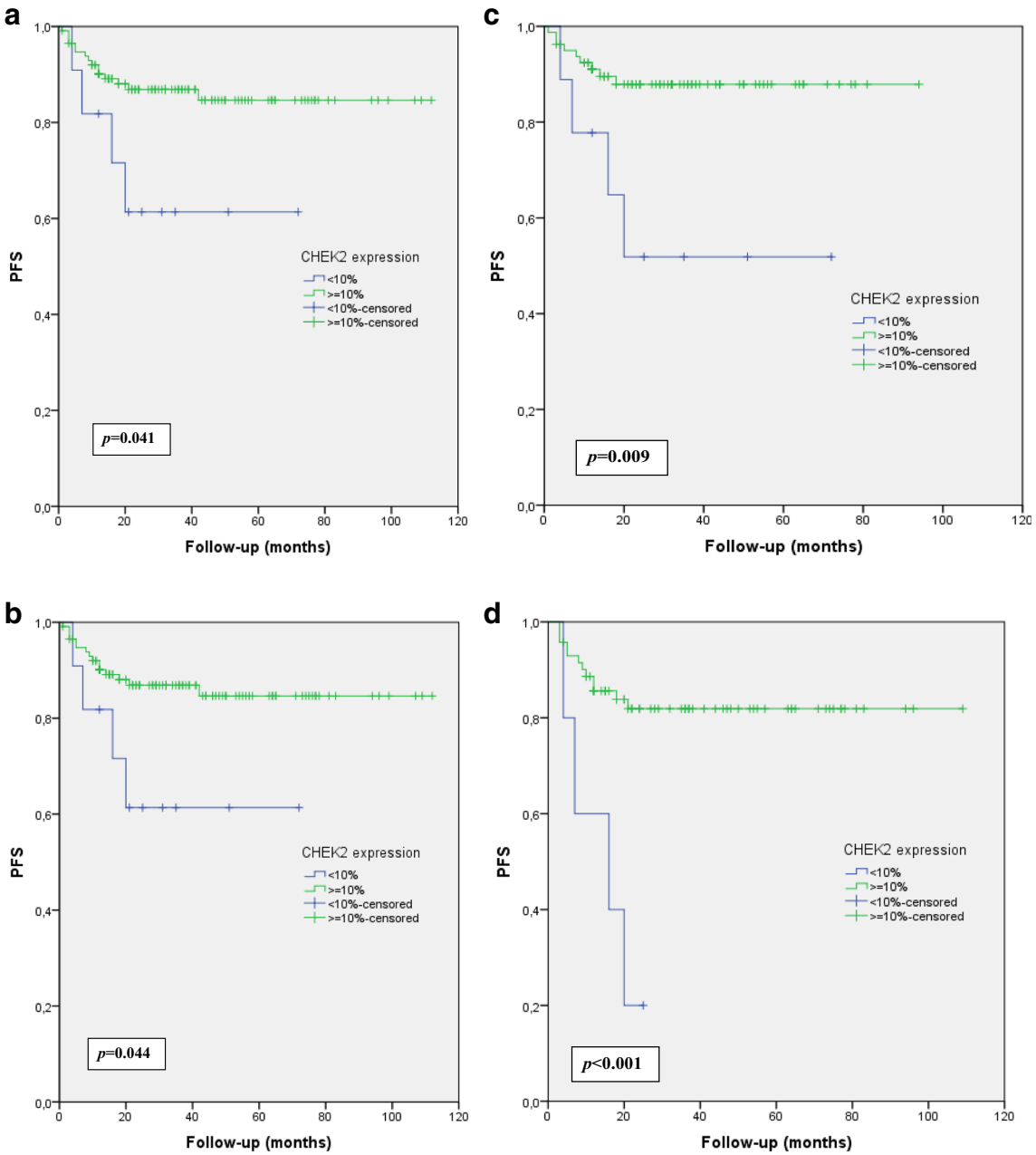

Fig. 2 Kaplan-Meier analysis of CHEK2 expression (<10% vs. ≥10%) with regard to progression-free survival (PFS) in the total cohort (A; $n = 126$), patients with concomitant Cis (B; $n = 53$), WHO1973 Grade 3 tumors (C; $n = 89$) and multifocal tumors (D; $n = 76$)

Moreover, loss of CHEK2 expression identified a highest risk group within the high-risk groups of pT1G3 tumors, tumors with concomitant Cis and multifocal tumors. The EORTC score was developed to improve prediction of recurrence and progression of NMIBC and the abovementioned parameters (concomitant Cis, Grading G3 WHO1973, multifocality) resemble the most important risk factors in stage pT1 [3]. Thus, adding CHEK2 expression especially in patients with these risk factors could help with the decision between a bladder-sparing approach or radical cystectomy.

Table 3 Multivariable Cox Regression Analysis of PFS in the pT1 NMIBC study cohort ($n = 126$). Significant results indicated in bold

	HR	95% CI	*p* value
Focality *multifocal* vs. *unifocal*	4.53	1.29–15.92	**0.018**
CHEK2 expression <10% vs. ≥10%	4.18	1.35–12.93	**0.013**

Besides the prognostic effect of CHEK2 expression, first studies investigated on specific inhibitors of CHEK2 [25–27]. Ghelli et al. could show that a specific CHEK1/2 inhibitor increases the effectiveness of conventional therapy in B−/T-

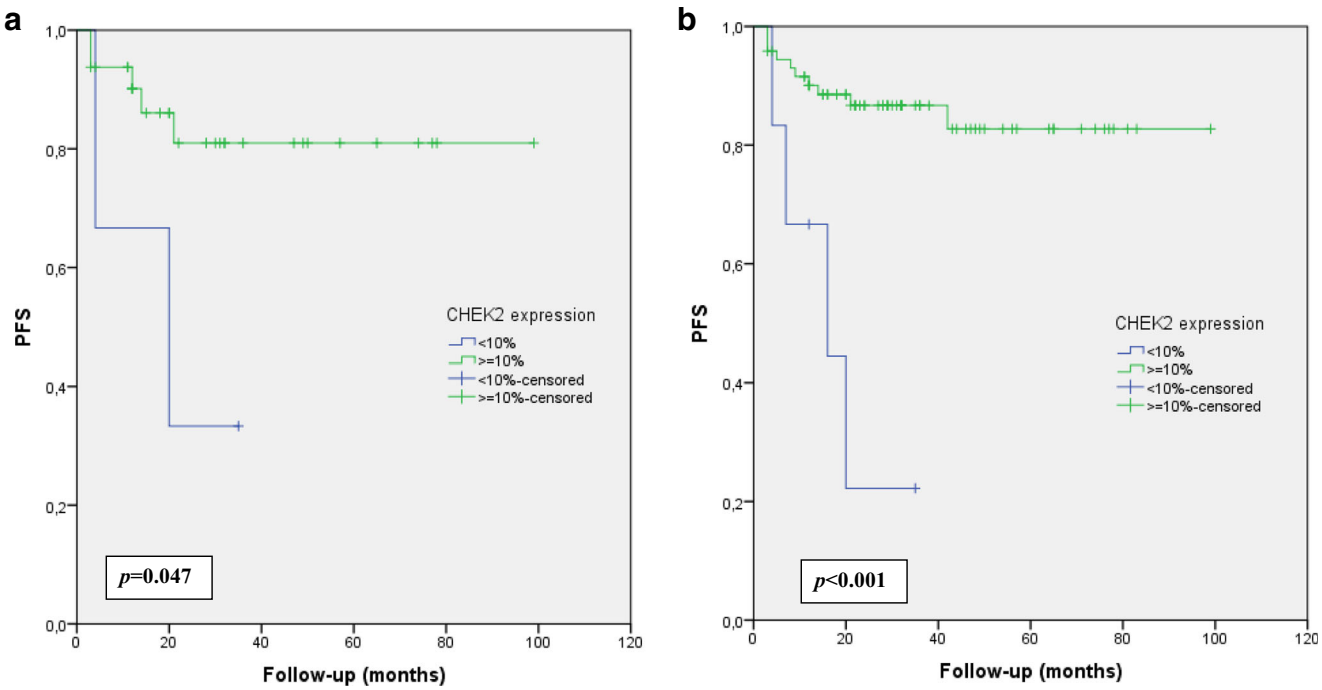

Fig. 3 Kaplan-Meier analysis of CHEK2 expression (<10% vs. ≥10%) with regard to progression-free survival (PFS) in active smokers (**a**; $n = 35$) and active and former smokers (**b**; $n = 78$)

cell progenitor acute lymphoblastic leukemia [25]. Furthermore, an enhanced toxicity of radiation on head and neck squamous cell carcinoma after CHEK1/2 inhibition could be shown in vitro and in vivo [26]. For urothelial carcinoma, first in vitro studies could prove that urothelial carcinoma cells can be sensitized to gemcitabine under treatment with a checkpoint kinase inhibitor [27]. This could offer novel potential treatment options for NMIBC in a bladder-sparing approach or for MIBC in a palliative setting. Herein it would be also of interest, if CHEK2 expression plays a role in treatment response as it would be supposable that those tumors with loss of CHEK2 expression would not benefit from this treatment.

CHEK2 is known to interact with BRCA1, PI3K and p53 to facilitate DNA repair, cell-cycle arrest and apoptosis and thus acts as a tumor suppressor [6]. It could be shown that DNA damage repair genes like CHEK2, p53 or ATM are induced by cigarette smoking [28]. In a first study on the role of CHEK2 IHC expression, we could show that it is downregulated in normal urothelium of healthy (no urothelial carcinoma of the bladder) male tobacco smokers [29]. Although this is contradictory to the findings of Zhao et al., this might be the first step in development of bladder cancer. However, this was a small cohort and we investigated on the association of CHEK2 expression with smoking status in the present larger cohort of pT1 NMIBC. There was no correlation between smoking status and CHEK2 expression but CHEK2 also identified a high-risk group with worse PFS in active smokers (33% vs. 81% 5-years PFS) and in ever-smokers (22% vs. 83% 5-years PFS) in the present study. This indicates to an increased risk of those smokers with loss of CHEK2 and taken together with the previous finding of no loss of CHEK2 in normal urothelium of former smokers a potential benefit of smoking cessation concerning aggressive NMIBC.

However, the retrospective nature of this study is a major limitation as seen in 17.5% of the patients without information on smoking status. Furthermore, we do not have information on CHEK2 mutations in our cohort. Further prospective and multicenter studies also investigating the prognostic effect on MICB would be necessary.

Conclusions

pT1 NMIBC is a challenging tumor stage in UCB. Stratifying risk for recurrence and progression of tumor is important in choosing between bladder sparing and early CE. The present retrospective study could identify loss of IHC expression of CHEK2 as a predictive factor for progression to muscle-invasive disease. This could help to identify high-risk patients at stage pT1 NMIBC who would benefit from early cystectomy.

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Author's Contribution Protocol/project development: Denzinger, Burger, Otto, Breyer.

Data collection or management: Azzolina, Weber, Evert, Eckstein, Breyer.

Data analysis: Spachmann, Otto, Breyer.
Manuscript writing/editing: Spachmann, Breyer.

Compliance with Ethical Standards

Conflict of Interest No potential conflicts of interest must be reported.

Research Involving Human Participants and/or Animals Research did not involve human participants or animals.

Informed Consent Informed consent was obtained from all individual participants included in the study. All the findings, data acquisition and processing in this study comply with the ethical standards laid down in the latest declaration of Helsinki. The study was approved by the local ethics committee of the University of Regensburg (Nr. 16–321-101).

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424
2. Babjuk M, Böhle A, Burger M, Capoun O, Cohen D, Compérat EM, Hernández V, Kaasinen E, Palou J, Rouprêt M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R (2017) EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 71(3):447–461
3. Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, Newling DW, Kurth K (2006) Predicting recurrence and progression in individual patients with stage ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 49:466–475 discussion 475–477
4. Denzinger S, Fritsche HM, Otto W, Blana A, Wieland WF, Burger M (2008) Early versus deferred cystectomy for initial high-risk pT1G3 urothelial carcinoma of the bladder: do risk factors define feasibility of bladdersparing approach? Eur Urol 53:146–152
5. Heeke AL, Pishvaian MJ, Lynce F, Xiu J, Brody JR, Chen WJ, Baker TM, Marshall JL, Isaacs C (2018) Prevalence of homologous recombination-related gene mutations across multiple Cancer types. JCO Precis Oncol 2018:1–13. https://doi.org/10.1200/PO.17.00286. Epub 2018 Jul 23
6. Bartek J, Lukas J (2003) Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 3(5):421–429
7. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ, Mak TW (2000) DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science 287(5459):1824–1827
8. Suchy J, Cybulski C, Wokołorczyk D, Oszurek O, Górski B, Debniak T, Jakubowska A, Gronwald J, Huzarski T, Byrski T, Dziuba I, Gogacz M, Wiśniowski R, Wandzel P, Banaszkiewicz Z, Kurzawski G, Kładny J, Narod SA, Lubiński J (2010) CHEK2 mutations and HNPCC-related colorectal cancer. Int J Cancer 126(12):3005–3009. https://doi.org/10.1002/ijc.25003
9. Havranek O, Spacek M, Hubacek P, Mocikova H, Markova J, Trneny M, Kleibl Z (2011) Alterations of CHEK2 forkhead-associated domain increase the risk of Hodgkin lymphoma. Neoplasma 58(5):392–395
10. Havranek O, Kleiblova P, Hojny J, Lhota F, Soucek P, Trneny M, Kleibl Z (2015) Association of Germline CHEK2 gene variants with risk and prognosis of non-Hodgkin lymphoma. PLoS One 10(10):e0140819. https://doi.org/10.1371/journal.pone.0140819eCollection 2015
11. Wang Y, Dai B, Ye D (2015) CHEK2 mutation and risk of prostate cancer: a systematic review and meta-analysis. Int J Clin Exp Med 8(9):15708–15715 eCollection 2015
12. Złowocka E, Cybulski C, Górski B, Debniak T, Słojewski M, Wokołorczyk D, Serrano-Fernández P, Matyjasik J, van de Wetering T, Sikorski A, Scott RJ, Lubiński J (2008) Germline mutations in the CHEK2 kinase gene are associated with an increased risk of bladder cancer. Int J Cancer 122(3):583–586
13. Lee HE, Han N, Kim MA, Lee HS, Yang HK, Lee BL, Kim WH (2014) DNA damage response-related proteins in gastric cancer: ATM, Chk2 and p53 expression and their prognostic value. Pathobiology 81(1):25–35. https://doi.org/10.1159/000351072 Epub 2013 Aug 21
14. Ow GS, Ivshina AV, Fuentes G, Kuznetsov VA (2014) Identification of two poorly prognosed ovarian carcinoma subtypes associated with CHEK2 germ-line mutation and non-CHEK2 somatic mutation gene signatures. Cell Cycle 13(14):2262–2280. https://doi.org/10.4161/cc.29271 Epub 2014 May 30
15. Słojewski M, Złowocka E, Cybulski C, Górski B, Debniak T, Wokołorczyk D, Matyjasik J, Sikorski A, Lubiński J (2008) CHEK2 germline mutations correlate with recurrence rate in patients with superficial bladder cancer. Ann Acad Med Stetin 54(3):115–121
16. Shahin O, Thalmann GN, Rentsch C, Mazzucchelli L, Studer UE (2003) A retrospective analysis of 153 patients treated with or without intravesical bacillus Calmette-Guerin for primary stage T1 grade 3 bladder cancer: recurrence, progression and survival. J Urol 169(1):96–100
17. Aziz A, May M, Burger M, Palisaar RJ, Trinh QD, Fritsche HM, Rink M, Chun F, Martini T, Bolenz C, Mayr R, Pycha A, Nuhn P, Stief C, Novotny V, Wirth M, Seitz C, Noldus J, Gilfrich C, Shariat SF, Brookman-May S, Bastian PJ, Denzinger S, Gierth M, Roghmann F (2014) PROMETRICS 2011 research group. Prediction of 90-day mortality after radical cystectomy for bladder cancer in a prospective European multicenter cohort. Eur Urol 66(1):156–163
18. Ge Y, Wang Y, Shao W, Jin J, Du M, Ma G, Chu H, Wang M, Zhang Z (2016) Rare variants in BRCA2 and CHEK2 are associated with the risk of urinary tract cancers. Sci Rep 6:33542. https://doi.org/10.1038/srep33542
19. Muranen TA, Blomqvist C, Dörk T, Jakubowska A, Heikkilä P, Fagerholm R, Greco D, Aittomäki K, Bojesen SE, Shah M, Dunning AM, Rhenius V, Hall P, Czene K, Brand JS, Darabi H, Chang-Claude J, Rudolph A, Nordestgaard BG, Couch FJ, Hart SN, Figueroa J, García-Closas M, Fasching PA, Beckmann MW, Li J, Liu J, Andrulis IL, Winqvist R, Pylkäs K, Mannermaa A, Kataja V, Lindblom A, Margolin S, Lubinski J, Dubrowinskaja N, Bolla MK, Dennis J, Michailidou K, Wang Q, Easton DF, Pharoah PD, Schmidt MK, Nevanlinna H (2016) Patient survival and tumor characteristics associated with CHEK2:p.I157T - findings from the Breast Cancer Association Consortium. Breast Cancer Res 18(1):98
20. Kilpivaara O, Bartkova J, Eerola H, Syrjäkoski K, Vahteristo P, Lukas J, Blomqvist C, Holli K, Heikkilä P, Sauter G, Kallioniemi OP, Bartek J, Nevanlinna H (2005) Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. Int J Cancer 113(4):575–580
21. de Bock GH, Schutte M, Krol-Warmerdam EM, Seynaeve C, Blom J, Brekelmans CT, Meijers-Heijboer H, van Asperen CJ, Cornelisse CJ, Devilee P, Tollenaar RA, Klijn JG (2004) Tumour characteristics and prognosis of breast cancer patients carrying the germline CHEK2*1100delC variant. J Med Genet 41(10):731–735
22. Ribeiro-Silva A, Moutinho MA, Moura HB, Vale FR, Zucoloto S (2006) Expression of checkpoint kinase 2 in breast carcinomas:

correlation with key regulators of tumor cell proliferation, angiogenesis, and survival. Histol Histopathol 21(4):373–382. https://doi.org/10.14670/HH-21.373

23. Kriege M, Hollestelle A, Jager A, Huijts PE, Berns EM, Sieuwerts AM, Meijer-van Gelder ME, Collée JM, Devilee P, Hooning MJ, Martens JW, Seynaeve C (2014) Survival and contralateral breast cancer in CHEK2 1100delC breast cancer patients: impact of adjuvant chemotherapy. Br J Cancer 111(5):1004–1013. https://doi.org/10.1038/bjc.2014.306 Epub 2014 Jun 10
24. Teodorczyk U, Cybulski C, Wokołorczyk D, Jakubowska A, Starzyńska T, Lawniczak M, Domagała P, Ferenc K, Marlicz K, Banaszkiewicz Z, Wiśniowski R, Narod SA, Lubiński J (2013) The risk of gastric cancer in carriers of CHEK2 mutations. Familial Cancer 12(3):473–478. https://doi.org/10.1007/s10689-012-9599-2
25. Ghelli Luserna Di Rorà A, Iacobucci I, Imbrogno E, Papayannidis C, Derenzini E, Ferrari A, Guadagnuolo V, Robustelli V, Parisi S, Sartor C, Abbenante MC, Paolini S, Martinelli G (2016) Prexasertib, a Chk1/Chk2 inhibitor, increases the effectiveness of conventional therapy in B−/T- cell progenitor acute lymphoblastic leukemia. Oncotarget 7(33):53377–53391. https://doi.org/10.18632/oncotarget.10535
26. Zeng L, Beggs RR, Cooper TS, Weaver AN, Yang ES (2017) Combining Chk1/2 inhibition with Cetuximab and radiation enhances *In Vitro* and *In Vivo*Cytotoxicity in head and neck squamous cell carcinoma. Mol Cancer Ther 16(4):591–600. https://doi.org/10.1158/1535-7163.MCT-16-0352 Epub 2017 Jan 30
27. Isono M, Hoffmann MJ, Pinkerneil M, Sato A, Michaelis M, Cinatl J Jr, Niegisch G, Schulz WA (2017) Checkpoint kinase inhibitor AZD7762 strongly sensitises urothelial carcinoma cells to gemcitabine. J Exp Clin Cancer Res 36(1):1. https://doi.org/10.1186/s13046-016-0473-1
28. Zhao H, Albino AP, Jorgensen E, Traganos F, Darzynkiewicz Z (2009) DNA damage response induced by tobacco smoke in normal human bronchial epithelial and A549 pulmonary adenocarcinoma cells assessed by laser scanning cytometry. Cytometry A 75(10):840–847. https://doi.org/10.1002/cyto.a.20778
29. Breyer J, Denzinger S, Hartmann A, Otto W (2016) Downregulation of checkpoint protein kinase 2 in the Urothelium of healthy male tobacco smokers. Urol Int 97(4):480–481 Epub 2016 Jun 2

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