Journal on Surgery

www.journalonsurgery.org

Research Article

Open Access, Volume 2

IHC-Based Gene Expression Analysis Predicts Response to Neoadjuvant Chemotherapy for Bladder Cancer

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Abstract

Objectives: To explore the related genes and clinical characteristics of neoadjuvant chemotherapy (NAC) in bladder cancer, and to establish the prediction model of NAC response.

Methods: We downloaded the clinical information and gene expression detected by IHC of 149 patients from the attachment of published paper. Patients were divided into three groups according to different pathological reactions of NAC. Then, Chi-square test and independent T test were used to compare the clinical features. Kruskal-Wallis test and Kaplan-Meier (K-M) survival analysis were used to identify genes that are differentially expressed and associated with prognosis. Lasso regression is used to further screen for significant variables. Neural network (NN), support vector machine (SVM) and random forest (RF) algorithms were used to construct NAC response prediction models for bladder cancer.

Results: Among all clinical characteristics, age, T, N, TURB path LVI, keratinization, CIS, Necrosis, RB_alt status, and P16_alt_status are strongly correlated with NAC efficacy. CDH1, EPCAM, FOXA1, CCND1, P16, and ZEB2 are related to different NAC pathological reactions and tumor prognosis. Ten variables were screened by Lasso regression for further model construction. NN, SVM and RF prediction models were constructed, and the NN model has higher accuracy (93.8%) and AUC (0.984).

Conclusions: Our study not only identified some genes and clinical features closely associated with bladder cancer NAC and constructed prediction models for the efficacy of NAC, but also provided new ideas for the treatment of bladder cancer.

Keywords: Bladder cancer; Machine learning; Neoadjuvant chemotherapy; Prediction model.

Manuscript Information: *Received: Sep 01, 2022; Accepted: Sep 19, 2022; Published: Sep 28, 2022*

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Citation: *Cao J, Chen S, Wang J, Fan X, Yang L, et al. IHC-Based Gene Expression Analysis Predicts Response to Neoadjuvant Chemotherapy for Bladder Cancer. J Surgery. 2022; 2(2): 1054.*

Introduction

Bladder cancer is the tenth most commonly diagnosed cancer worldwide [1]. The age-standardized incidence rate (per 100,000 person-years) is 9.5 for men and 2.4 for women worldwide [1]. Smoking is the most important risk factor for bladder cancer, accounting for slightly less than 50% of cases [2]. Although the majority of patients present with non-muscle-invasive disease, approximately 30-40% of patients already have MIBC at the time of initial diagnosis due to the insignificance of early clinical symptoms, with a poor prognosis [3]. The traditional treatments for MIBC mainly include radical cystectomy (RC) and chemotherapy, but there is a high recurrence rate, and the 5-year overall survival (OS) rate remains at 15–20% [4]. Nearly 50% of MIBC patients who underwent radical bladder resection still developed metastatic bladder cancer [5]. However, neoadjuvant chemotherapy (NAC) can reduce the risk of death in patients with MIBC after RC and improve the OS [6,7]. At present, it has become the standard treatment recommended by multiple MIBC guidelines [8].

Although RC has historically been the cornerstone of treatment for MIBC, optimizing outcomes with NAC and alternative options for bladder preservation strategies have also emerged as treatment options [8,9]. The choice of treatment regimen for MIBC patients is directly related to the final prognosis, and preoperative NAC can create opportunities for subsequent tumor resection. There are many NAC schemes for MIBC, among which gemcitabine combined with cisplatin (GC) and methotrexate, vincristine, doxorubicin combined with cisplatin (MVAC) are the two most widely used schemes in clinical application [8]. Preoperative NAC for MIBC can help control local lesions, reduce tumor stage, reduce surgical difficulty, eliminate micro-metastases, and improve post-operative long-term survival rate. Compared with RC alone, NAC and RC resulted in a 16% increase in the 5-year OS rate [10]. As the utilization of NAC for MIBC has increased and post-NAC ypT0 rates have remained steady at 35–40%, many have questioned the need for RC at all [11]. In fact, a recent study concluded that while patients who achieved pathological complete response (pCR) lived longer, patients who did not achieve pCR had a worse prognosis than those who underwent direct RC without NAC [12]. As a result, insensitivity to NAC in some patients leads to tumor progression, overtreatment, and delayed surgery. Therefore, it is an urgent and difficult problem to predict the sensitivity of NAC for bladder cancer [13]. Many tentative efforts have been made to solve this problem. Soichiro Yoshida et al. investigated whether Diffusion-weighted MRI (DW-MRI) could predict NAC sensitivity of MIBC and concluded DW-MRI is a potential biomarker [14]. But the sample size was too small to draw a wide conclusion. Recent evidence suggests that bladder urothelial cancers harboring mutations in DNA damage response (DDR) pathways are associated with improved pathologic responses to NAC [15] and prolonged survival [16]. Contrary to this, Russell E.N.Baker et al. argued that clinical restaging and tumor sequencing are inaccurate indicators of response to NAC for MIBC [11]. Thus, predicting the sensitivity of NAC for bladder cancer patients has not been solved yet, which is a clinical problem to be solved urgently.

In the current study, we sought to understand whether the clinical and pathologic tools currently available in clinical practice are sufficient to determine individual patient responses to NAC accurately and reliably in a retrospective population. We downloaded the clinical data and the immunohistochemical results of 149 bladder cancer patients from the attachment of the paper published by Gottfrid Sjodahl et al. [17]. Multiple machine learning algorithms were used to screen and identify some genes and clinical features that were differentially expressed in different pathological reactions. Finally, the prediction model of NAC therapy for bladder cancer was constructed according to the 10 factors included. Our study identified some genes closely associated with NAC in bladder cancer and constructed predictive models for the efficacy of NAC. Furthermore, this study also provides ideas and clues to bladder cancer therapy, and the identified genes could also be considered as NAC biomarkers for bladder cancer.

Materials and methods

Data source and Pre-processing

We downloaded the clinical data of 149 bladder cancer patients and the immunohistochemical results of some genes from the attachments of the paper published by Gottfrid Sjodahl et al [17], and the data used in this study is attached in supplementary data 1. According to the pathological response of NAC, patients were divided into pCR, pathological partial response (pPR) and no pathological response (no pR) groups, and the clinical baseline data of patients in different groups were statistically analyzed and sorted out. The independent T test was used for measurement data and the Chi-square test was used for enumeration data. Among them, the clinical characteristics with $P<0.1$ were incorporated into the factors of subsequent model construction. Then, R software (R Foundation for Statistical Computing, Vienna, Austria) was used for subsequent analyses.

Influence of NAC pathological response on prognosis

There is a close relationship between the efficacy of NAC and the prognosis of bladder cancer. To clarify the relationship between pathological response of NAC and prognosis of bladder cancer, the Kaplan-Meier (K-M) analysis for OS, cancer-specific survival (CCS) and recurrence free survival (RFS) were proceeded based on the NAC pathological response groups with the aid of R software and the Log-Rank was utilized to test. The survival curve was plotted using the survminer R package.

Identification of genes associated with NAC efficacy

We compared the expression of the 16 genes detected by immunohistochemistry (IHC) among the three groups via the Kruskal-Wallis test to explore genes closely related to NAC. Finally, genes with $P<0.1$ were selected for mapping and demonstration using ggplot2 R package.

Survival analysis for genes related to NAC

To clarify the effect of genes associated with NAC efficacy on prognosis, six genes with $P<0.1$ were selected for K-M survival analysis. During the process, the K-M analysis for OS were proceeded based on the IHC expression of genes whose cut-off level was set at the median value with the aid of R software and the Log-Rank was utilized to test.

Lasso regression and correlation analysis

To prevent the over-fitting of the prediction model and further screen the clinical factors and genes expression, we selected the

significant factors for Lasso regression analysis, and analyzed the correlation of IHC expression levels of the selected genes. Lasso regression was completed using glmnet R package, and the correlation analysis was completed by using corrplot R package. The selected factors were used for the next step of model construction.

Predictive model construction and comparison

The significant factors screened via Lasso regression was used for model construction to predict the pathological response of NAC in patients with bladder cancer. Model construction methods include random forest (RF), support vector machine (SVM) and neural network (NN). Among which, RF analysis was completed via randomForest R package, SVM analysis via e1071 R package, and NN analysis via neuralnet R package. All 149 samples were used for the training set, and 30% of them were randomly selected as internal validation for the model validation. Then, we compared the accuracy, sensitivity, specificity and the area under curve (AUC) of the three different prediction models. Finally, we select the NN model with better prediction performance, and demonstrate the importance of screening factors and the prediction accuracy of the NN model.

Results

Clinical baseline data

We compared the clinical characteristics of three different pathological response groups to NAC, and the detailed results were shown in (Table 1). Among them, age, T, N, TURB path LVI, keratinization, CIS, Necrosis, RB_alt_status, and P16_alt_status are strongly correlated with NAC efficacy. These variables were used for further screening and analysis in subsequent Lasso regression.

Influence of NAC pathological reaction on prognosis

Survival analysis and comparison among the no pR, pPR and pCR groups showed that the prognosis of patients in the pCR and pPR groups was much better than that of patients with no pR for OS (Figure 1A), RFS (Figure 1B) and CSS (Figure 1C). This also indicates that the pathological response of NAC plays a very important role in the prognosis of bladder cancer patients, so predicting the efficacy of NAC therapy is also an urgent problem to be solved.

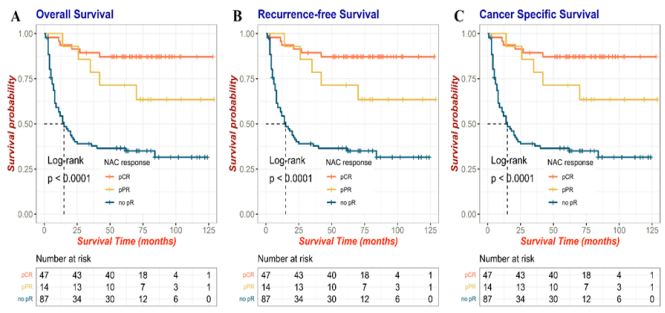


Figure 1: Kaplan-Meier analysis for bladder cancer NAC in different pathological response groups. **(A)** Overall survival; **(B)** Recurrence-free survival; **(C)** Cancer specific survival. NAC: neoadjuvant chemotherapy; pCR: pathological complete response; pPR: pathological partial response; no pR: no pathological response.

Table 1: Clinical baseline data.

	no pR	pCR	pPR	*P*
Total	87	48	14	
Age (years)				0.125
<60	20	6	5	
≥60	67	42	9	
Sex (Male)	68	37	11	0.987
T stage				0.0008
T1	0	1	0	
T2	24	31	4	
T3	49	15	8	
T4	14	1	2	
N stage				0.254
N0	75	42	12	
N1	9	1	1	
N2	3	3	1	
N3	0	2	0	
M				0.347
M0	87	47	14	
M1	0	1	0	
TURB path LVI (yes)	37	16	1	0.03
TURB path keratinization (yes)	25	7	1	0.051
TURB path CIS (yes)	19	10	6	0.201
TURB path necrosis (yes)	56	28	5	0.094
Histologic Variant (yes)	16	7	1	0.535
RB_alt_status (mutation)	30	24	4	0.147
P16_alt_status (mutation)	44	13	7	0.026
LundTax IHC subtype				0.388
Ba/Sq	15	3	0	
GU	27	19	5	
Mes-like	2	1	0	
NE-like	5	4	0	
Uro	37	21	9	
Consensus.subtype (%)				0.779
BASQ	23	8	2	
LumNS	10	4	2	
LumP	20	16	5	
LumU	13	10	2	
NE_like	7	2	0	
StromaRich	14	8	3	
NAC protocol				0.818
GC	51	24	7	
MVAC	34	23	7	
Other	2	1	0	
NAC courses				0.815
2	5	1	0	
3	57	34	10	
4	16	10	4	
5	4	1	0	
6	5	2	0	

Identification of genes associated with NAC efficacy

The IHC expression values of 16 genes were analyzed and compared among different pathological response groups, and the six genes with *P*<0.1 were selected and showed in (Figure 2A-F). These six genes are CDH1, EPCAM, FOXA1, CCND1, P16 and ZEB2, respectively. These genes are associated with NAC response in bladder cancer, and can be used to predict NAC response. Moreover, these genes are also potential NAC therapy targets for bladder cancer.

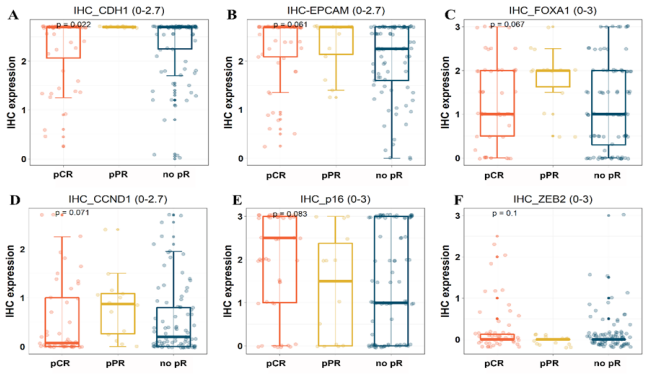


Figure 2: Comparison of 6 NAC-related genes with the most significant differences among three different pathological response groups. **(A-F)** are CDH1, EPCAM, FOXA1, CCND1, P16, and ZEB2 respectively.

Survival analysis for six genes related to NAC

K-M analysis for OS was used to explore the prognostic value of the six genes associated with NAC and the results were shown in (Figure 3). We found that although these six genes were not significant differences with prognosis, there were obvious differences between high and low expression groups. Therefore, these six genes are related to prognosis, and were used for further screening and analysis in subsequent Lasso regression.

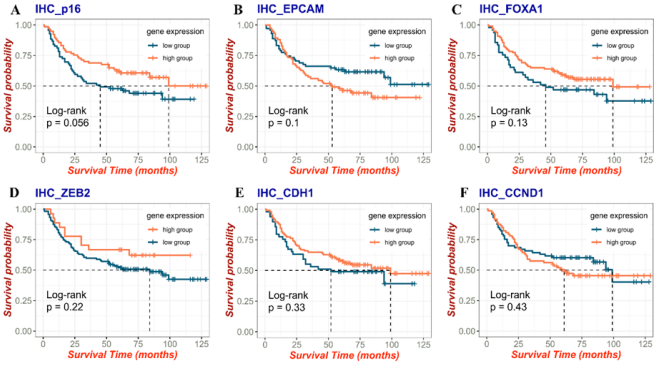


Figure 3: Kaplan-Meier analysis for 6 NAC-related genes. Kaplan–Meier survival analysis and log-rank test for the 6 NAC-related genes were conducted, and patients were divided into high and low groups based on the median value of gene expressions detected by IHC.

Lasso regression and correlation analysis

The results of Lasso regression (Figure 4A-B) showed that 10 of the 15 NAC-related factors can be used for model construction, while the other 5 factors may have potential collinearity, which would affect the fitting degree of the model. The correlation analysis of the 6 NAC-related genes is shown in Figure 4C, and we could find that CCND1 was significantly correlated with FOXA1 and P16, while CDH1 was significantly correlated with FOXA1 and ZEB2. Therefore, CCND1 and CDH1 were excluded by Lasso regression analysis. Figure 4D shows the importance ranking of the 10 genes included in lasso regression.

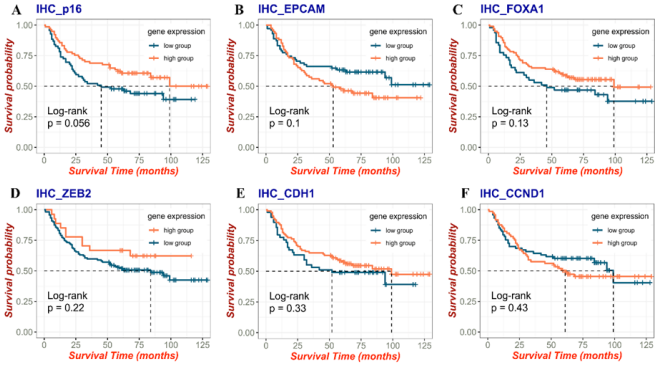


Figure 4: Lasso regression and correlation analysis for the 15 significant factors. **(A)** The trajectory of each independent variable. The horizontal axis shows the logarithm of lambda, and the vertical axis displays the coefficient of lambda. **(B)** The confidence interval under each lambda. **(C)** The correlation analysis for 6 NAC-related genes. Blue represents negative correlation, and red represents positive correlation. **(D)** The importance ranking for Lasso regression analysis screened independent variables.

Predictive model construction and comparison

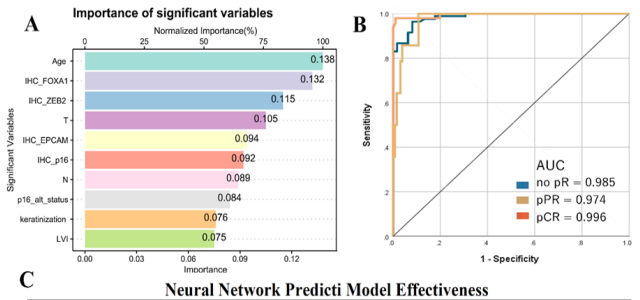
The 10 clinical characteristics or genes expression were used to construct the prediction model. The main analysis results of RF/SVM/NN models are shown in supplementary data 2, and the comparison results of the three models are shown in (Table 2). Among the three models, the NN model has higher accuracy (93.8%) and AUC (0.984). It can predict the efficacy of NAC for bladder cancer more accurately.

Table 2: Comparison of prediction results of three prediction models in train set.

	Correct percentage				AUC
Train set	overall	no pR	pCR	pPR	
NN	93.8%	96.3%	97.9%	64.3%	0.984
RF	81.3%	79.3%	83.3%	85.7%	na
SVM	89.6%	95.1%	85.4%	71.4%	0.948

Demonstration of prediction results of NN model

The NN model has the best prediction accuracy, so we present the main results of the NN model. (Figure 5A) shows the importance of various predictive factors in the NN model, among which age and IHC_FOXA1 were of higher importance. (Figure 5B) shows ROC curves of different pathological reactions, and the AUC value of no pR, pPR and pCR were 0.985, 0.974 and 0.996, respectively. (Figure 5C) shows the confusion matrix of training set and verification set. It can be seen that the NN model has high accuracy in both training set and internal validation set.



C

Neural Network Predicti Model Effectiveness

Samples	True	prediction			
		no pR	pCR	pPR	Correct percentage
Train set	no pR	79	1	2	96.3%
	pCR	1	47	0	97.9%
	pPR	4	1	9	64.3%
	Overall percentage	58.3%	34.0%	7.6%	93.8%
Test set	no pR	22	1	2	88.0%
	pCR	0	12	0	100.0%
	pPR	1	0	3	75.0%
	Overall percentage	56.1%	31.7%	12.2%	90.2%

dependent variable: Pathologic response

Figure 5: Demonstration of the results of the neural network model. (A) The importance ranking for the significant variables in NN analysis. (B) ROC curves of different pathological reactions, and the AUC value of no pR, pPR and pCR were 0.985 0.974 and 0.996 respectively. (C) The confusion matrix of training set and verification set.

Discussion

Currently, the clinical guidelines of the European Association of Urology and the American Society of Clinical Oncology have recommended NAC for the preoperative treatment of MIBC [18-20]. Nonetheless, patients may be ineligible for either chemotherapy [21] or RC, and selection of the most appropriate therapy depends on a staging system that may not accurately determine the eligibility of a patient for a specific treatment plan [22]. Thus, a more personalized approach to bladder NAC therapy management is warranted [13].

The results of our study showed that the prognosis of bladder cancer patients whose NAC efficacy reached pCR was much better than that of patients without pathological reaction, which was consistent with previous studies [6,7]. However, for patients with no pR, NAC therapy may cause delay of operation time, delay of surgery and overtreatment, resulting in the progression and poor prognosis of bladder cancer. Therefore, in this study, available clinical features and pathological data were used to explore the genes and clinical features closely related to NAC for bladder cancer, and to construct a prediction model for the efficacy of NAC for bladder cancer patients.

The clinical factors included in this study are age, T stage, N stage, TURB path LVI (lymphatic invasion) and keratinization. Age is one of the most important risk factors for cancer, and the occurrence and prognosis of cancer patients is highly influenced by age and ageing [19,23]. Studies have shown that older patients are less likely to receive NAC [24], and this may be one reason for the poor prognosis in older patients with bladder cancer. Clinical stages are associated with the indications of NAC therapy and RC in bladder cancer patients [9]. NAC with cisplatin-based combinations has been standard for decades in patients with resectable N0M0-invasive bladder cancer [18]. Moreover, LN-positive patients previously treated with NAC have a poor prognosis, significantly worse than LN-positive patients subsequently treated with AC, and should be considered for protocols using sandwich chemotherapy approaches or novel agents [25]. Therefore, NAC therapy should not be used in patients with positive lymph nodes. Intracavitary keratosis of bladder cancer was also one of the factors associated with NAC in this study. Hendrik Jütte et.al [20] retrospectively analyzed the association of basal and luminal mRNA expression patterns such as Keratin 20 (KRT 20), Keratin 5 (KRT5), as well as ESR1 and ERBB2 in patients with MIBC at transurethral resection (TUR-BT) with pCR at RC after platinum-based NAC, and found that tumors with elevated expression of markers associated with intracavity differentiation (KRT20, ERBB2, ESR1) was associated with a higher incidence of pCR. Therefore, all the clinical factors included in this study have a certain relationship with bladder cancer NAC.

In this study, the four genes screened into the prediction model are P16, EPCAM, ZEB2 and FOXA1. These genes are closely associated with NAC efficacy and prognosis of bladder cancer. P16 is a tumor suppressor gene directly involved in cell cycle regulation, and deletion or mutation of P16 is very common in tumors [26,27]. In bladder cancer, the expression of P16 is significantly reduced, and it is associated with various molecular subtypes of bladder cancer [28]. The molecular subtypes of bladder cancer are closely related to the efficacy of NAC therapy [17]. EPCAM expression is increased in bladder cancer and is associated with lymph node metastasis [29]. EPCAM is mainly involved in bladder cancer metastasis and chemotherapy resistance through PI3K/AKT signaling pathway [30]. Moreover, antibody-drug conjugations targeting EPCAM represent a novel therapeutic approach for urothelial carcinoma [31]. Expression of ZEB2 correlates with histopathological grade in papillary urothelial tumors of the urinary bladder [32]. In addition, ZEB2 is involved in the invasion and metastasis of bladder cancer [33]. FOXA1 is expressed throughout the urothelium, and associated with the urothelial differentiation process and the molecular subtypes of bladder cancer. There is a near relationship between MIBC tumor subtypes (identified by IHC) and the response to NAC and survival [12]. In brief, P16, EPCAM, ZEB2 and FOXA1 are well correlated with NAC efficacy and prognosis of bladder cancer.

In this study, three different machine learning algorithms were used to construct prediction models for the efficacy of NAC for bladder cancer. Among them, the neural network prediction model has high accuracy. The advantage of this study lies in the use of a variety of clinical characteristics and genes expression tested by IHC to predict the efficacy of NAC. Secondly, the prediction model is highly reliable by comparing various methods. However, this study also has shortcomings. The sample size of this study is small, and the model is only verified internally, but not externally. A larger sample size and more scientific research methods are needed to predict the efficacy of NAC for bladder cancer in the future.

Conclusion

In this study, available clinical features and gene expression detected by IHC were used to explore factors closely related to NAC for bladder cancer, and to construct prediction models to predict the efficacy of NAC for bladder cancer patients. This study not

only finds several targets for NAC and provides reference for the feasibility of NAC therapeutic prediction model for bladder cancer, but also provides a new clue for other researchers and clinical workers to predict the efficacy of NAC.

Declarations

Conflict of interest: All the authors declare no conflicts of interest.

Funding: This work was supported by the Open Foundation of Gansu Key Laboratory of Functional Genomics and Molecular Diagnostics and Gansu Province Intellectual Property Planning project (21ZSCQ012).

Data availability: All data and material analyzed can be obtained from the corresponding author.

References

1. Organization WH, Cancer IAfRo. Estimated number of new cases in 2018, worldwide, both sexes, all ages. In: 2018.
2. van Osch FH, Jochems SH, van Schooten FJ, Bryan RT, Zeegers MP. Quantified relations between exposure to tobacco smoking and bladder cancer risk: a meta-analysis of 89 observational studies. International journal of epidemiology. 2016; 45(3): 857-870.
3. Lewis GD, Haque W, Verma V, Butler EB, Teh BS. The Role of Adjuvant Radiation Therapy in Locally Advanced Bladder Cancer. Bladder cancer (Amsterdam, Netherlands). 2018; 4(2): 205-213.
4. Aggen DH, Drake CG. Biomarkers for immunotherapy in bladder cancer: a moving target. Journal for immunotherapy of cancer. 2017; 5(1): 94.
5. Stenzl A, Cowan NC, De Santis M, et al. The updated EAU guidelines on muscle-invasive and metastatic bladder cancer. European urology. 2009; 55(4): 815-825.
6. Petrelli F, Coinu A, Cabiddu M, Ghilardi M, Vavassori I, Barni S. Correlation of pathologic complete response with survival after neoadjuvant chemotherapy in bladder cancer treated with cystectomy: a meta-analysis. European urology. 2014; 65(2): 350-357.
7. Sherif A, Holmberg L, Rintala E, et al. Neoadjuvant cisplatinum based combination chemotherapy in patients with invasive bladder cancer: a combined analysis of two Nordic studies. European urology. 2004; 45(3): 297-303.
8. Cathomas R, Lorch A, Bruins HM, et al. The 2021 Updated European Association of Urology Guidelines on Metastatic Urothelial Carcinoma. European urology. 2022; 81(1): 95-103.
9. Aragon-Ching JB, Werntz RP, Zietman AL, Steinberg GD. Multidisciplinary Management of Muscle-Invasive Bladder Cancer: Current Challenges and Future Directions. American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting. 2018; 38: 307-318.
10. Anan G, Hatakeyama S, Fujita N, et al. Trends in neoadjuvant chemotherapy use and oncological outcomes for muscle-invasive bladder cancer in Japan: a multicenter study. Oncotarget. 2017; 8(49): 86130-86142.
11. Becker REN, Meyer AR, Brant A, et al. Clinical Restaging and Tumor Sequencing are Inaccurate Indicators of Response to Neoadjuvant Chemotherapy for Muscle-invasive Bladder Cancer. European urology. 2021; 79(3): 364-371.
12. Bhindi B, Frank I, Mason RJ, et al. Oncologic Outcomes for Patients with Residual Cancer at Cystectomy Following Neoadjuvant Chemotherapy: A Pathologic Stage-matched Analysis. European urology. 2017; 72(5): 660-664.
13. Shah JB, McConkey DJ, Dinney CP. New strategies in muscle-invasive bladder cancer: on the road to personalized medicine. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011; 17(9): 2608-2612.
14. Yoshida S, Koga F, Kobayashi S, et al. Role of diffusion-weighted magnetic resonance imaging in predicting sensitivity to chemoradiotherapy in muscle-invasive bladder cancer. International journal of radiation oncology, biology, physics. 2012; 83(1): e21-27.
15. Plimack ER, Dunbrack RL, Brennan TA, et al. Defects in DNA Repair Genes Predict Response to Neoadjuvant Cisplatin-based Chemotherapy in Muscle-invasive Bladder Cancer. European urology. 2015; 68(6): 959-967.
16. Teo MY, Bambury RM, Zabor EC, et al. DNA Damage Response and Repair Gene Alterations Are Associated with Improved Survival in Patients with Platinum-Treated Advanced Urothelial Carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017; 23(14): 3610-3618.
17. Sjödahl G, Abrahamsson J, Holmsten K, et al. Different Responses to Neoadjuvant Chemotherapy in Urothelial Carcinoma Molecular Subtypes. European urology. 2021.
18. Witjes JA, Bruins HM, Cathomas R, et al. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. European urology. 2021; 79(1): 82-104.
19. Chang SS, Bochner BH, Chou R, et al. Treatment of Non-Metastatic Muscle-Invasive Bladder Cancer: AUA/ASCO/ASTRO/SUO Guideline. The Journal of urology. 2017; 198(3): 552-559.
20. Jütte H, Reike M, Wirtz RM, et al. KRT20, KRT5, ESR1 and ERBB2 Expression Can Predict Pathologic Outcome in Patients Undergoing Neoadjuvant Chemotherapy and Radical Cystectomy for Muscle-Invasive Bladder Cancer. Journal of personalized medicine. 2021; 11(6).
21. Galsky MD, Hahn NM, Rosenberg J, et al. A consensus definition of patients with metastatic urothelial carcinoma who are unfit for cisplatin-based chemotherapy. The Lancet Oncology. 2011; 12(3): 211-214.
22. Compérat EM, Burger M, Gontero P, et al. Grading of Urothelial Carcinoma and The New "World Health Organisation Classification of Tumours of the Urinary System and Male Genital Organs 2016". European urology focus. 2019; 5(3): 457-466.
23. Kendal WS. Dying with cancer: the influence of age, comorbidity, and cancer site. Cancer. 2008; 112(6): 1354-1362.
24. Gan T, Chen Q, Huerta CT, Huang B, Evers BM, Patel JA. Neoadjuvant Therapy in Stage II/III Rectal Cancer: A Retrospective Study in a Disparate Population and the Effect on Survival. Diseases of the colon and rectum. 2021; 64(10): 1212-1221.
25. Cha EK, Sfakianos JP, Sukhu R, Yee AM, Sjoberg DD, Bochner BH. Poor prognosis of bladder cancer patients with occult lymph node metastases treated with neoadjuvant chemotherapy. BJU international. 2018; 122(4): 627-632.
26. Dizdar O, Demirci U. p53 status in locally advanced bladder cancer. Journal of clinical oncology : official journal of the American Soci-

ety of Clinical Oncology. 2012; 30(3): 339; author reply 339-340.

27. Carneiro FP, Amorim RF, de Vasconcelos Carneiro M, et al. P16 as a marker of carcinoma in effusions and peritoneal washing. BMC cancer. 2020; 20(1): 225.

28. Olkhov-Mitsel E, Hodgson A, Liu SK, Vesprini D, Xu B, Downes MR. Three-antibody classifier for muscle invasive urothelial carcinoma and its correlation with p53 expression. Journal of clinical pathology. 2021.

29. van der Fels CAM, Rosati S, de Jong IJ. EpCAM Expression in Lymph Node Metastases of Urothelial Cell Carcinoma of the Bladder: A Pilot Study. International journal of molecular sciences. 2017; 18(8).

30. Zuo Y, Xu X, Chen M, Qi L. The oncogenic role of the cerebral endothelial cell adhesion molecule (CERCAM) in bladder cancer cells in vitro and in vivo. Cancer medicine. 2021; 10(13): 4437-4450.

31. Lattanzi M, Rosenberg JE. The emerging role of antibody-drug conjugates in urothelial carcinoma. Expert review of anticancer therapy. 2020; 20(7): 551-561.

32. Lee H, Jun SY, Lee YS, Lee HJ, Lee WS, Park CS. Expression of miRNAs and ZEB1 and ZEB2 correlates with histopathological grade in papillary urothelial tumors of the urinary bladder. Virchows Archiv: an international journal of pathology. 2014; 464(2): 213-220.

33. Sun DK, Wang JM, Zhang P, Wang YQ. MicroRNA-138 Regulates Metastatic Potential of Bladder Cancer Through ZEB2. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2015; 37(6): 2366-2374.