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Biomarkers in Non-Schistosomiasis-related squamous cell carcinoma of the urinary bladder: A review

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ABSTRACT

Non-urothelial (NU) histologies represent less than 10% of bladder cancers, with squamous cell carcinoma (SCC) being the most common subtype (approximately 5%). Bladder SCCs are subdivided into Schistosoma-related or non-Schistosoma-related tumors, with the latter being the most frequent subtype in the western world. Typically, these patients have been excluded or under-represented in clinical trials and thus little is known about treatment efficacy in bladder SCC. To address this paucity of data, extrapolation from urothelial carcinoma (UC) trials can be performed but this approach has inherent limitations. In the era of precision medicine, efforts to characterize the genomic and molecular profiles of bladder tumors may yield evidence to support new targets for effective therapies. We reviewed the available data on biomarkers of bladder SCC and provide suggestions on how these may influence therapeutic testing and clinical trials in the future.

1. Introduction

Over 430,000 new cases per year of bladder cancer are estimated worldwide, making it the ninth most common cancer (Antoni et al., 2017). Despite the treatment options available, the 5-year survival rate of urinary bladder cancer (all stages) is 77%, which decreases to 34% for locally advanced disease; a trend that has not changed significantly for the past 30 years (Jemal et al., 2010; Motzer et al., 2015).

Urothelial carcinoma (UC) represents the most common histologic type of bladder cancer, accounting for 90% of all cases. UC can originate less frequently from other sites within the urinary system, such as renal pelvis and ureter, also known as upper tract urothelial carcinoma. Non-urothelial bladder tumors account for less than 10% of all cases and are particularly relevant in other regions of the world, where it may account for up to 75% of bladder cancer diagnoses (Dahm and Gschwend, 2003). A spectrum of rare subtypes, including squamous cell carcinoma [SCC] (2%–5%), adenocarcinoma (0.5%–2%), and small cell carcinoma (< 1%) comprise the non-urothelial histologies (Shokeir, 2004; Chalasani et al., 2009).

SCC may be subdivided into two categories: Bilharzial squamous cell carcinoma (B-SCC), which is associated with Schistosomiasis infection, and non-bilharzia squamous cell carcinoma (NB-SCC). These two types differ in their epidemiology, risk factors, pathogenesis, disease biology and prognosis (Martin et al., 2016). Given the rare incidence of non-urothelial bladder tumors, most studies have focused solely on UC histology. Thus scant data exist to guide treatment approaches for both types of SCC of the bladder and the cornerstone is still surgery for localized disease (Abol-Enein et al., 2007). For those patients diagnosed with advanced or metastatic disease, extrapolation of data from UC has been the rule, although results are generally inferior (Shokeir, 2004; Chalasani et al., 2009).

In this review, we focused on the epidemiology, pathogenesis and disease biology, with special considerations for the genomic landscape and key molecular drivers of NB-SCC of the bladder.

2. Epidemiology

NB-SCC of the bladder is more prevalent among African-Americans (ratio of 2:1 compared to the White population) and males (male to female ratio of 3:2) (Porter et al., 2002). Compared to B-SCC, which is a disease of younger age, NB-SCC typically occurs in the seventh decade of life and diagnosed at a more advanced stage, and therefore associated with worse prognosis (Abol-Enein et al., 2007).

The risk factors associated with NB-SCC of the bladder in this

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population are neurogenic bladder dysfunction and chronic bladder irritation due to prolonged indwelling catheters. In patients with spinal cord injury and catheterization for more than 10 years, reports have estimated the incidence of SCC of the bladder approaches 10% (Shokeir, 2004; Locke et al., 1985). A 15 to 28-fold increased risk of this disease among the paraplegic population has been estimated, although most data were retrospective and from single center experiences and therefore subject to bias (Welk et al., 2013). More recent publications have challenged this incidence and reported numbers similar to the overall population (Pannek, 2002).

Other risk factors for SCC of the bladder are tobacco exposure, repetitive urinary tract infections (UTIs) and bladder irritants (including chemotherapy, such as cyclophosphamide). Notably, these risk factors share a common pathway for tumor formation: an environment of chronic inflammation, where growth factors, cytokines and immune cell infiltration promote cell proliferation and migration, ultimately leading to metaplasia, dysplasia and cancer (Youssef et al., 2011; Stein et al., 1993).

More recently, other risk factors have been suggested for bladder SCC. Human papilloma virus (HPV) which has been associated with anogenital and oropharynx tumors. Some data implicate HPV in SCC of the bladder (Shaker et al., 2013). However, conflicting results have been reported and at this point no definitive causation of HPV and bladder SCC has been established (Westenend et al., 2001).

In contrast, Schistosoma infection has been demonstrated in B-SCC of the bladder (Westenend et al., 2001). As mentioned, this subtype is rarely found in the Western world, being more prevalent in the middle east and east Africa (Ghoneim et al., 1997). Control of schistosomiasis infection can result in lower frequency of B-SCC, which has been demonstrated in Egypt (Felix et al., 2008).

3. Pathology

The histologic features of SCC of the bladder are characterized by classical squamous appearance, such as pearl formation, intracellular bridges and keratohyalingranules (Manunta et al., 2005). Although some bladder tumors may present with mixed urothelial and squamous components, the diagnosis of SCC of the bladder requires it to be the only histology (Youssef et al., 2011; Stein et al., 1993). In addition, compared to B-SCC, NB-SCC tend to be poorly differentiated and have a higher histological grade (Shokeir, 2004).

At cystoscopy, SCC tumors are typically large solitary and often necrotic masses affecting the trigone or lateral walls of the bladder and usually associated with leukoplakia (Martin et al., 2016; Manunta et al., 2005). Advanced T stage at diagnosis is generally seen (≥T2), with superficial cases (e.g. Ta or T1) rarely reported. Case series (Lagwinski et al., 2007) and population based studies have reported a high rate of T3 tumors (60% and 43%, respectively) as well as a higher prevalence of grade 2 (37.3%) and 3 (42.5%) tumors (Abdollah et al., 2012). Surprisingly, NB-SCC tumors have a lower incidence of both lymphovascular invasion and lymph node metastasis (Spradling et al., 2016).

4. Disease biology and molecular features

Most studies about molecular biomarkers of SCC bladder tumors have been limited to bilharzia-associated disease or were conducted in a patient population enriched with bilharzia-associated tumors (Youssef et al., 2011; Hammam et al., 2014; Eissa et al., 2005; Badr et al., 2004). In addition, most studies have focused on a prognostic role for the biomarkers rather than a predictive association.

Since chronic inflammation appears to have a causative role in the pathogenesis of bladder SCC, many studies have focused on studying inflammation markers in this disease. Youssef et al reported that COX-2 expression (assessed by immunohistochemistry - IHC), as a single biomarker, was an independent predictor of clinical outcomes after surgery and associated with higher pathological stage and grade in B-SCC, but not in NB-SCC of the bladder (Youssef et al., 2011).

Another study reported by the same group evaluated a panel of multiple biomarkers in archival tissues from radical cystectomy specimens in a population of 151 SCC patients treated in Egypt (80% were related to Bilharzial infestation). The proteins assessed by IHC were those related to cell cycle regulation, proliferation, apoptosis, angiogenesis, signal transduction and inflammation. From a panel of 14 biomarkers tested, 5 of them [Cyclooxygenase-2 (COX-2), p53, Bax, Fibroblast growth factor-2 (FGF-2) and Epidermal Growth Factor Receptor (EGFR)] were significantly correlated with oncologic outcomes, particularly disease recurrence and bladder cancer-specific mortality (BCSM) (Youssef et al., 2015a). Among them, FGF-2 showed the strongest association with recurrence (HR 3.1; 95%CI 1.6–5.9; $p = 0.001$) and Bax the strongest association with BCSM (HR 3.9, 95% CI 1.708–8.952, $p = 0.001$), which was also corroborated by another study (Youssef et al., 2015b). The authors created a prognostic score based on the number of altered markers and showed that it was an independent predictor of recurrence and survival after adjusting for clinicopathological variables (Youssef et al., 2015a).

Cell cycle regulators have also been explored as prognostic biomarkers. For instance, p53 staining, as assessed by IHC, was shown to be prognostic and associated with T stage (Youssef et al., 2015a). Interestingly, there appears to be an interrelationship between p53, COX-2 and Bax expression, with the former inducing the expression of the latter (Gallo et al., 2003; Subbaramaiah et al., 1999).

Other biomarkers, such as HER-2 overexpression, have been investigated by other groups. In a study reported by Hamman et al in a population of both UC and SCC of the bladder, HER-2 expression was correlated with a higher tumor grade and stage in UC, but not in SCC, although the overall prevalence of HER-2 overexpression was higher among SCC (52% vs 33%, as assessed by fluorescence in situ hybridization - FISH) (Hammam et al., 2015). Importantly, all SCC cases in this study were associated with Schistosoma infestation.

In contrast, Eissa et al reported differing results for HER2 overexpression (Eissa et al., 2005). Although the authors found a higher HER2 expression in malignant bladder tumors as compared to non-malignant lesions, no difference in HER2 expression between UC (90.3%) and SCC (84.2%) tumors; and between B-SCC (92.9%) and NB-SCC (84%) tumors was demonstrated. Of note, the authors showed that there is a correlation between HER2 expression and ploidy, synthetic phase fraction and DNA index, which are markers of higher proliferation and usually confer a worse prognosis (Eissa et al., 2005). The lack of association between tumor stage and grade was corroborated by Badr et al, but not by Hamman et al. (Badr et al., 2004). Importantly, the presence of worse cohorts of patients may have driven the differences found among the cited studies.

Unfortunately, these results are only hypothesis-generating for further drug development strategies. As of now, these biomarkers have yet not been tested or validated as predictive factors for treatment approaches.

Table 1 lists studies reporting on biomarkers of NB-SCC.

5. Mutational landscape

Limited data exist regarding the mutational landscape of SCC bladder tumors. Most of the work reported in this field pertain to UC specimens, although some authors have included variable percentages of non-UC, particularly SCC cases.

As previously discussed, bladder carcinomas expressing FGF-2 carries a worse prognosis, which correlates with the degree of expression. Exploring the molecular mechanisms behind this poor prognosis, McNiel et al analyzed TCGA data of primary bladder carcinomas. They showed that FGF-2 expression correlates positively with the expression of epithelial to mesenchymal transition (EMT), promoting transcription factors and with changes in gene expression that are characteristic of EMT. In addition, they demonstrated a positive correlation between

Table 1
Detailed summary of the body of evidence on biomarkers of SCC of urinary bladder.

References	No. of patients	SCC patient population	Biomarker	Frequency	Influence on outcome
Schistosomiasis-related - SCC predominant					
Youssef et al. (2011)	315 patients with SCC and UC	Schistosoma associated (n = 205), non-Schistosoma associated SCC (n = 110, mainly UC).	COX-2 by IHC		COX-2 expression was an independent predictor of disease recurrence (HR 1.9, CI 0.99-3.626 and P = 0.05) and cancer specific mortality (HR 2.8, CI 1.155-6.73 and P = 0.023) only in Schistosoma associated tumors.
Badr et al. (2004)	15 patients with SCC	All are Schistosoma related.	IHC for p53, bcl-2, HER2/neu, and MIB-1	p53 (73%), MIB-1 (87%), bcl-2 (20%), and HER2/neu (27%).	No correlation of any marker with grade or stage. The effect on survival was not assessed.
Youssef et al. (2015a)	151 patients with SCC	Schistosoma associated in the majority (81%). T2 in 50%, T3 in 38%, T1 in 6% and T4 in 6%	COX-2, FGF-2, p53, Bax and EGFR	NR	Prognostic score based on marker alterations was an independent predictor of intermediate and high risk of recurrence (HR 3.2, $p = 0.008$ and HR 15.5, $p \leq 0.001$) and bladder cancer specific mortality (HR 5.2, $p = 0.009$ and HR 19.4, $p \leq 0.001$)
Youssef et al. (2015b)	151 patients with SCC	Schistosoma associated in the majority (81%). T2-T3 in 88%	FGF2 by IHC	NR	Altered FGF2 was associated with tumor grade ($P = 0.014$), LN invasion, and LVI ($P = 0.042$), cancer recurrence (HR 2.561, $P = 0.009$) and cancer-specific mortality (HR 2.679, $P = 0.033$)
Ramchurren et al. (1995)	21 patients with SCC	All with Schistosomiasis	IHC for P53, Rb, EGFR and c-erbB-2 proteins, and sequencing for mutations in RAS (H, N, K) codon hotspots (12,13,61) and p53 (exons 4-9) genes	38% were positive for p53, 67% for EGFR, 28% for c-erbB-2, and 95% for Rb. Three (14%) had H-RAS mutation, 12 (57%) had p53 mutations.	Not examined
Haitel et al. (2001)	109 patients; 60 with SCC and 49 with UC	Non-metastatic disease, all patients had schistosomiasis	IHC for Bcl-2, Bcl-x, Bax, Bak, p53, E-cadherin, c-erbB-2, and Ki-67		p53 overexpression and loss of Bak positivity predicted shorter PFS in UC and SCC. In UC, Bcl-x expression and c-erbB-2 positivity predicted poor prognosis. P53 remains significant in multivariate analysis for UC and SCC
Abdulamir et al. (2009)	148 (84 with bladder cancer)	Schistosoma and non-Schistosoma bladder tumors (SCC and UC) the rest are patients with Schistosomal chronic cystitis, non-Schistosomal cystitis, and normal controls.	p53, p16, bcl-2, ki-67, c-myc, Rb and EGFR by IHC	The expression of p53, bcl-2, c-myc, and EGFR was higher in Schistosoma bladder tumors (SBT) than in non-Schistosoma bladder tumors (NSBT), while Rb was higher in NSBT than in SBT. However, p16 and ki-67 were not different between SBT and NSBT.	p53 was associated with high grade SCC in both SBT and NSBT. Bcl-2 was associated with high grade invasive tumors in SBT and NSBT. Rb was associated with SCC in SBT. Correlation of markers with outcomes was not reported
El-Kenawy Ael et al. (2003)	50 patients with SCC	All are Schistosoma related	IHC for p53 and MDM2	p53 in 48% and MDM2 in 20%.	Significant correlation between p53 and survival (p = 0.01), while the correlation between MDM2 and survival was not significant (p = 0.72).
El-Meghawry El-Kenawy et al. (2006)	81 patients with SCC	Most with Schistosoma – associated	IHC for MK-1 expression	Forty-six cases (56.8%) were positive for MK-1	Grade (p = 0.004), Schistosoma (p = 0.031), DNA ploidy (p = 0.001), and tumor recurrence ($p < 0.001$).
Schistosomiasis-related – UC predominant					
Hammam et al. (2015)	10 chronic cystitis, 19 SCC with schistosomiasis, 33 UC Schistosomal and non-Schistosomal, and ten healthy individuals	Schistosoma and non-Schistosoma associated	HER2 by IHC and FISH	HER2 over expression was detected in57.7% of bladder cancer cases, and was more common in SCC ($p < 0.01$) compared to UC	Not examined
Eissa et al. (2005)	22 with SCC and 31 with UC	Schistosomal and non-Schistosomal			Not examined.

(*continued on next page*)

Table 1 (*continued*)

References	No. of patients	SCC patient population	Biomarker	Frequency	Influence on outcome
			HER2/neu expression by enzyme immunoassay (EIA) and Western blot (WB).	HER2/neu is overexpressed in malignant compared to benign lesions, with no significant difference between SCC and UC.	
Non-Schistosomiasis-related - SCC predominant					
Guo et al. (2009)	16 patients with SCC	Non-Schistosomial	IHC for EGFR (all 16 patients), and p53 (n = 11)	All patients (100%) over expressed EGFR. 11 patients (69%) over expressed P53	Not reported.
Non-Schistosomiasis-related - UC predominant					
Omran (2012)	144 patients with bladder carcinoma (UC and SCC).	72 UC (30 bilharzial and 42 non-bilharzial) and 72 with SCC (38 bilharzial and 34 non-bilharzial).	CD10 and E-cad by IHC	CD10 tumor cells, CD10 stromal cells, and E-cad were expressed in 56%, 58%, and 51% of cancer bladder cases, respectively	Significant correlation between tumor cells CD10 and grade, stage, and lymph node metastasis of both UC and SCC. E-cad correlated with grade, stage, and LN metastasis in UC but not in SCC.
Molitor et al. (2015)	n = 29 SCC, n = 35 with UC and squamous differentiation, and n = 23 with UC	Non-Schistosoma-associated	Loss of 3p is more frequent in SCC	NR	Not examined
Makboul et al. (2015)	104 patients with bladder ca: 52 UC, 20 UC with squamous differentiation, and 32 SCC	Majority of SCC patients (56%) had Schistosoma infection	Survivin and Ki67 by IHC	Altered survivin 78% of UC, 38% SCC, and 40 % of UC with squamous differentiation, $p < 0.0001$.	Altered survivin had no effect on outcome of SCC, but predicted higher proliferative index, higher stage and higher recurrence in UC.

FGF-2, *Akt3* and *Fibroblast Growth Factor Receptor-2* (*FGFR-2*) alternative splicing. These observations suggest that FGF-2 induces EMT, angiogenesis, cell proliferation and invasion, contributing to poor prognosis (McNiel and Tsichlis, 2017). Interestingly, the same authors showed that expression of FGF-2 has also been correlated with low mutational burden and decreased gene copy number variations in bladder cancer (McNiel and Tsichlis, 2017). On the other hand, overexpression of FGF-2 positively correlated with elevated expression of immune checkpoints, such as cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), programmed cell death 1(PD-1) and programmed cell death ligand 1 (PD-L1), which may suggest why immune checkpoint inhibitions have improved outcomes in this disease (McNiel and Tsichlis, 2017).

In an attempt to better characterize *FGFR* alterations among squamous carcinoma of the bladder, Baldia et al analyzed 73 such cases for *FGFR1–3* protein expression, copy number variations, *FGFR* chromosomal rearrangements (using fluorescence *in situ* hybridization - FISH) and *FGFR3* mutations (using SNapShot analysis). They reported a reduced overall frequency of *FGFR* 1 and 2 expression compared to normal tissue and high expression of *FGFR3*. *FGFR3* mutations were found in 8.5% of samples, consistent with TCGA findings, also being correlated with *FGFR3* protein expression and poor clinical outcomes (both p values < 0.001) (Baldia et al., 2016). These findings support evaluation of such markers in the drug development for bladder cancers harboring these alterations.

Regarding structural genetics, Molitor et al compared the cytogenetic changes among NB-SCC, UC and mixed histology tumors using comparative genomic hybridization (CGH) and FISH. The authors showed that pure SCC tend to have a lower frequency of polysomy and genetic alterations as compared to mixed and pure UC tumors (means: 5.37 vs 6.75 vs 7.64 changes, respectively) (Molitor et al., 2015), suggesting NB-SCC is genetically more stable. Although SCC tumors showed a higher frequency of chromosome 3p loss compared to other histologic subtypes (51.4% vs 37.5% vs 13.9% for SCC, mixed and UC, respectively), no specific alterations were characteristic of any group. Of note, squamous components of mixed histology tumors tend to carry genetic alterations similar to pure UC tumors, highlighting that *"pure"* SCC tumors could potentially be a separate entity (Molitor et al., 2015).

In an attempt to correlate the number of chromosomal variations with T stage and grade in the same study, no statistically significant difference was found, although a trend towards a positive correlation was observed with advanced stage and undifferentiated tumors having a higher mean of chromosomal variation (Molitor et al., 2015). Although these findings are in line with those reported by other authors (Pycha et al., 1999), the study acknowledged that the lower number of specimens analyzed may have reduced statistical power to detect a meaningful difference (Molitor et al., 2015).

Another group has studied differences in copy number changes between Schistosoma and non-Schistosoma-associated tumors (including both UC and SCC cases). El Rifai et al reported a higher mean of DNA copy number changes among Schistosoma-associated tumors (7 *vs* 4), whereas no difference was detected between SCC and UC tumors. The authors observed changes in similar frequencies in both SCC and UC, irrespective of Schistosoma status, such as gains and high-level amplifications at 1q, 8q, and 20q and losses in 9p and 13q. On the other hand, specific changes were more prevalent in one of the subtypes: losses in 3p and gains at 5p were seen only in SCC ($P < 0.01$), while gains at 17q ($P < 0.01$) and losses in 4q ($P < 0.05$) and 6q ($P < 0.01$) were more frequent in UC. These findings highlight important considerations that while bladder cancer subtypes share common pathways for tumor development, at some point they diverge along distinct molecular pathways (El-Rifai et al., 2000).

More recently, some studies have used whole-genome mRNA expression profiling to better characterize different clusters of bladder tumors. While these important studies have focused in pure or predominant urothelial carcinomas (UC component of ≥50%), they are

informative for other histologies as a proportion of these were mixed with SCC. These important studies have led to the discovery that muscle-invasive bladder cancer is highly heterogeneous at the genomic level and may indeed be classified into different intrinsic subgroups similar to breast cancer. Although different authors have distinct classifications, a direct comparison of subtype assignments by different authors revealed they are highly similar (Cancer Genome Atlas Research, 2014; Choi et al., 2014a; Damrauer et al., 2014; Choi et al., 2014b). In broad terms, most groups mainly divided bladder tumors into basal and luminal subtypes.

The basal subtype is enriched with squamous as well as sarcomatoid features (a morphological consequence of EMT) (Choi et al., 2014a). This subtype predominantly harbors biomarkers characteristic of epithelial-to-mesenchymal transition, stemness and cancer progression, particularly ΔNp63α and STAT3 gene expression signatures. Additionally, epidermal growth factor receptor (EGFR) is also overexpressed in this subtype, as seen in other squamous tumors from head and neck and lung origin (Choi et al., 2014b).

On the other hand, the luminal tumors are enriched with papillary histopathological features, highlighting the observation that these cancers arise from non-muscle-invasive bladder cancers. They can be further subdivided in p53-like and luminal, which differ from one another by distinct levels of biomarkers related to cell cycle progression, stromal infiltration and proliferation. In contrast to basal subtypes, luminal subtypes are enriched with *FGFR3* and *HER3* mutations, HER2 amplifications, estrogen receptor, peroxisome proliferator activator receptors (PPARs) and uroplakin expression (Cancer Genome Atlas Research, 2014; Choi et al., 2014a,b).

Perhaps the most important implications from this bladder cancer clustering into subtypes are the relationships in regard to treatment response. The TCGA study has demonstrated differential chemotherapy sensitivity among the distinct subtypes, with basal tumors being more likely to respond to chemotherapy and some luminal subtypes more likely to be chemotherapy-resistant (particularly p53-like subtype). However, the genomic characterization of these distinct subtypes offers a unique opportunity to explore alternative molecular targets. As examples, basal subtypes might be particularly sensitive to inhibitors of *EGFR* (justified by its inherent *EGFR* amplification), STAT3, Hypoxia-Inducible Factor 1 (HIF1) and Vascular endothelial growth factor (*VEGF*) and its receptor (*VEGFR*). On the other hand, interesting targets for luminal subtypes include: *FGFR, HER2, HER3*, estrogen receptor and PPARs.

Another important study reported on gene expression patterns in bladder carcinomas, specifically focusing on differentiating UC from SCC using a prediction analysis of microarrays (PAM) classifier of 30 genes. No relationship between Schistosomal infection and histology was reported. However, the authors showed a successful classification into UC versus SCC based in microarray analysis in 89% of the cases. According to this study, the top predictive gene to separate histologic subtypes into different groups was Parathyroid hormone–related protein *(PTHrp)* (Blaveri et al., 2005). Interestingly, other genes also expressed in higher levels among SCC tumors are linked to inflammation, such as *S100A7*, *S100A8*, *S100A9* and *SKALP* (Ostergaard et al., 1999; Celis et al., 2000). *PTHrp* has been implicated in differentiation, proliferation, epithelial to mesenchymal transition and apoptosis in different cancer types, including bladder cancer (Vaidyanathan et al., 2002; Wysolmerski and Stewart, 1998; Hirasawa et al., 2002).

6. Treatment approaches for SCC of the bladder

6.1. Local treatments

SCC of the bladder is a rare cancer subtype and as such suffers from limited number of patients enrolled in clinical trials. As a consequence, there is lack of high quality randomized prospective data and little evidence supports treatment decisions in this disease. Most of the data is derived from small observational studies, as detailed below.

Older studies have established radical cystectomy (RC) as an effective treatment for localized SCC of the bladder, which has shown better disease control and survival rates compared with other therapies alone, such as partial cystectomy, radiotherapy and chemotherapy (Martin et al., 2016; Kassouf et al., 2007; Kwon et al., 2014). A 5-year survival rate of 48% with RC has been reported in a retrospective analysis (Kwon et al., 2014). In this context, the literature supports radical cystectomy as the gold-standard treatment for localized disease (Martin et al., 2016).

The largest retrospective study to date assessing outcomes of patients with NB-SCC was reported by the MD Anderson Cancer Center group. They retrospectively analyzed the data of 27 patients presenting with localized disease treated between 1988 and 2003. The 2-year overall survival reported was 47.6% and recurrence-free survival 32.8%. With a wide variety of treatments being employed either before or after surgery and even non-surgical approaches (only 50% of them were amenable to RC), the main conclusion of the study was that NB-SCC is associated with a high local recurrence rate, which is the primary cause of death among these patients (Kassouf et al., 2007). Similar findings have been reported by other groups. In a retrospective analysis of NU carcinomas of the bladder from the SEER database, SCC had a 5-year OS of 37% (Aragon-Ching and Henson, 2018). Importantly, among mixed histology bladder cancers, the proportion of squamous differentiation appears to correlate with outcomes, with the higher proportion having worse outcomes ($\geq$50% vs $<$ 50% of the tumor, progression free survival [PFS]: 6.3 months vs 60.2 months, $p = 0.014$, pelvic recurrence 72% vs 17% $p = 0.049$) (Slim et al., 2018).

Unfortunately, patients with SCC of the bladder frequently present with locally advanced disease at diagnosis, which is often unresectable. Additionally, there is a high incidence of local recurrence (Kassouf et al., 2007). In this context, multimodal approaches adding radiotherapy and/or chemotherapy have been studied in both the neoadjuvant and adjuvant settings. For instance, there are conflicting data about benefit of neoadjuvant therapy. In the largest series of patients with NB-SCC of urinary bladder, 8 of 27 patients received initial chemotherapy and/or radiotherapy with the intention to be followed by surgical resection (4 in neoadjuvant setting and 4 with initially unresectable disease). Unfortunately, in 5 of the 8 patients, surgery was not performed due to non-conversion to a surgically resectable status or rapid progression of disease (Kassouf et al., 2007). A prospective study has shown an improvement in disease-free survival (DFS) with preoperative radiation followed by RC compared to RC alone (Ghoneim et al., 1985) and a retrospective analysis suggested that neoadjuvant radiation followed by RC is better than radiation alone (5-year disease free survival [DFS] of 40% vs 16%, respectively) (Swanson et al., 1990; Prempree and Amornmarn, 1984).

Similar to UC patients, those with NB-SCC of the bladder are at high risk for increased mortality and morbidity with RC, and alternative approaches for these patients have been explored. For instance, the SWOG 8733 trial, which included a small proportion of SCC patients ineligible for cisplatin-based chemotherapy or with unresectable localized disease, showed that chemotherapy with 5-fluouracil (5-FU) and radiation followed by RC versus chemotherapy and radiation alone performed relatively well (Response Rate [RR] of 49%, PFS of 13 months and OS of 20 months for the inoperable cases) (Higano et al., 2008). Data from the phase III BC2001 trial also showed efficacy results for chemotherapy and radiation (5-FU and mitomycin C) compared to RT alone in bladder cancer, with improved locoregional control and a trend toward better survival. Importantly, no difference in outcomes was found between non-UC and UC histologies, although only 2.7% of patients had adenocarcinoma or squamous cell carcinoma (James et al., 2012). Comparable outcomes between NU and UC of the bladder treated with multimodality approaches were reported (chemoradiation followed by surgery), showing similar RR (71% *vs* 84%; $p = 0.13$), 5-year muscle-invasive bladder cancer recurrence-free survival rates

(92% *vs* 100%; p = 0.21) and 5-year cancer specific survival (93% *vs* 94%; p = 0.64). A total of 11 patients with SCC were included (Kijima et al., 2018). Thus, in patients not suitable for RC, combination treatment without surgery may represent a valid alternative.

6.2. Systemic treatments

SCC has long been considered as a chemotherapy resistant tumor. Notwithstanding, some small studies have shown good outcomes with chemotherapy. As an example, a retrospective analysis comparing outcomes of patients with UC, mixed histologies or SCC treated with various platinum-based regimens showed a RR of 27%, PFS of 7.2 months and OS of 13.6 months in SCC, which is somewhat comparable to the results seen between UC and mixed bladder tumors (RR of 44% and 34%, PFS of 7.7 and 7.2 months, and OS of 11.3 and 10.4 months, respectively) (Kastritis et al., 2006). Importantly, only a small fraction of patients included in the analysis had SCC (3.5%).

A small prospective study (n = 20) assessed the combination of paclitaxel, ifosfamide and cisplatin in NU tumors, including a total of 8 SCC cases (40%), 11 adenocarcinomas (55%) and 1 small cell carcinoma (5%). The authors showed a RR of 25% and OS of 8.9 months among SCC patients (Galsky et al., 2007). Although outcomes were inferior compared to the other tumor types, this study provides evidence of some activity of this regimen among SCC cases. Khaled et al also explored a cisplatin-doublet in locally advanced and metastatic Bilharzial-associated tumors, including cases of UC (59%), SCC (38%) and adenocarcinoma (3%). A RR of 60% and 50% was seen in UC and SCC, respectively, a difference that did not reach statistical significance (p = 0.5) (Khaled et al., 2000). Other regimens, such as carboplatin-based schemes, have been tested and comparable outcomes were seen in UC and SCC cases (Hussain et al., 2001).

In the second-line setting, post-failure of cisplatin-based chemotherapy, vinflunine is the only chemotherapeutic agent to show improvement in clinical outcomes over best supportive care in a phase 3 trial, leading to its approval by the European Medicines Authority. However, flaws in trial design did not allow for a FDA approval. Notwithstanding, this trial included UCC patients only and therefore its activity among SCC of the bladder is unclear (Oing et al., 2016).

Targeted therapies have also been evaluated in bladder carcinoma, specifically in UC. Nonetheless, disappointing results were often seen with limited benefit. As discussed previously, some studies demonstrated variable overexpression and amplification of EGFR in bladder carcinomas. A small trial explored lapatinib, an EGFR and HER2 inhibitor, in bladder carcinoma patients with no significant improvement in outcomes (PFS and OS) (Powles et al., 2017a). Similarly, everolimus failed to show better outcomes in a single-arm, non-randomized study in UC patients (Milowsky et al., 2013).

More recently, other targets have been explored and some encouraging results were seen in early phase clinical trials. Perhaps the most successful and promising is BGJ398, a selective FGFR1–3 inhibitor that appears to be active in *FGFR*-amplified and *FGFR* mutated tumors, including UC (Nogova et al., 2017). In the phase 1 study, which included 8 patients with FGFR3-mutant bladder cancer, the disease control rate (DCR) was 75%. In its subsequent phase 2 study, a 64% DCR and 36% ORR was reported (Pal et al., 2016). Similarly, clinical trials testing erdafitinib, a pan-FGFR inhibitor, also showed promising activity among pre-selected bladder cancer patients harboring FGFR2-3 alterations, with a ORR of 43.5%, median duration of response of 7.2 months and PFS of 5.1 months in the phase 1 study, with RR up to 34% recently reported in a phase 2 study (Loriot et al., 2018; Soria et al., 2016). In contrast, dovitinib showed no activity in advanced UC, regardless of *FGFR3* mutation status (Milowsky et al., 2014).

Given the overall good results of new immunotherapeutic agents in UC of the bladder and in SCC of other sites of origin, such as lung and head and neck, immunotherapies appear promising in SCC of the bladder (Rosenberg et al., 2016; Brahmer et al., 2015; Ferris et al., 2016). However, there is no data at the moment of its activity in SCC of the bladder, as these patients have been generally excluded from IO clinical trials. The Keynote-045, which evaluated pembrolizumab in the second-line setting against investigator's choice of chemotherapy, and the IMVigor 211, which evaluated atezolizumab in the same context, were the only trials that allowed mixed histologies as long as the predominant subtype was UC. Pure NU histologies were not allowed. In the former, pembrolizumab was associated with a survival benefit (OS 10.3 v 7.3 months; HR, 0.70; $P < 0.001$) and superior safety profile compared to chemotherapy. Of note, as reported in the subset analysis, the magnitude of OS benefit among the mixed histologies subgroup (which comprised approximately 30% of patients) was higher as compared to pure UC histology (HR 0.58 *vs* 0.80) (Bellmunt et al., 2017, 2018). Atezolizumab, however, did not confirm the findings from the previous phase 2 study and failed to improve OS when compared to chemotherapy in the overall study population (11.1 months vs 10.6 months; HR 0.87; 95%CI 0.63–1.21; p = 0.41) (Powles et al., 2017b). On the other hand, the trials of nivolumab, durvalumab and avelumab did not account for mixed or NU histologies, therefore the clinical benefit of these particular agents among this population is not available. Nevertheless, these agents have now been approved and hold great promise for the treatment of UC patients (Sharma et al., 2017; Powles et al., 2017c; Apolo et al., 2017).

In the first-line setting, checkpoint inhibitors, such as pembrolizumab and atezolizumab have also been evaluated in the context of cisplatin-ineligibility. In the Keynote-052, a single-arm phase 2 trial which evaluated pembrolizumab among locally advanced or metastatic bladder cancer patients, non-urothelial histologies were allowed per protocol. Pembrolizumab resulted in a response rate of 24% in the overall study population, although no subgroup analysis have been reported at this point (Balar et al., 2016). In the atezolizumab trial, however, NU histologies were excluded (Balar et al., 2017).

7. Future directions

Further characterization of NU bladder cancer subtypes is needed to further targeted drug development in this disease. Bladder cancer classification according to pathological features should be augmented by comprehensive molecular subtyping. High throughput technologies have greatly expanded our understanding of the molecular classification and pathogenesis of bladder cancer and have facilitated the identification of potential therapeutic targets and predictive biomarkers (Iyer et al., 2012; Wagle et al., 2014). In this context, there are an increasing number of observational studies designed to interrogate the mutational profile of the TCGA defined molecular subtypes of bladder cancer.

Clinical trial designs that are agnostic of tumor type but focus on the inclusion of tumors with certain molecular alterations may address the limitations of rare tumor subtypes. This has been demonstrated in mismatch repair deficient tumors and pembrolizumab (Le et al., 2015).

Table 2 lists selected ongoing trials exploring targeted therapies in bladder carcinoma.

8. Conclusion

SCC of the bladder is a rare bladder cancer subtype. Advanced stage at presentation is usual and currently the cornerstone of treatment remains radical cystectomy for localized disease and systemic chemotherapy for metastatic tumors. Limited data are available on the efficacy of systemic therapies in the neoadjuvant, adjuvant or metastatic settings, although activity has been noted with some regimens. Increasing evidence suggests a distinct genetic and molecular landscape behind the different bladder cancer phenotypes, with tremendous implications on prognosis as well as treatment selection and response. Further understanding of bladder cancer tumor biology may facilitate drug development with either new drugs or the application of existing

Table 2
Selected ongoing clinical trials exploring biomarkers in bladder cancer.

Clinical Trial Number	Study Type	Study Setting	Subtype/histology	Study Name	Comments
NCT03123055	Phase 1b, single-arm	Second-line	Urothelial carcinoma	A Study of B-701 in Combination With Pembrolizumab in treatment of Locally Advanced or Metastatic Urothelial Cell Carcinoma (U22)	B-702 is aFGFR3 inhibitor.
NCT01827943	Phase 2, single-arm	Second-line	Any bladder cancer histology	Phase II Evaluating Efficacy of Temsirolimus in 2nd Line Therapy for Patients With Advanced Bladder Cancer (VESTOR)	
NCT02648100	Non-interventional	Biomarker study (assay validation)	Basal bladder cancer subtype	Basal Like Bladder Cancer: Signature and Therapeutic (DIATRIBBE)	
NCT02465060	Phase 2, non-randomized	Second and latter lines	Any bladder cancer subtype or histology.	NCI-MATCH: Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma	Different targeted agents according to the specific genetic alterations found (crizotinib, afatinib, dabrafenibetc).
NCT02643043	Non-interventional	Biomarker study	Urothelial carcinoma.	UC-GENOME: Urothelial Cancer-GENOmic Analysis to iMprove Patient Outcomes and rEsearch (observational – biospecimen collection and NGS)	NGS will be applied in archival tumor tissue of metastatic urothelial specimens for a comprehensive genetic characterization.
NCT02197897	Phase 2, single-arm	First or second-line (after local resection)	Urothelial carcinoma.	Phase II Trial of Estrogen Receptor Targeted Treatment of Non-Muscle Invasive Bladder Cancer With Tamoxifen (BCTamoxifen)	Papillary, non-muscle invasive tumors.
NCT02496208	Phase 1, non-randomized	Second and latter lines	Urothelial carcinoma, adenocarcinoma or squamous cell carcinoma of the bladder	A Phase 1 Study of Cabozantinib Plus Nivolumab (CaboNivo) Alone or in Combination With Ipilimumab (CaboNivoIpi) in Patients With Advanced/Metastatic Urothelial Carcinoma and Other Genitourinary Tumors	

drugs towards specific actionable mutations or molecular subtypes, which may improve clinical outcomes for distinct subsets of bladder cancer.

Conflicts of interest

The authors declare no conflict of interest.

References

Abdollah, F., et al., 2012. Survival after radical cystectomy of non-bilharzial squamous cell carcinoma vs urothelial carcinoma: a competing-risks analysis. BJU Int. 109, 564–569. https://doi.org/10.1111/j.1464-410X.2011.10357.x.

Abdulamir, A.S., et al., 2009. Tumor markers of bladder cancer: the schistosomal bladder tumors versus non-schistosomal bladder tumors. J. Exp. Clin. Cancer Res. 28, 27. https://doi.org/10.1186/1756-9966-28-27.

Abol-Enein, H., Kava, B.R., Carmack, A.J., 2007. Nonurothelial cancer of the bladder. Urology 69, 93–104. https://doi.org/10.1016/j.urology.2006.08.1107.

Antoni, S., et al., 2017. Bladder cancer incidence and mortality: a global overview and recent trends. Eur. Urol. 71, 96–108. https://doi.org/10.1016/j.eururo.2016.06.010.

Apolo, A.B., et al., 2017. Avelumab, an anti-programmed death-ligand 1 antibody, in patients with refractory metastatic urothelial carcinoma: results from a Multicenter, Phase Ib Study. J. Clin. Oncol. 35, 2117–2124. https://doi.org/10.1200/jco.2016.71.6795.

Aragon-Ching, Jeanny B., Henson, D., 2018. Differences in survival among non-urothelial bladder cancers: analyses of SEER 1988–2008. J. Clin. Oncol. 36 (suppl 6S; abstr 425) (2018).

Badr, K.M., et al., 2004. Muscle invasive schistosomal squamous cell carcinoma of the urinary bladder: frequency and prognostic significance of p53, BCL-2, HER2/neu, and proliferation (MIB-1). Hum. Pathol. 35, 184–189.

Balar, A., et al., 2016. Pembrolizumab (pembro) as first-line therapy for advanced/unresectable or metastatic urothelial cancer: preliminary results from the phase 2 KEYNOTE-052 study. Ann. Oncol. 27https://doi.org/10.1093/annonc/mdw435.25. LBA32_PR-LBA32_PR.

Balar, A.V., et al., 2017. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet 389, 67–76. https://doi.org/10.1016/S0140-6736(16)32455-2.

Baldia, P.H., et al., 2016. Fibroblast growth factor receptor (FGFR) alterations in squamous differentiated bladder cancer: a putative therapeutic target for a small subgroup. Oncotarget 7, 71429–71439. https://doi.org/10.18632/oncotarget.12198.

Bellmunt, J., et al., 2017. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N. Engl. J. Med. 376, 1015–1026. https://doi.org/10.1056/NEJMoa1613683.

Bellmunt, J., et al., 2018. Two-year follow-up from the phase 3 KEYNOTE-045 trial of pembrolizumab (pembro) vs investigator's choice (paclitaxel, docetaxel, or vinflunine) in recurrent, advanced urothelial cancer (UC). J. Clin. Oncol. 36 (suppl 6S; abstr 410).

Blaveri, E., et al., 2005. Bladder cancer outcome and subtype classification by gene expression. Clin. Cancer Res. 11, 4044–4055. https://doi.org/10.1158/1078-0432.CCR-04-2409.

Brahmer, J., et al., 2015. Nivolumab versus docetaxel in advanced squamous-cell non–-small-cell lung cancer. N. Engl. J. Med. https://doi.org/10.1056/NEJMoa1504627.150531143008000-150531143008000.

Cancer Genome Atlas Research, N, 2014. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 507, 315–322. https://doi.org/10.1038/nature12965.

Celis, J.E., Wolf, H., Ostergaard, M., 2000. Bladder squamous cell carcinoma biomarkers derived from proteomics. Electrophoresis 21, 2115–2121. https://doi.org/10.1002/1522-2683(20000601)21:11<2115::AID-ELPS2115>3.0.CO;2-K.

Chalasani, V., Chin, J.L., Izawa, J.I., 2009. Histologic variants of urothelial bladder cancer and nonurothelial histology in bladder cancer. Can. Urol. Assoc. J. 3, S193–198.

Choi, W., et al., 2014a. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 25, 152–165. https://doi.org/10.1016/j.ccr.2014.01.009.

Choi, W., et al., 2014b. Intrinsic basal and luminal subtypes of muscle-invasive bladder cancer. Nat. Rev. Urol. 11, 400–410. https://doi.org/10.1038/nrurol.2014.129.

Dahm, P., Gschwend, J.E., 2003. Malignant non-urothelial neoplasms of the urinary bladder: a review. Eur. Urol. 44, 672–681.

Damrauer, J.S., et al., 2014. Intrinsic subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. Proc. Natl. Acad. Sci. U. S. A. 111, 3110–3115. https://doi.org/10.1073/pnas.1318376111.

Eissa, S., et al., 2005. HER2/neu expression in bladder cancer: relationship to cell cycle kinetics. Clin. Biochem. 38, 142–148. https://doi.org/10.1016/j.clinbiochem.2004.09.004.

El-Kenawy Ael, M., El-Kott, A.F., Khalil, A.M., 2003. Prognostic value of p53 and MDM2 expression in bilharziasis-associated squamous cell carcinoma of the urinary bladder. Int. J. Biol. Markers 18, 284–289.

El-Meghawry El-Kenawy, A., et al., 2006. Prognostic value of carcinoma-associated antigen MK-1 in urinary bladder carcinoma. Int. J. Biol. Markers 21, 170–174.

El-Rifai, W., et al., 2000. DNA copy number changes in Schistosoma-associated and non-Schistosoma-associated bladder cancer. Am. J. Pathol. 156, 871–878. https://doi.org/10.1016/S0002-9440(10)64956-5.

Felix, A.S., et al., 2008. The changing patterns of bladder cancer in Egypt over the past 26 years. Cancer Causes Control 19, 421–429. https://doi.org/10.1007/s10552-007-9104-7.
Ferris, R.L., et al., 2016. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N. Engl. J. Med. 375, 1856–1867. https://doi.org/10.1056/NEJMoa1602252.
Gallo, O., et al., 2003. Down-regulation of nitric oxide synthase-2 and cyclooxygenase-2 pathways by p53 in squamous cell carcinoma. Am. J. Pathol. 163, 723–732. https://doi.org/10.1016/S0002-9440(10)63699-1.
Galsky, M.D., et al., 2007. Prospective trial of ifosfamide, paclitaxel, and cisplatin in patients with advanced non-transitional cell carcinoma of the urothelial tract. Urology 69, 255–259. https://doi.org/10.1016/j.urology.2006.10.029.
Ghoneim, M.A., et al., 1985. Randomized trial of cystectomy with or without preoperative radiotherapy for carcinoma of the bilharzial bladder. J. Urol. 134, 266–268. https://doi.org/10.1016/S0022-5347(17)47119-9.
Ghoneim, M.A., et al., 1997. Radical cystectomy for carcinoma of the bladder: critical evaluation of the results in 1,026 cases. J. Urol. 158, 393–399.
Guo, C.C., et al., 2009. Squamous cell carcinoma of the urinary bladder: a clinicopathologic and immunohistochemical study of 16 cases. Hum. Pathol. 40, 1448–1452. https://doi.org/10.1016/j.humpath.2009.03.005.
Haitel, A., et al., 2001. Bilharzial related, organ confined, muscle invasive bladder cancer: prognostic value of apoptosis markers, proliferation markers, p53, e-cadherin, epidermal growth factor receptor and c-erbb-2. J. Urol. 165, 1481–1487. https://doi.org/10.1016/S0022-5347(05)66332-X.
Hammam, O., et al., 2014. Expression of cytokeratin 7, 20, 14 in urothelial carcinoma and squamous cell carcinoma of the Egyprian urinary bladder cancer. J. Egypt. Soc. Parasitol. 44, 733–740.
Hammam, O., et al., 2015. The clinical significance of HER2 protein amplification/expression in urinary bladder lesion. Arab. J. Urol. 13, 146–152. https://doi.org/10.1016/j.aju.2015.01.004.
Higano, C.S., et al., 2008. Treatment options for muscle-invasive urothelial cancer for patients who were not eligible for cystectomy or neoadjuvant chemotherapy with methotrexate, vinblastine, doxorubicin, and cisplatin. Cancer 112, 2181–2187. https://doi.org/10.1002/cncr.23420.
Hirasawa, K., et al., 2002. Bladder tumor producing granulocyte colony-stimulating factor and parathyroid hormone related protein. J. Urol. 167, 2130.
Hussain, M., et al., 2001. Combination paclitaxel, carboplatin, and gemcitabine is an active treatment for advanced urothelial cancer. J. Clin. Oncol. 19, 2527–2533. https://doi.org/10.1200/jco.2001.19.9.2527.
Iyer, G., et al., 2012. Genome sequencing identifies a basis for everolimus sensitivity. Science 338, 221. https://doi.org/10.1126/science.1226344.
James, N.D., et al., 2012. Radiotherapy with or without chemotherapy in muscle-invasive bladder cancer. N. Engl. J. Med. 366, 1477–1488. https://doi.org/10.1056/NEJMoa1106106.
Jemal, A., Siegel, R., Xu, J., Ward, E., 2010. Cancer statistics, 2010. CA Cancer J. Clin. 60, 277–300. https://doi.org/10.3322/caac.20073.
Kassouf, W., et al., 2007. Outcome and patterns of recurrence of nonbilharzial pure squamous cell carcinoma of the bladder: a contemporary review of The University of Texas M D Anderson Cancer Center experience. Cancer 110, 764–769. https://doi.org/10.1002/cncr.22853.
Kastritis, E., et al., 2006. The outcome of patients with advanced pure squamous or mixed squamous and transitional urothelial carcinomas following platinum-based chemotherapy. Anticancer Res. 26, 3865–3869.
Khaled, H.M., et al., 2000. A phase II study of gemcitabine plus cisplatin chemotherapy in advanced bilharzial bladder carcinoma. Eur. J. Cancer 36, 34–37. https://doi.org/10.1016/S0959-8049(00)00079-4.
Kijima, T., et al., 2018. Clinical outcomes of patients with histologic variants of urothelial carcinoma treated with tetra-modality bladder-sparing protocol incorporating consolidative partial cystectomy. J. Clin. Oncol. 36 (suppl 6S; abstr 490) (2018).
Kwon, E.D., et al., 2014. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. 15, 700–712. https://doi.org/10.1016/S1470-2045(14)70189-5.
Lagwinski, N., et al., 2007. Squamous cell carcinoma of the bladder: a clinicopathologic analysis of 45 cases. Am. J. Surg. Pathol. 31, 1777–1787. https://doi.org/10.1097/PAS.0b013e31805c9cd9.
Le, D.T., et al., 2015. PD-1 blockade in tumors with mismatch-repair deficiency. N. Engl. J. Med. 372, 2509–2520. https://doi.org/10.1056/NEJMoa1500596.
Locke, J.R., Hill, D.E., Walzer, Y., 1985. Incidence of squamous cell carcinoma in patients with long-term catheter drainage. J. Urol. 133, 1034–1035.
Loriot, Y., et al., 2018. Erdafitinib (ERDA; JNJ-42756493), a pan-fibroblast growth factor receptor (FGFR) inhibitor, in patients (pts) with metastatic or unresectable urothelial carcinoma (mUC) and FGFR alterations (FGFRa): phase 2 continuous versus intermittent dosing. J. Clin. Oncol. 36 (suppl 6S; abstr 411) (2018).
Makboul, R., et al., 2015. Expression of survivin in squamous cell carcinoma and transitional cell carcinoma of the urinary bladder: a comparative immunohistochemical study. Korean J. Urol. 56, 31–40. https://doi.org/10.4111/kju.2015.56.1.31.
Manunta, A., et al., 2005. Non-transitional cell bladder carcinomas. BJU Int. 95, 497–502. https://doi.org/10.1111/j.1464-410X.2005.05327.x.
Martin, J.W., et al., 2016. Squamous cell carcinoma of the urinary bladder: systematic review of clinical characteristics and therapeutic approaches. Arab. J. Urol. 14, 183–191. https://doi.org/10.1016/j.aju.2016.07.001.
McNiel, E.A., Tsichlis, P.N., 2017. Analyses of publicly available genomics resources define FGF-2-expressing bladder carcinomas as EMT-prone, proliferative tumors with low mutation rates and high expression of CTLA-4, PD-1 and PD-L1. Signal Transduct. Target. Ther. 2. https://doi.org/10.1038/sigtrans.2016.45.
Milowsky, M.I., et al., 2013. Phase II study of everolimus in metastatic urothelial cancer. BJU Int. 112, 462–470. https://doi.org/10.1111/j.1464-410X.2012.11720.x.
Milowsky, M.I., et al., 2014. Phase 2 trial of dovitinib in patients with progressive FGFR3-mutated or FGFR3 wild-type advanced urothelial carcinoma. Eur. J. Cancer 50, 3145–3152. https://doi.org/10.1016/j.ejca.2014.10.013.
Molitor, M., et al., 2015. Comparison of structural genetics of non-schistosoma-associated squamous cell carcinoma of the urinary bladder. Int. J. Clin. Exp. Pathol. 8, 8143–8158.
Motzer, R.J., et al., 2015. Nivolumab versus everolimus in advanced renal-cell carcinoma. N. Engl. J. Med. 1803–1813. https://doi.org/10.1056/NEJMoa1510665.
Nogova, L., et al., 2017. Evaluation of BGJ398, a fibroblast growth factor receptor 1–3 kinase inhibitor, in patients with advanced solid tumors harboring genetic alterations in fibroblast growth factor receptors: results of a global phase I, dose-escalation and dose-expansion study. J. Clin. Oncol. 35, 157–165. https://doi.org/10.1200/jco.2016.67.2048.
Oing, C., et al., 2016. Second line chemotherapy for advanced and metastatic urothelial carcinoma: vinflunine and beyond-A comprehensive review of the current literature. J. Urol. 195, 254–263. https://doi.org/10.1016/j.juro.2015.06.115.
Omran, O.M., 2012. CD10 and E-cad expression in urinary bladder urothelial and squamous cell carcinoma. J. Environ. Pathol. Toxicol. Oncol. 31, 203–212.
Ostergaard, M., et al., 1999. Psoriasin (S100A7): a putative urinary marker for the follow-up of patients with bladder squamous cell carcinomas. Electrophoresis 20, 349–354. https://doi.org/10.1002/(SICI)1522-2683(19990201)20:2<349::AID-ELPS349>3.0.CO;2-B.
Pal, S.K., et al., 2016. Efficacy of BGJ398, a fibroblast growth factor receptor (FGFR) 1-3 inhibitor, in patients (pts) with previously treated advanced/metastatic urothelial carcinoma (mUC) with FGFR3 alterations. J. Clin. Oncol. 34 (suppl; abstr 4517) (2016).
Pannek, J., 2002. Transitional cell carcinoma in patients with spinal cord injury: a high risk malignancy? Urology 59, 240–244.
Porter, M.P., et al., 2002. Racial variation in the incidence of squamous cell carcinoma of the bladder in the United States. J. Urol. 168, 1960–1963. https://doi.org/10.1097/01.ju.0000034384.91899.67.
Powles, T., et al., 2017a. Phase III, double-blind, randomized trial that compared maintenance lapatinib versus placebo after first-line chemotherapy in patients with human epidermal growth factor receptor 1/2-positive metastatic bladder cancer. J. Clin. Oncol. 35, 48–55. https://doi.org/10.1200/jco.2015.66.3468.
Powles, T., et al., 2017b. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. Lancet. https://doi.org/10.1016/S0140-6736(17)33297-X.
Powles, T., et al., 2017c. Efficacy and safety of durvalumab in locally advanced or metastatic urothelial carcinoma: updated results from a phase 1/2 open-label study. JAMA Oncol. e172411. https://doi.org/10.1001/jamaoncol.2017.2411.
Prempree, T., Amornmarn, R., 1984. Radiation management of squamous cell carcinoma of the bladder. Acta Radiol.: Oncol. 23, 37–42. https://doi.org/10.3109/02841868409135983.
Pycha, A., et al., 1999. Numerical chromosomal aberrations in muscle invasive squamous cell and transitional cell cancer of the urinary bladder: an alternative to classic prognostic indicators? Urology 53, 1005–1010.
Ramchurren, N., Cooper, K., Summerhayes, I.C., 1995. Molecular events underlying schistosomiasis-related bladder cancer. Int. J. Cancer 62, 237–244.
Rosenberg, J.E., et al., 2016. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 387, 1909–1920. https://doi.org/10.1016/s0140-6736(16)00561-4.
Shaker, O.G., Hammam, O.A., Wishahi, M.M., 2013. Is there a correlation between HPV and urinary bladder carcinoma? Biomed. Pharmacother. 67, 183–191. https://doi.org/10.1016/j.biopha.2012.10.019.
Sharma, P., et al., 2017. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. Lancet Oncol. 18, 312–322. https://doi.org/10.1016/S1470-2045(17)30065-7.
Shokeir, A.A., 2004. Squamous cell carcinoma of the bladder: pathology, diagnosis and treatment. BJU Int. 93, 216–220.
Slim, M., et al., 2018. Prognostic impact of percentage of squamous differentiation in patients with nonbilharzial squamous cell carcinoma and transitional cell carcinoma treated with radical cystectomy. J. Clin. Oncol. 36 (suppl 6S; abstr 498) (2018).
Soria, J.-C., et al., 2016. Safety and activity of the pan–fibroblast growth factor receptor (FGFR) inhibitor erdafitinib in phase 1 study patients with advanced urothelial carcinoma. Ann. Oncol. 27 (6), 266–295. https://doi.org/10.1093/annonc/mdw373.
Spradling, K., et al., 2016. Lymphovascular invasion is associated with oncologic outcomes following radical cystectomy for squamous cell carcinoma of the urinary bladder. Urol. Oncol. 34 (417), e411–418. https://doi.org/10.1016/j.urolonc.2016.03.023.
Stein, J.P., et al., 1993. Squamous cell carcinoma of the bladder associated with cyclophosphamide therapy for Wegener's granulomatosis: a report of 2 cases. J. Urol. 149, 588–589.
Subbaramaiah, K., et al., 1999. Inhibition of cyclooxygenase-2 gene expression by p53. J. Biol. Chem. 274, 10911–10915.
Swanson, D.A., Liles, A., Zagars, G.K., 1990. Preoperative irradiation and radical cystectomy for stages T2 and T3 squamous cell carcinoma of the bladder. J. Urol. 143, 37–40. https://doi.org/10.1016/S0022-5347(17)39857-9.
Vaidyanathan, S., et al., 2002. Problems in early diagnosis of bladder cancer in a spinal cord injury patient: report of a case of simultaneous production of granulocyte colony stimulating factor and parathyroid hormone-related protein by squamous cell carcinoma of urinary bladder. BMC Urol. 2, 8.

Wagle, N., et al., 2014. Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. Cancer Discov. https://doi.org/10.1158/2159-8290.cd-13-0353.

Welk, B., et al., 2013. Bladder cancer in individuals with spinal cord injuries. Spinal Cord 51, 516–521. https://doi.org/10.1038/sc.2013.33.

Westenend, P.J., Stoop, J.A., Hendriks, J.G., 2001. Human papillomaviruses 6/11, 16/18 and 31/33/51 are not associated with squamous cell carcinoma of the urinary bladder. BJU Int. 88, 198–201.

Wysolmerski, J.J., Stewart, A.F., 1998. The physiology of parathyroid hormone-related protein: an emerging role as a developmental factor. Annu. Rev. Physiol. 60, 431–460. https://doi.org/10.1146/annurev.physiol.60.1.431.

Youssef, R., et al., 2011. Bilharzial vs non-bilharzial related bladder cancer: pathological characteristics and value of cyclooxygenase-2 expression. BJU Int. 108, 31–37. https://doi.org/10.1111/j.1464-410X.2010.09854.x.

Youssef, R.F., et al., 2015a. Utility of biomarkers in the prediction of oncologic outcome after radical cystectomy for squamous cell carcinoma. J. Urol. 193, 451–456. https://doi.org/10.1016/j.juro.2014.08.109.

Youssef, R.F., et al., 2015b. Role of fibroblast growth factor in squamous cell carcinoma of the bladder: prognostic biomarker and potential therapeutic target. Urol. Oncol. 33 (111), e111–117. https://doi.org/10.1016/j.urolonc.2014.09.020.