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Clinical-Bladder cancer

Catalog of prognostic tissue-based biomarkers in patients treated with neoadjuvant systemic therapy for urothelial carcinoma of the bladder: a systematic review.

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Abstract

PURPOSE: The present systematic review aimed to identify prognostic values of tissue-based biomarkers in patients treated with neoadjuvant systemic therapy (NAST), including chemotherapy (NAC) and checkpoint inhibitors (NAI) for urothelial carcinoma of the bladder (UCB).

MATERIAL AND METHODS: The PubMed, Web of Science, and Scopus databases were searched in August 2020 according to the PRISMA statement. Studies were deemed eligible if they compared oncologic or pathologic outcomes in patients treated with NAST for UCB with and without detected pretreatment tissue-based biomarkers.

RESULTS: Overall, 44 studies met our eligibility criteria. Twenty-three studies used immunohistochemistry (IHC), 19 − gene expression analysis, three - quantitative polymerase chain reaction (QT PCR), and two − next-generation sequencing (NGS). According to the currently available literature, predictive IHC-assessed biomarkers, such as receptor tyrosine kinases and DNA repair pathway alterations, do not seem to convincingly improve our prediction of pathologic response and oncologic outcomes after NAC. Luminal and basal tumor subtypes based on gene expression analysis showed better NAC response, while claudin-low and luminal-infiltrated tumor subtypes did not. In terms of NAI, PD-L1 seems to maintain value as a predictive biomarker, while the utility of both tumor mutational burden and molecular subtypes remains controversial. Specific genomic alterations in DNA repair genes have been shown to provide significant predictive value

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in patient treated with NAC. QT PCR quantification of specific genes selected through microarray analysis seems to classify cases regarding their NAC response.

CONCLUSION: We believe that the present systematic review may offer a robust framework that will enable the testing and validation of predictive biomarkers in future prospective clinical trials. NGS has expanded the discovery of molecular markers that are reflective of the mechanisms of the NAST response.

Keywords: Biomarkers; UCB; bladder cancer; Neoadjuvant systemic therapy; NAC; systematic review

1. Introduction

Urothelial carcinoma of the bladder (UCB) is one of the most frequently diagnosed and harmful cancers worldwide [1]. Neoadjuvant cisplatin based combination chemotherapy (NAC) prior to radical cystectomy is the preferred first treatment in cisplatin eligible patients with muscle-invasive UCB [2, 3]. However, multiple reasons impeded the widespread uptake of NAC such as the fear of unnecessary chemotoxicity, its perceived relatively modest survival benefit, and/or the fear of a delay to radical treatment [4, 5]. Moreover, UCB is a highly heterogeneous disease with varied response rates when therapies are given in unselected patient populations. Identification of the patients who are unlikely to respond to NAC could allow better selection of patients to immediate radical cystectomy or allocation of different systemic therapies such as checkpoint inhibitors (CPI).

Modern medical decisions can be tailored to the individual patient based on predicted response or risk of disease. Understanding the molecular basis of disease has ushered in a new age of precision medicine. Molecular markers are promising tools that may give insight into which UCB patients will or will not benefit from neoadjuvant systemic therapy (NAST) and which have the potential to overcome the limitations of conventionally used prognostic risk factors. In addition, a biomarker-based strategy to identify patients who should undergo NAC is more cost-effective compared to the current unselected use of NAC or radical cystectomy alone [6]. Numerous publications provided data on potential molecular markers associated with NAC response in UCB patients; however, none is yet validated or widely used in the clinical practice [7−9].

In this systematic review we aimed to summarize the available evidence as well as to determine whether pretreatment tissue-based biomarkers may help predict oncologic and pathologic outcomes in patients treated with NAST for UCB. This review is a benchmark for future developments.

2. Evidence acquisition

2.1. Literature search

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [10]. This study's protocol was registered a priori on the International Prospective Register of Systematic Reviews (PROSPERO; Registration ID CRD42020208417).

The PubMed, Web of Science, and Scopus databases were searched in August 2020 to identify studies reporting on the prognostic value of tissue-based biomarkers in patients treated with NAST for UCB. A comprehensive systematic literature search was independently performed by two authors. The keywords used in our search strategy included: (NAC OR neoadjuvant) AND (bladder OR urothelial) AND (cancer OR tumor OR malignancy OR carcinoma) AND (biomarker). In addition, we manually searched for potentially relevant trials from the references of selected studies. The primary outcome of interest was both oncologic and pathologic outcomes in patients treated with NAST for UCB.

After removing duplicates, two independent reviewers screened the titles and abstracts. Any citation which either reviewer thought should be included or unclear for inclusion was identified for full text screening. Subsequently, reviewers reviewed full texts of eligible articles for final inclusion and data extraction. In cases of disagreement, the authors consulted with the co-authors, and final decisions were reached by consensus.

2.2. Inclusion and exclusion criteria

We included all non-randomized observational studies that reported on the prognostic value of tissue-based biomarkers in UCB.

The PICO in this study was the following: patients treated for UCB with detected pretreatment tissue-based biomarkers. Intervention included NAST for UCB. Control group included those patients without pretreatment tissue-based biomarkers. The outcome included any measure of association between oncologic and pathologic outcomes and the candidate biomarker, the diagnostic performance of the biomarker.

We excluded reviews, letters, editorials, animal studies, study protocols, case reports, meeting abstracts, replies from authors, brief correspondence, and articles not published in English. Furthermore, we excluded the studies

that did not provide data regarding the oncologic or pathologic outcomes. References of all papers included were scanned for additional studies of interest.

2.3. Data extraction

Data extracted from each study were independently extracted by two independent reviewers. Extracted data included the following: first author's name, publication year, study design, demographics characteristics including age range, sample size, pathological T stage, follow-up duration, NAC regime, definition of response, type of biomarkers, methods of biomarkers detection, % of patients with high expression, and Main results. Subsequently, the hazard ratios (HR) and 95% confidence intervals (CI) of tissue-based biomarkers associated with each outcome were retrieved.

2.4. Evidence synthesis

The literature search identified 624 unique references. Among them, 233 records were removed due to duplication, and 261 articles were excluded due to unrelated outcomes during the screening process (Figure 1). Of the 130 full-text articles assessed for eligibility, 86 were excluded based on the selection criteria.

Overall, 44 studies were finally included in the present systematic review. Characteristics of the studies are shown in Table 1. Fifteen of the included studies had a prospective study design, and twenty-nine were retrospective.

3. Immunohistochemistry (IHC)

Twenty-three studies provided data on the pretreatment biomarkers detected at IHC.

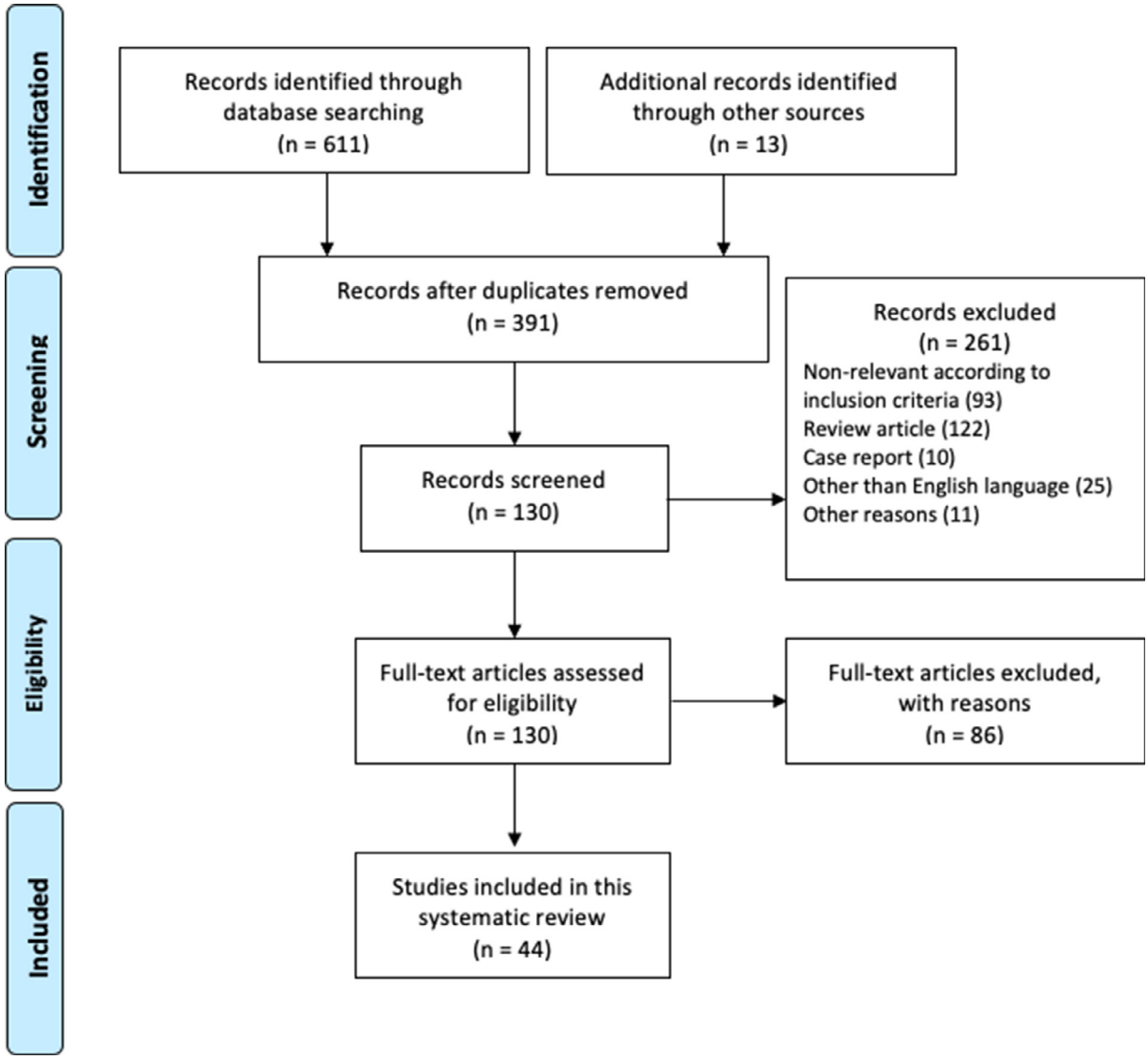

Figure 1. Flow diagram of the study selection procedure for the systematic review.

Table 1
Characteristics of included studies reporting biomarker predictive models of response to neoadjuvant systemic therapy in patients with bladder cancer.

Author, publication year	Study design	Number of NAC patients	Age, years (median, range)	Stage	Follow-up, median (range)	NAC	Definition of response	Type of markers evaluated(cut off values)	Methods	% of high expression (%)	Main results
Bandini, 2020 [27]	P	112	66 (IQR 61-73)	T2-T4, N0	NR	Pembrolizumab	pT0N0	TMB (11 mut/Mb)	CGP	TMB (12.5)	TMB was not associated with NAC response on multivariable analysis (OR 1.04, 0.98-1.10, p=0.09)
Baras, 2015 [42]	R	37	63 (44−83)	T2-T4	NR	GC	<ypT2	mRNAs (10%), Ki67, p53, GDPD3, and SPRED1	IHC	NR	The combination of GDPD3 and SPRED1 predicted NAC response (p<0.001)
Baras, 2016 [43]	R	41	64 (45-82)	T2-T4, N0/N+	NR	NR	<ypT2	PD-L1, CD8, FOXP3, the ratio of CD8/FOXP3	IHC	NR	The ratio of CD8/FOXP3 TIL densities was strongly associated with response (p=0.0003)
Choi, 2014 [23]	R	18	NR	T2-T4, N0/N+, M0/+	NR	Platinum-based	<pT1	Molecular subtypes: basal-like, luminal- like and p53-like	Whole genome mRNA expression profiling	basal (22), luminal (25), p53-like (27)	Response was 0% in p53-like, 40% - basal-like and 67% - luminal-like subtypes (p=0.018)
Choueiri, 2014 [16]	P	31	NR	T2-T4, N0-1, M0	2 years	ddMVAC	<pT1	ERCC1 (H score>0.1)	IHC	ERCC1 (39)	43% of ERCC1-positive and 60% of ERCC1-negative patients achieved PR
de Jong, 2019 [44]	R	223	62 (56−71)	T2−4, N0-3, M0	NR	NR	NR	lncRNA (LC1, LC2, LC3, LC4 clusters) and mRNA subtypes (luminal-papillary, luminal, luminal- infiltrated, basal squamous and neuronal)	Gene expression analysis	FGFR3+ (16%)	The luminal-papillary lncRNA cluster (LC3) tumors had favorable prognosis and had enhanced FGFR3, SHH, and wild-type p53 pathway activity.
Efstathiou, 2019 [24]	R	223	61.7	T2-T4, N0, M0	3.5 year (IQR 2.1 - 5.0)	NR	NR	Molecular subtypes: luminal, luminal- infiltrated, basal, claudin-low	Transcriptome-wide gene expression profiles	NR	DSS and OS were worse among patients with claudin-low tumors (p=0.01 and p=0.068, respectively). A stromal signature was associated with worse DSS and OS (p=0.006 and p=0.015, respectively).
Font, 2011 [33]	R	57	64 (41-80)	T2-T4, N0/+, M0/+	45 mo (14−190)	GC, CMV	pT0-1	BRCA1 (>26.77)	RT-PCR	BRCA1 (32)	60% of patients with low/intermediate BRCA1 levels atteined PR vs 22% of those with high levels (p=0.01). Median OS was 168 mo in patients with low/intermediate levels and 34 mo in patients with high BRCA1 levels (P = 0.002).
Garcia del Muro, 2004 [18]	R	82	61 (30−74)	T2-T4, N0, M0	55 mo	MVAC, CMV, CbMV + radiotherapy	≤ T1	p53 (20%), p21 (20%), pRB (10%)	IHC	p53 (47), p21 (52), pRB (67)	Positive p53 and p21 were independently associated with decreased survival with bladder preservation (both p<0.02). DFS: positive p53 and p21 were independently associated with decreased DFS (p<0.005 and p<0.009, respectively). OS: p53 overexpression was associated with poor OS (p<0.03). The positive expression of combination p53 and p21 was a strong and unfavorable prognostic factor for survival with bladder preservation (p<0.006), DFS (p<0.003), and OS (p<0.02).
Groenendijk, 2016 [36]	P	94	NR	NR	NR	GC, GCb, MVAC	ypT0N0	178 cancer- associated genes	NGS	NR	ERBB2 mutations are strongly associated with response (p=0.006), whereas ERCC2 mutations are not.
Grossman, 2006 [12]	P	94	64 (39-80)	T2-T4a, N0, M0	NR	MVAC	NR	Ki67 (1000 cells), p53 (20%), angiogenesis	IHC	NR	Ki67 expression was not associated with PFS (HR 0.62; 95% CI 0.37-1.03; p=0.063) and OS (HR 0.74; 95% CI 0.44-1.24; p=0.25). p53 expression was not associated with worse PFS (HR=1.02; 95% CI 0.61-1.71; p=0.93) and OS (HR 1.48; 95% CI 0.87-2.53; p=0.15). Angiogenesis was not associated with PFS (HR 1.0; 95% CI 0.62-1.64; p=0.99) and OS (HR1.04; 95% CI 0.63-1.70; p=0.89).
Hemdan, 2015 [19]	R	125	66	T1G3, T2−T4, Nx, M0	NR	Cisplatin/ methotrexate or doxorubicin + radiotherapy	pT0 or Ta/CIS	Emmprin and survivin	IHC	Emmprin (28), surviving (50)	OS: negative emmprin expression had significantly greater OS (71% vs 38%, p <0.001). CSS: in negative and positive emmprin expression was 76% vs 56% (p=0.027).
Hemdan, 2018 [45]	R	177	NR	T1G3, T2−T4, Nx, M0	NR	Cisplatin/ methotrexate	pT0 or Ta/CIS	CCT-α (20%)	IHC	CCT-α (24)	Improved OS with NAC treatment only in the CCT-α-negative group (p=0.006). No difference was found in the CCT-α-positive group (p=0.9).
Hensley, 2019 [46]	R	69	NR	T2	NR	MVAC, GC	ypT0N0	E-cadherin (125), N-cadherin (34.7), b-catenin (125), vimentin (50.3),	IHC	NR	Extravesical disease showed increased N-cadherin (p= 0.004), increased vimentin (p=0.028), increased b-catenin (p= 0.019), decreased P-cofilin

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Table 1 (*Continued*)

Author, publication year	Study design	Number of NAC patients	Age, years (median, range)	Stage	Follow-up, median (range)	NAC	Definition of response	Type of markers evaluated(cut off values)	Methods	% of high expression (%)	Main results
								α-tubulin (181), cofilin (214), P-cofilin (223), Zeb-1 (82.8), TUNEL (1.82)			(P = 0.036), increased α-tubulin (p=0.007). cPR: low N-cadherin (p=0.044), low vimentin (p=0.013), low P-cofilin (p=0.037), and low Zeb-1 (p=0.030) expression. Better CSS: low N-cadherin expression (p=0.016) and high TUNEL (p=0.003).
Kato, 2010 [34]	P	37	67 (52-78)	T2-T4, N0, M0	NR	GCb	NR	Numerical prediction scoring system including 12 genes	Genome- wide expression profiling, PT-PCR	NR	12 genes separated responders (9 patients) from non-responders (9 patients). Among these genes IPO-7 and SLC22A18 were up-regulated in non-responders.
Kilari, 2016 [47]	R	44	68 (42-81)	T2-T4, N0-1, M0	NR	GC, MVAC, GCb, Cis/ Etoposide, or Carbo/ Etoposide	≤pT1	CTR-1	IHC	CTR-1 (43)	Higher CTR-1 expression score correlated with PR in pre-NAC and post NAC specimens (p=0.0076 and p=0.023, respectively).
Kiss, 2017 [17]	R	127	NR	NR	NR	GC	<ypT2N0	Her2 (10%), ERBB2 gene	IHC and FISH	Her2 (19), ERBB2 gene (19)	None of the Her2 alterations were related to PR and OS.
Liu, 2017 [32]	R	101	NR	NR	NR	GC, MVAC	≤pT1	Cell-cycle and immune checkpoint regulation genes	DNA exome sequencing	TP53 (68%), KMT2D (28%), CDKN2A (23%), ARID1A (22%), PIK3CA (22%), and RB1 (20%)	Genetic alterations in genes associated with cell cycle checkpoints and regulators (E2F3, JUN, FBXW7) suggests potential resistance.
Miron, 2019 [37]	P	58	65 (44−83)	T2-4, N0-1, M0	74 mo	GC, MVAC	T0N0M0	DNA damage repair genes	NGS	NR	Mutations in ATM, RB1, or FANCC were significantly associated with improved OS (p=0.0043) and DSS (p=0.0015). The 5-yr survival rates were also higher for both OS (85%, 95% CI 60.4−94.9% vs 46%, 95% CI 29.5 − 61.7%) and DSS (90%, 95% CI 64.8−97.3% vs 49%, 95% CI 31.6− 64.9%) in patients with one or more mutations compared to those without.
Necchi, 2020 [26]	P	114	66 (60−71)	T2-T4, N0, M0	13.2 mo	Pembrolizumab	pT0	PD-L1 CPS (≥10), TMB	CGP	PD-L1 CPS (67)	TMB and CPS were associated with both the pT0 and the pT1 response (all p<0.03).
Necchi, 2020 [25]	R	140 (NAC)	62 (54−70)	T2-T4, N0, M0	8 mo (IQR: 5 −13.5 mo)	NR	ypT0N0	Molecular subtypes: basal squamous, luminal nonspecified, luminal papillary, luminal unstable, stroma-rich, and NE-like. TMB, PD-L1 CPS (≥10), IFNγ, IFNα.	CGP	NR	The molecular subtypes were not significantly associated with response (all p>0.2).
		84 (PURE-01)	68 (62−74)	T2-T4, N0, M0	18.4 mo (IQR 12 −22.4 mo)	Pembrolizumab					The Immune 190 signature was significant for cPR (p = 0.02) in PURE-01, but not in the NAC cohort (p = 0.7). Hallmark signatures for IFNγ (p= 0.004) and IFNα response (p= 0.006) were also associated with cPR for PURE-01, but not for NAC (IFNγ: p=0.9 and IFNα: p = 0.8).
Nomura, 2013 [48]	R	44	70 (43-84)	T1G3, T2N0M0	47 mo	Cisplatin/ methotrexate/ doxorubicin	NR	Snail (H-score > 10)	IHC	Snail (34.1)	DFS was significantly shorter for the Snail-positive group (p=0.014). DSS was significantly shorter for the Snail-positive group (p=0.039). In multivariate analysis, Snail expression level was identified as an independent prognostic factor for DSS (p = 0.020).
Nomura, 2015 [49]	R	44	70 (43-84)	T1G3, T2N0M0	47 mo	Cisplatin/ methotrexate/ doxorubicin	pT0 or Ta/CIS	DYRK2	IHC	DYRK2 (47.7)	Sensitivity and specificity of DYRK2 expression in terms of complete response were 62.5 % and 91.7 %, respectively (p=0.0018). DSS was significantly higher for DYRK2-positive patients (p=0.017).
Ornstein, 2018 [50]	P	36	68 (44−87)	T0-T4, N0/N+, M0	NR	GC, GCb, MVAC, or others	pT0N0	MDSC	NR	MDSC (34.8)	Tumor MDSC subtypes were not significantly associated with response.
Pal, 2016 [51]	R	36	65 (36−76)	T0-T4, N0/N+, M0	38 mo	GC, MVAC	NR	CD15 (105 cells/hpf), pSTAT3 (254 cells/hpf), IL-17 (8 cells/hpf)	IHC (LN)	NR	No differences in OS were noted
Pichler, 2019 [52]	R	23	66.5 (48-76)	T0-T4, N0-N3, M0	8 mo (6 - 89	GC	ypT0-T1N0	TMB (≥10 mut/Mb), chromosomal aberrations, CD3, CD8, PD-L1, FoxP3, Cytokeratin. Molecular subtypes: luminal, basal.	WES, IHC	DNA damage repair alterations (38.1), TP53 (45), ARID1A/B (40), and KMT2B/C/ D/E (35)	Chromosomal 7p12 amplification (HUS1, EGFR, ABCA13, and IKZF1) predicted non-response with a sensitivity and specificity of 71.4% and 100%, respectively and was associated with RFS (HR 4.0; 95% CI 0.16-100.9; p<0.001). Total count of CD3+ T tumor was a significant predictor of NAC response (p<0.001).
Plimack, 2014 [29]	P	39	64 (44-83)	T2-T4, N0-1, M0	20 mo	MVAC	pT0	p53	DNA sequencing (Illumina)	p53 (48.7)	No correlation between altered p53 and response to NAC

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Table 1 (*Continued*)

Author, publication year	Study design	Number of NAC patients	Age, years (median, range)	Stage	Follow-up, median (range)	NAC	Definition of response	Type of markers evaluated(cut off values)	Methods	% of high expression (%)	Main results
Plimack, 2015 [30]	P	34 24	64 (44−83) 68 (55−82)	T2-4, N0-1, M0	28.3 mo 16.75 mo	MVAC Dose dense GC	pT0, pN0, cM0	287 cancer- related genes	DNA sequencing (Illumina)	NR	In the discovery set, ATM, RB1, and FANCC alterations predicted PR (p<0.001; 87% sensitivity, 100% specificity) and better OS (p = 0.007). In the validation set ATM, RB1, and FANCC alterations predicted PR (p = 0.033), with a trend towards better OS (p = 0.055).
Powles, 2019 [28]	P	95	73 (68−77)	T2-T4, N0, M0	13.1 mo	Atezolizumab	NR	CD8+ T, TMB (≥10 mut/Mb), TGF-β, PD-L1	IHC, RNA and DNA sequencing (Illumina)	TMB (31), PD-L1 (41)	High CD8+ was associated with a cPR rate of 40% (95% CI: 26−57%) compared to a rate of 20% (95% CI: 9−35%) with absence of CD8 (p<0.05). TMB-high was not associated with cPR.
Rubino, 2020 [13]	R	130	65 (33−84)	NR	NR	MVAC, ddMVAC, and other	NR	Ki−67 and PD−L1	IHC	Ki-67 (81.6), PD−L1 (43.8)	Positive Ki-67 expression was associated with poor OS (HR 2.412, 95% CI, 1.076−5.408), the absence of cPR (p<0.001) and tumor downstaging (p<0.001). Positive PD-L1 was associated with lack of cPR response (OR = 0.16; 95% CI, 0.05−0.59; p=0.006) and tumor downstaging (OR = 0.29; 95% CI, 0.13−0.67; p=0.003)
Sarkis, 1995 [11]	R	111	64 (30-79)	T2-T4, NO, MO	5.8 years	MVAC	≤ pT1	p53 (20%)	IHC	p53 (52)	p53 overexpression had independent significance for survival (p=0.001; relative risk ratio, 3.1). Long-term survival was evident in 41% of patients with p53 overexpression vs. 77% - with no overexpression (p=0.007).
Seiler, 2017 [21]	R	269	61	T2-T4, N0-3, M0	NR	GC, MVAC, and other	ypT<2N0	Molecular subtypes: luminal, luminal-infiltrated, basal, claudin-low, and p53-like	Whole transcriptome analysis	NR	Claudin-low (HR 2.16, 95% CI 1.22−3.81, p=0.008) and luminal-infiltrated (HR 2.46, 95% CI 1.29−4.7, p=0.006) subtypes were associated with OS.
Seiler, 2018 [22]	R	134	61	NR	35.4 mo	Platinum-based	pT0N0	Molecular subtypes: luminal, luminal-infiltrated, basal, claudin-low.	Whole transcript analysis, IHC	NR	Basal or luminal tumors had a favorable prognosis compared to claudin-low or luminal- infiltrated tumors (p<0.05). Higher expression of genes that were consistent with wound healing/scarring (MYH11, CNN1, DES) or with epithelial-to- mesenchymal transition (EMT; i.e. ZEB1, ZEB2, VIM), suggesting these patients had response to therapy.
Takata, 2005 [53]	P	27	66 (53-77)	T2a-3b, N0, M0	NR	MVAC	NR	Numerical prediction scoring system including 14 genes	Genome- wide expression profiling	NR	14 gene separated the responders from non-responder group. Among these genes Topoisomerase 2, was downregulated in non-responder group. The scoring system correctly identified response for 8 of 9 cases.
Takata, 2007 [54]	P	22	66.7 (58−75)	T2a-3b, N0, M0		MVAC	NR	Numerical prediction scoring system including 14 genes	Genome- wide expression profiling	NR	The scoring system correctly identified response for 19 of 22 cases.
Tervahartiala, 2017 [55]	R	68	65 (47-76)	T0-T4, N0/N+, M0	3.6 year (0.25 −7.7)	GC, GCb	pT0N0	CD68 (60), MAC387 (79), CLEVER-1 (54)	IHC		MAC387+ cells (HR 3.76, 95% CI 1.10−12.82, p=0.034) and CLEVER-1+ (HR 2.78, 95% CI 1.00−7.67, p=0.049) macrophages associated with poor NAC response, while CLEVER-1+ vessels associated with more favorable response to NAC (p = 0.01). OS: higher counts of CLEVER-1+ macrophages associated with poorer OS (HR 3.17, 95% CI 1.01−9.97, p=0.048).
Turker, 2019 [20]	R	119	NR	T1G3 or T2-T4, N0/N+, M0	NR	Cisplatin/ doxorubicin or methotrexate + radiotherapy	NR	Bcl-2 (10%)	IHC	Bcl-2 (38)	Bcl-2 negative expression had a significant increased OS (p=0.009), while Bcl-2 positive -showed no difference (p=0.4).
Van Allen, 2014 [31]	P	50	62.5±8.9	T2-T4, N0/N+, M0	351 ± 363.2 days (± SD)	GC, ddMVAC, ddGC, or GC and sunitinib	pT0 or pTis	ERCC2	WES	NR	ERCC2 was the only significantly mutated gene enriched in the cisplatin responders compared with non-responders (p<0.01).
Vinall, 2016 [35]	P	41	NR	≥ pT2	NR	Gemcitabine, carboplatin/ cisplatin, taxol	pT0	let-7c	MiRNA expression profiling, RT-PCR	NR	Higher let-7c expression had higher odds of responding (OR 2.493, 95% CI 1.121-5.546, p=0.023). Let-7c levels allowed for prediction of patient response (AUC 0.72, positive predictive value 59%).
Wahlin, 2019 [14]	R	65		T2-T4, N0/N+, M0-1		NR	pT0 or Ta/CIS		IHC		

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Table 1 (*Continued*)

Author, publication year	Study design	Number of NAC patients	Age, years (median, range)	Stage	Follow-up, median (range)	NAC	Definition of response	Type of markers evaluated(cut off values)	Methods	% of high expression (%)	Main results
			64.7 (43.1-75.9)		4.3 years (0.2 −7.9)			CD8 (20%), FoxP3 (4%), CD20 (6.75%), PD-1 (10%), PD-L1^{IC} (5%), PD-L1^{TC} (0%)		CD8 (43.8), FoxP3 (49.5), CD20(50.0), PD-1 (44.2), PD-L1^{IC} (49.4), PD-L1^{TC} (48.1)	High infiltration of CD8, FoxP3, CD20, PD-1, PD-L1^{IC}, and PD-L1^{TC} were associated with the longest TTR (all<0.05) On Cox proportional hazards analysis: CD8 (HR 0.41, 95% CI 0.08-2.04, p=0.91); FoxP3 (HR 0.27, 95% 0.05-1.34, p=0.26); CD20 (HR 0.42, 95% 0.10-1.76, p=0.71); PD-1 (HR 0.41, 95% 0.10-1.70, p=0.89); PD-L1^{IC} (HR 0.51, 95% 0.11-2.28, p=0.98); PD-L1^{TC} (HR 0.51, 95% 0.11-2.28, p=0.95).
Williams, 2009 [56]	R	89	NR	NR	NR	MVAC	NR	Gene expression models	In vitro drug sensitivities evaluation and microarray analyses	NR	The 3-years OS for patients with favourable gene expression model score was 81% vs 33% for those with un-favourable score (p=0.002).
Winters, 2018 [57]	R	62	61.5 (56-69)	T2−4, N0/N+, M0-1	36.5 mo (IQR 8 −55)	GC, MVAC, ddMVAC, other	ypT0	mTOR, pmTOR, Ki67	IHC, mRNA expression analysis	NR	mTOR (p=0.01) and pmTOR (p=0.03) expression was decreased in complete responders.
Xylinas, 2016 [58]	R	18	71 (60-77)	T2−4, N0/+	NR	GC	pT0	Cancer- related genes	RNA sequencing and DNA methylation assays	NR	H3XA9 promoter methylation status is associated with response (p < 0.001).
Yang, 2018 [15]	R	52	62.6	T0-4, N0/N+,	NR	GC	ypT0N0	EGFR, RRM1, PD-L1, BRCA1, TUBB3, ERCC, ERCC1, AG integrin α3β1 and CK5/6. Six cancer-associated genes (TERT, FGFR3, TP53, PIK3CA, ERBB2 and TSC1).	IHC	NR	ERBB2, FGFR3 and PIK3CA exclusively altered in the responders (p<0.01), in which FGFR3 mutations were significantly enriched in patients with a response (p=0.01). Strong expression of ERCC1 was associated with PR (p=0.01)

AG = abernantly glycosylated integrin $\alpha 3\beta 1$; ATM = ataxia telangiectasia mutated; Cas3 = cleaved caspase-3; CGP = comprehensive genomic profiling; CMV = cisplatin, methotrexate, vinblastine; ChMV = arboplatin, methotrexate, vinblastine; cPR = complete pathologic response; CPS=combined positive score; ctDNA = circulating tumor DNA; ddGC = dose-dense GC; ddMVAC = dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin; DFS - disease-free survival; DSM = disease-specific mortality; DSS = disease-specific survival; DYRK2 = dual-specificity tyrosine phosphorylation- regulated kinase 2; ERBB2 = erb-b2 receptor tyrosine kinase 2, ERCC2 = excision repair cross- complementation group 2; FANCC = Fanconi anaemia complementation group C; FISH = Fluorescence in-situ hybridization; IHC = immunohistochemistry; GC = gemcitabine/cisplatin; GCb = gemcitabine/carboplatin; IFN = interferon; LN = lymph nodes; lncRNAs = long non-coding RNAs; MDSC = myeloid-derived suppressors cells; mTOR = the mechanistic target of rapamycin; MVAC = methotrexate, vinblastine, doxorubicin (Adriamycin), cisplatin; NAC = neoadjuvant chemotherapy; NGS = next-generation sequencing; PD-1 = programmed death-1; PD-L1 = programmed death-ligand 1; PD-L1^{IC} = immune cells expressing PD-L1; PD-L1^{TC} = tumor cells expressing PD-L1; PFS = progression-free survival; pmTOR = phosphorylated mTOR; PR = pathologic response; pSTAT3 = phosphorylated signal transducer and activator of transcription-3; RB1 = retinoblastoma 1; RT-PCR = real-time quantitative polymerase chain reaction; TGF-β = transforming growth factor (TGF)-β; TMB = tumor mutational burden; TTR = time to recurrence; WES = Whole Exome Sequencing.

3.1. Cell-cycle and proliferation markers

Several studies in patients undergoing NAC demonstrated a correlation between pretreatment p53 (cell-cycle marker) overexpression at IHC and worse survival outcomes. For example, Sarkis et al. found that at 5.8 years after NAC, 41% of patients with p53 overexpression and 77% - without overexpression (p=0.007) experienced death [11]. In contrast, Grossman et al. [12] reported that p53 expression was not associated with progression free (PFS) (HR=1.02; 95% CI 0.61-1.71; p=0.93) or overall (OS) survival (HR 1.48; 95% CI 0.87-2.53; p=0.15). Similarly, Ki-67 (proliferation marker) expression was associated with neither PFS (HR 0.62; 95% CI 0.37-1.03; p=0.063) nor OS (HR 0.74; 95% CI 0.44-1.24; p=0.25). Conversely, in a study comprising 130 patients, Rubino et al. [13] found that positive Ki-67 expression was associated with worse OS (HR 2.412, 95% CI, 1.076−5.408) as well as the absence of complete pathological response (p<0.001) and tumor downstaging (p<0.001). Interestingly, positive PD-L1 was associated with a lack of complete pathological response (OR = 0.16; 95% CI, 0.05−0.59; p=0.006) and tumor downstaging (OR = 0.29; 95% CI, 0.13−0.67; p=0.003) in 130 patients treated with NAC [13]. High infiltration of PD-1 in tumor was shown to be associated with the longest time to recurrence (all<0.05) [14].

3.2. DNA repair pathway alterations

A study assessing DNA repair pathway alterations found that a strong expression of ERCC1 was associated with pathological response in patients treated with neoadjuvant gemcitabine and cisplatin (GC) (p=0.01) [15]. Choueiri et al. [16] reported a pathological response (<pT1) rate of 43% in ERCC1-positive and 60% in ERCC1- negative UCB patients treated with dose dense MVAC.

3.3. Receptor tyrosine kinases

Yang et al. [15] reported that receptor tyrosine kinases (ERBB2, FGFR3, and PIK3CA) were more commonly altered in the responders (p<0.01) compared to the non-responders; FGFR3 mutations were significantly enriched in patients with a response to GC based regimen (p=0.01). In contrast, Kiss et al. [17] failed to report on the association between ERBB2 alterations and both pathological response (<ypT2N0) or OS.

3.4. Biomarkers for combination of NAC and radiotherapy

Three studies reported IHC biomarkers in patients treated with combination of NAC and radiotherapy [18] −[20]. Positive p53 and p21 were independently associated with decreased disease free survival (DFS) in a retrospective study of 82 patients (p<0.005 and p<0.009, respectively); additionally, p53 overexpression was associated

with poor OS (p<0.03) [18]. Alteration of the combination of p53 and p21 was a strong and unfavorable prognostic factor for both DFS (p<0.003) and OS (p<0.02). Hemdan et al. [19] demonstrated that patients with negative emmprin (extracellular matrix metalloproteinase inducer) expression had significantly greater OS in 125 UCB patients treated with radiotherapy and NAC (71% vs. 38%, p <0.001); cancer specific survival (CSS) in patients with negative and positive emmprin expression was 76% and 56%, respectively (p=0.027). Turker et al. [20] reported that patients exhibiting Bcl-2 negative expression had a significantly increased OS (p=0.009). In summary, pretreatment p53, p21, emmprin, and Bcl-2 have been suggested to exhibit predictive value in UCB patients treated with NAC and radiotherapy. However, further studies are needed to improve our understanding of the radiotherapy impact on inflammation status, which could affect biomarker expression.

According to the currently available literature, IHC biomarkers, including receptor tyrosine kinases and DNA repair pathway alterations, do not seem to clearly improve our prediction of pathological response or oncologic outcomes in UCB patients treated with NAC.

4. Gene expression and genomic DNA analyses

Nineteen studies provided data on the pretreatment biomarkers detected using gene expression analysis.

Over the last decade, molecular subtyping has led to distinct or partially overlapping molecular classifications of UCB. The arising molecular subtypes based on these classifications have been shown to be clinically useful in predicting the likelihood of therapy response. Whole transcriptome analysis suggests that luminal and basal tumors, compared to claudin-low or luminal-infiltrated tumors, might have the best response to platinum-based NAC (p<0.05) [21, 22]. Supporting this data, Choi et al. [23] reported response rate of 0% in p53-like, 40% - basal-like, and 67% - luminal-like subtypes (p=0.018). Efstathiou et al. [24] detected worse DSS and OS among patients with claudin-low tumors at transcriptome-wide gene expression profile analysis (p=0.01 and p=0.068, respectively). Taking together, luminal and basal tumor subtypes showed better NAC response, while claudin-low and luminal-infiltrated tumor subtypes did not.

Surprisingly, during comprehensive genomic profiling, molecular subtypes were not significantly associated with response (ypT0N0) in both studies assessing NAC and Pembrolizumab (all p>0.2) [25]. Notably, immune signatures explored in this study had a significant association with the pathologic response in the PURE-01 cohort (all p<0.02), but not in the NAC cohort (p>0.7) [25]. Among other studies on predictive biomarkers for neoadjuvant immunotherapy, Necchi et al. [26] reported an association of tumor mutational burden (TMB) and PD-L1 combined positive score with both the pT0 and the pT1 response to Pembrolizumab (all p<0.03). In contrast, Bandini et al. [27] found that TMB was not associated with response (pT0N0) to Pembrolizumab on multivariable analysis (OR 1.04, 0.98-1.10, p=0.09). These results were supported by Powles et al. [28] in a study of 95 patients treated with neoadjuvant Atezolizumab. Summing up, in terms of neoadjuvant immune-checkpoint inhibitors (CPI), PD-L1 seems to maintain value as a predictive biomarker, while the utility of TMB and molecular subtypes is still controversial.

Among other predictive biomarkers detected with gene expression analysis, Plimack et al. analyzed molecular alterations in baseline tumor samples and did not find a correlation between p53 deleterious mutations and response to NAC [29]. Defects in DNA repair genes (ATM, RB1, and FANCC) were shown to predict pathological response in both MVAC (p<0.001) and dose dense GC (p = 0.033) cohorts and at the same time with better OS after MVAC (p = 0.007) [30]. Another DNA repair pathway alteration (ERCC2) was also significantly mutated in cisplatin responders compared to non-responders (p<0.01) [31]. In contrast, genetic alterations in genes associated with cell cycle checkpoints and regulators (E2F3, JUN, FBXW7) suggested potential resistance [32].

Summing up, according to the currently available literature, alterations in DNA repair genes seem useful to predict pathological response and even oncologic outcomes in UCB patients treated with NAC. However, these data should be supported by future large-scale trials.

5. Polymerase chain reaction (PCR)

Three studies provided data on the pretreatment biomarkers detected at quantitative PCR [33]−[35].

In order to investigate the predictive role of the breast cancer susceptibility gene 1 (BRCA1) mRNA expression in UCB, tumor samples of 57 patients treated with GC or CMV (cisplatin, methotrexate, vinblastine) for UCB were retrospectively analyzed using quantitative PCR [33]. 66% of patients with low/intermediate BRCA1 levels attained a pathological response (pT0-1) compared to 22% of those with high BRCA1 levels. Furthermore, median survival was longer in patients with low BRCA1 expression (168 and 34 months, respectively, p=0.002). Thus, BRCA1 expression could be a useful tool for selecting UCB patients who are likely to benefit from cisplatin-based NAC. The authors suggested that taxane-based therapy for patients with high BRCA1 expression could be explored in further studies.

Among studies on other tissue-based biomarkers detected with PCR, Kato et al. [34] identified 12 candidates genes tested in tissue microarrays derived from baseline biopsies of 37 patients treated with NAC. Among these genes, IPO-7 and SLC22A18 were upregulated in non-responders. Vinall et al. [35] found that higher let-7c expression had higher odds of responding (OR 2.493, 95% CI 1.121-5.546, p=0.023), and let-7c levels allowed predicting

response (pT0) with an accuracy of 72%. Nevertheless, larger scale studies are certainly warranted to confirm and validate these results.

In general, quantitative PCR results for the expression of genes selected through microarray analysis might correctly classify cases with regard to their NAC response.

6. Next-generation sequencing (NGS)

Two studies provided data on the pretreatment biomarkers detected at NGS [36, 37].

In a study of Groenendijk et al. [36], ERBB2 was strongly associated with NAC response, defined as ypT0N0 (p=0.006), whereas ERCC2 mutations were not. Miron et al. [37] found that mutations in ATM, RB1, or FANCC were significantly associated with improved OS (p=0.0043) and DSS (p=0.0015) in 58 patients treated with NAC (GC or MVAC). The authors hypothesized that, based on understanding the function of ATM, RB1, and FANCC and their involvement in DNA damage repair, mutations in these genes sensitize tumors to cisplatin because of a baseline deficiency in DNA repair.

7. Discussion

This review on the impact of using pretreatment tissue-based biomarkers to select patients who are most likely to benefit from NAST generated several important findings.

First of all, there is no clear benefit of using predictive biomarkers, including receptor tyrosine kinases and DNA repair pathway alterations, detected at IHC to predict pathologic response or oncologic outcomes in UCB patients treated with NAC. The controversial results can be explained by the small sample size as well as the retrospective nature of most included studies, leading to heterogeneity between NAST cohorts, differences between NAC settings, and definitions such as that of pathologic response as well as non-standardized sample collections and arbitrary cut-offs during assay analysis. Moreover, we believe that for the initial development of a putative marker model as well as markers with combinations, it is essential to reflect the molecular understanding of the tumor and its microenvironment.

We found out that specific genomic alterations in DNA repair genes (e.g., ATM, RB1, FANCC, and ERCC2) provide predictive value for predicting pathologic response and oncologic outcomes after NAC. Quantitative PCR results for the expression of genes selected through microarray analysis (e.g., BRCA1) could correctly classify cases with regard to their NAC response. However, it should be stressed that the utility of genetic profiling has historically been limited to small gene panels and costly molecular diagnostics. Hence, biomarkers detected at IHC can still be a simple and less expensive alternative. To facilitate inclusion into routine urological practice, precise identification of tissue-based biomarkers with accurate detection technology seems to be of necessity. The continuous improvement in high throughput technologies, the development of novel analytical tools based on artificial intelligence need for biomarker-driven preclinical and clinical trials. Nowadays, NGS is becoming a complementary diagnostic tool, guiding the decision-making progress with the goal of facilitating precision medicine. We believe that with the incorporation of NGS, physicians will have the ability to obtain a more comprehensive understanding of the molecular alterations driving an individual urothelial cancer [38].

In terms of predicting the likelihood of responding to neoadjuvant CPI, TURBT PD-L1 seems to have value as an accurate but not ideal biomarker [39]. Indeed, a higher pathologic response rate was shown in patients with PD-L1 positive tumors compared to those with PD- L1 negative tumors; while the utility of TMB or molecular subtypes in patients treated with neoadjuvant CPI is still unclear, at best. Moreover, it was recently shown that indicate molecular subtypes may not be useful due to tumor heterogeneity and various models of changes in molecular profiles before or during progression [40, 41]. Understanding the stability of molecular subtypes over time and the subtype heterogeneity within tumors and patients remains challenging. Future areas certainly include conceptual molecular pathways (e.g., FGFR3 pathway) that would allow for targeted therapy approaches. New clinical trials that use molecularly guided therapy selection will determine the clinical efficacy of the integration of genomics and other molecular predictive biomarkers to guide daily therapeutic decision-making.

Our systematic review is not free from limitations. First, the inconsistencies in evaluation of the tissue-based biomarkers among the enrolled trials could lead to potential confounding and bias. The second limitation is the retrospective and heterogeneous nature of most included studies which also suffered from single-center designs. Third, the small cohort size of most of the included studies may have limited their power to detect a statistically and/or clinically significant associations. Therefore, well-designed comparative trials with larger cohorts are required to validate some of the most promising findings inherent to the present systematic review.

8. Conclusions

Pretreatment tissue-based biomarkers still hold promise in selecting the ideal UCB patient who is most likely to benefit from NAST. However, due to the lack of prospective, well-designed, large scale data, no molecular biomarkers could be recommended for the routine use. The present systematic review offers a robust framework to enable the testing and validation of predictive biomarkers in future prospective clinical trials.

ETHICAL STANDARDS

Not applicable.

Declaration of Competing Interest

None

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