CLINICAL INVESTIGATION

Personalized therapy for urothelial cancer: review of the clinical evidence

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Despite a detailed understanding of the molecular aberrations driving the development of urothelial cancers, this knowledge has not translated into advances for the treatment of this disease. Urothelial cancers are chemosensitive and platinum-based combination chemotherapy remains the standard of care for advanced disease, as well as neoadjuvant and adjuvant therapy for locally advanced disease. However, nearly half of patients who undergo resection of locally advanced urothelial cancer will relapse and eventually develop platinum-resistant disease. Clinical trials of targeted agents against angiogenesis and growth factors, as well as novel chemotherapeutics, have generally been unsuccessful in urothelial cancers. Improvements in the therapeutic arsenal for urothelial cancer depend upon identification of new targets and strategies to overcome platinum resistance.

Keywords: bacillus Calmette-Guerin • bladder cancer • epithelial-to-mesenchymal transition • platinum resistance • targeted therapy • transitional cell carcinoma • urothelial carcinoma

The era of personalized medicine has already revolutionized the approach to a number of malignancies. For example, in breast cancer, from determining the benefit of adjuvant chemotherapy to selecting treatment for hEGF receptor 2 (HER2)-positive disease, molecular diagnostics has enabled oncologists to tailor therapy to an individual patient's cancer beyond clinicopathologic characteristics such as stage and histologic grade [1,2]. Ground-breaking work has emerged in the treatment of melanoma [3] and non-small-cell lung cancer [4] that has the potential to transform fatal malignancies into treatable conditions. The hope is that this same possibility exists for urothelial cancers for which the only treatment options remain standard chemotherapy and for whom the majority of patients derive limited benefit.

While our understanding of the molecular changes in urothelial cancers has rapidly evolved over the last few decades, our therapeutic arsenal has not. First-line treatment for advanced disease remains platinum-based combination chemotherapy, and no US FDA-approved second-line treatment exists. Attempts to improve current therapies have focused on dose intensity and combination doublet and triplet regimens without substantial gains, and unlike in other malignancies, targeted therapies have failed thus far to advance the standard of care beyond cytotoxic chemotherapy. Furthermore, it is unclear whether the multiple biomarkers that have been identified are responsible for the aggressive phenotype, or rather, are secondary to other driving mechanisms. Significant efforts to address these unmet needs are underway and range from identification of new molecular targets to testing of novel chemotherapeutic agents and targeted therapies to elucidating the mechanisms of cisplatin resistance.

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Diagnosis & treatment of urothelial carcinomas

An estimated 70,500 new cases and 14,500 deaths in 2010, will be attributed to bladder cancer in the USA alone, making it the fourth most common cancer and ninth leading cause of cancer-related deaths among men [5]. In addition to the human cost, multiple economic analyses demonstrate that bladder cancer is among the most expensive to treat due to the invasive nature of surveillance and treatment, with one group predicting a lifetime cost between US$99,000 and $120,000 [6]. Bladder cancer is three-times more common in men than women. The majority of patients are elderly, with a median age at presentation in men and women of 72 and 74 years, respectively [7]. Tobacco use is the strongest risk factor for development of urothelial cancer; other risk factors include occupational exposures to aniline dyes and aromatic amines, treatment with chemotherapy agents, including cyclophosphamide and acrolein, and pelvic irradiation [8].

The majority of patients present with superficial disease, and treatment of bladder cancer is based on the TNM staging system. Non-muscle-invasive, high-grade disease (carcinoma *in situ*, T1) is treated with transurethreal bladder resection and intravesical bacillus Calmette–Guerin (BCG) or intravesical chemotherapy. Radical cystectomy with or without neoadjuvant or adjuvant cisplatin-based chemotherapy or a bladder-preservation approach with chemoradiation is used in the management of locally advanced disease. Platinum-based cytotoxic combination regimens are used in advanced disease. While gemcitabine and cisplatin (GC) does not improve overall survival compared with the combination of methotrexate, vinblastine, doxorubidin and cisplatin (MVAC), MVAC is associated with increased toxicity, including granulocytopenia, nausea and vomiting. Therefore, GC is generally favored [9]. However, these strategies are clearly inadequate. Of patients with superficial disease, 70% relapse, between 10 and 30% will eventually progress to muscle-invasive disease, and half of all patients with resected, locally advanced disease die from metastatic disease within 2 years [10]. The prognosis of patients with advanced disease is extremely poor with median survival of 14 months despite optimal cisplatin-based combination chemotherapy [9].

Molecular pathways

Bladder cancer represents a unique opportunity to study the progression of genetic aberrations across stages as tissue is frequently accessible. A well-described signature of chromosomal aberrations exists between low-grade, noninvasive, papillary hyperplasia variants and high-grade, muscle-invasive disease. However, despite a detailed understanding of the molecular pathogenesis of urothelial carcinoma, translating this knowledge into clinical biomarkers and effective therapies has been challenging and elusive. As discussed by Bryan *et al.* in their review of molecular pathways in bladder cancer, the genetic changes in low-grade and high-grade urothelial carcinoma promote the six hallmarks of cancer outlined by Hanahan and Weinberg: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis [11–14].

The most frequent activating mutations detected in low-grade tumors constitutively upregulate the activity of the receptor-tyrosine kinase-Ras pathway and include overexpression of FGF receptor (FGFR)-3 in up to 70% of tumors [15], HRAS in 30–40% [16] and PIK3CA in 10% [17]. Chromosome 9 loss is seen in both low-grade and high-grade tumors [18]. The deletion or mutation of tumor suppressor genes *p53* [19] and *pRB* [20], both critical cell cycle regulators, are the most frequent abnormalities in high-grade tumors and contribute to tumor progression. High-grade lesions may also have *PTEN* [21] and *p16* [22] loss. Finally, changes in the microenvironment also promote invasion and progression though aberrant N- and E-cadherin expression [23] and production of VEGF [24].

Targeted therapy

The results of clinical trials of targeted agents for urothelial cancers published thus far have generally been disappointing and, to date, no biologic agents have been approved either as monotherapy or in combination with cytotoxic chemotherapy for advanced urothelial carcinoma. Despite the identification of genetic alterations thought to drive high-grade, muscle-invasive disease, these aberrations have not successfully predicted response to targeted treatment. Classes of agents in recent and ongoing clinical trials include antiangiogenic monoclonal antibodies; multitargeted tyrosine kinase inhibitors (TKIs) against VEGF receptor (VEGFR)-2 and PDGF receptor (PDGFR); EGF receptor (EGFR) and HER2 inhibitors; and other inhibitors targeted against mTOR, FGFR-3, IGF receptor 1 and Src. Novel vaccine strategies are also being employed.

Angiogenesis inhibitors

Angiogenesis is an attractive target in urothelial cancer given the roles of the proangiogenic factors VEGF, FGF and PDGF in cell cycle regulation and invasion [25]. In addition, VEGFR and EGFR inhibition may sensitize to cisplatin [26,27]. Hahn *et al.* recently reported the mature results of their Phase II study of bevacizumab with cisplatin and gemcitabine as first-line therapy for metastatic urothelial carcinoma (Supplementary Table 1) [28]. The overall response rate

was 72% with nine complete responses and 22 partial responses out of 43 patients. Median progression-free survival was 8.2 months with a median overall survival of 20.4 months; however, this study failed to meet its primary end point. Toxicities were consistent with recognized effects of antiangiogenesis treatment and included deep venous thrombosis/pulmonary embolus (21%), hemorrhage (7%), hypertension (5%) and proteinuria (2%). Based on these results, a Cancer and Leukemia Group B (CALBG) Phase III trial comparing first-line gemcitabine and cisplatin with or without bevacizumab is currently underway (NCT00942331), as well as a Phase II first-line study of gemcitabine, carboplatin and bevacizumab in patients who are ineligible for cisplatin (NCT00588666) (Supplementary Table 1). The triplet of gemcitabine, cisplatin and bevacizumab is also being studied in the neoadjuvant setting in a Phase II study for locally advanced urothelial cancer prior to radical cystectomy (NCT00268450) (Supplementary Table 1).

The VEGF receptor fusion protein VEGF-Trap (aflibercept) targets PDGF and has greater affinity for VEGF than bevacizumab. This agent was well-tolerated in platinum-pretreated patients with transitional cell carcinoma, but had limited single-agent activity with a response rate of 4.5% and progression-free survival of 3.5 months (Supplementary Table 1) [29].

Sunitinib is a multitargeted TKI with activity against VEGF, PDGFR, Kit, FLT3 and RET. It showed modest activity in a Phase II trial in previously treated urothelial cancer with clinical regression or stable disease in 43% of patients and which lasted longer than 3 months in 29% of patients (Supplementary Table 1); overall survival was approximately 7 months [30]. Given its activity against urothelial cancer, sunitinib was also studied in the first-line setting together with gemcitabine and cisplatin. While preliminary data suggest antitumor activity with nine of 15 patients having partial responses or stable disease, toxicity was limiting. Specifically, six of 15 patients discontinued treatment early, most commonly due to cytopenias, and 33% experienced a serious adverse event, including one death due to neutropenic sepsis (Supplementary Table 1) [31]. Sorafenib, a multikinase inhibitor with activity against VEGFR, PDGFR, Kit and RAF, showed no objective responses in first-line treatment of metastatic urothelial cancer with time to progression of 1.9 months and median survival of 5.9 months (Supplementary Table 1) [32]. In the second-line setting, sorafinib also failed to produce any objective responses (Supplementary Table 1) [33].

Pazopanib is a TKI that selectively targets VEGF, PDGFR and Kit to inhibit angiogenesis. Preliminary results of this agent in a Phase II trial of single-agent pazopanib in relapsed or refractory advanced urothelial carcinoma, which included eight cases of upper urinary tract disease, demonstrated four out of 18 patients had partial responses and 11 out of 18 had stable disease (83% clinical benefit) after a median follow-up of 3 months (NCT01031875) (Supplementary Table 1) [34]. Other ongoing trials are investigating the role of pazopanib in the second-line setting for advanced, platinum-refractory urothelial carcinoma in combination with vinflunine (NCT01265940) and weekly paclitaxel (NCT01108055) (Supplementary Table 1). A Phase II trial of single-agent pazopanib in second-line metastatic urothelial carcinoma was recently completed, but not yet reported (NCT00471536) (Supplementary Table 1).

■ Growth factor inhibitors

Members of the ErbB or EGFR protein family of receptors and their ligands, including EGF and FGF-3, as well as type 1 IGF and IGF receptor in the IGF axis, are potential targets in the treatment of urothelial cancers given reports of overexpression in advanced disease [35–37]. Results from *in vitro* and preclinical studies confirm the role of EGFR and HER2/neu in the proliferation of bladder cancer cells and support the rationale to target these receptors in clinical trials with small-molecule TKIs [38–41].

Petrylak *et al.* recently reported the disappointing results that ZD1839 (gefitinib), an oral EGFR TKI, was ineffective as second-line therapy for metastatic transitional cell carcinoma based on the results of their Southwest Oncology Group (SWOG) Phase II study (S0031) (Supplementary Table 2) [42]. They found that despite strong expression of EGFR staining in nearly half of the pretreatment biopsies reviewed, only two patients survived past 6 months without disease progression and the median progression-free survival was 2 months. Similarly, the results of CALGB 90102, a Phase II evaluation of cisplatin, gemcitabine and gefitinib as first-line treatment for advanced urothelial carcinoma, showed no improvement in response rates or survival compared with historical controls who received gemcitabine and cisplatin (Supplementary Table 2) [43].

Erlotinib, another EGFR TKI, has shown evidence of activity in a Phase II trial of neoadjuvant treatment in patients with muscle-invasive bladder cancer undergoing radical cystectomy (Supplementary Table 2) [44]. Five of 20 patients with clinical stage T2 were found to have pT0 disease following treatment, seven were clinically downstaged (≤pT1) and 15 had organ-confined disease at surgical pathology. At 24.8 months of follow-up, 14 remained alive, of whom ten had no evidence of disease, and four had disease progression. However, the 50% disease-free survival at 2 years with erlotinib was comparable with historical data from patients with clinical T2 disease who underwent surgery alone. No published data for erlotinib in the metastatic setting are available.

An alternative strategy to target EGFR employs the use of cetuximab, a chimeric monoclonal antibody against the receptor. Cetuximab is currently being studied in combination with gemcitabine and cisplatin in a Phase II trial of previously untreated patients with advanced urothelial carcinoma (NCT00645593) (Supplementary Table 2).

As with EGFR, HER2/neu overexpression has been targeted theraputically. Lapatinib, a dual TKI of EGFR and HER2/neu, was studied in a Phase II trial as second-line therapy for patients with advanced transitional cell carcinoma (Supplementary Table 2) [45]. The overall response rate was 1.7 and 31% of patients had stable disease. While the median overall survival was 17.9 weeks, patients with EGFR or HER2 overexpression appeared to benefit the most from therapy with a median overall survival of 30.3 weeks compared with 10.6 for those patients with low expression. A Phase II/III study of lapatinib as second-line therapy for advanced urothelial cancer is ongoing (NCT00949455), as well as a study sponsored by the EORTC in the first-line setting together with gemcitabine and cisplatin (NCT00623064) (Supplementary Table 2).

The HER2/neu monoclonal antibody trastuzumab was assessed in combination with paclitaxel, carboplatin and gemcitabine in a first-line, Phase II trial that required overexpression of HER2/neu by immunohistochemistry, gene amplification or serology (Supplementary Table 2) [46]. A total of 31 of 44 patients (70%) responded with five complete responses, and median time to progression and overall survival were 9.3 and 14.1 months, respectively. Of the patients, 22% experienced grade 1–3 cardiac toxicity, although only three patients out of 44 experienced grade 3 cardiac toxicity. Trastuzumab is being studied together with paclitaxel and radiation for patients ineligible for cystectomy (NCT00238420) and studies of a novel Fc-optimized monoclonal antibody that targets HER2, MGAH22, are in Phase I trials (NCT01195935, NCT01148849) (Supplementary Table 2).

Multiple groups have speculated on the discouraging lack of efficacy of gefitinib and lapatinib in urothelial carcinoma despite EGFR and HER2 overexpression. In the case of EGFR, Blehm *et al.* examined 11 urothelial bladder cancer cell lines and 75 patient tumors for the presence of mutations within the kinase domain and expression of EGFRvIII expression, which have been reported to affect patient response to gefitinib [47,48]. They failed to detect kinase domain mutations and expression of EGFRvIII and concluded that the rare presence of these alterations in bladder cancer could reduce the rate of response to TKI therapy. Gallucci *et al.* found a significantly lower rate of *HER2* gene amplification than HER2 protein expression or chromosome 17 polysomy [49] and suggested that the form of overexpression could affect response to targeted therapy [50], an issue that has been raised in the breast cancer literature as well [51].

The process of epithelial-to-mesenchymal transition (EMT) has also been suggested to play a role in promoting resistance of bladder cancer cells to EGFR inhibitors. Using global gene expression profiling, McConkey *et al.* showed that human urothelial carcinoma cell lines segregated into 'epithelial' and 'mesenchymal' subsets where the epithelial subset was sensitive to EGFR inhibitors [52]. Furthermore, EGFR resistance in the mesenchymal lines could be reversed by expression of the miRNA-200 family (see 'Future perspective' for description of miRNAs), which restored an epithelial phenotype characterized by increased levels of E-cadherin; decreased expression of ZEB1, ZEB2 and ERRFI-1; and decreased cell migration [53]. These findings warrant further clinical investigation into the role of EMT and miR200 targets as predictive markers for sensitivity to EGFR inhibition and may present a novel pathway to reverse resistance in urothelial carcioma.

As previously mentioned, FGFR-3 and IGF1R are known to be overexpressed in urothelial carcinoma with rates of 60–70% in some series and, thus, may represent clinically useful theraputic targets [36]. TKI12458 from Novartis is currently being studied in a Phase II trial (NCT00790426) of second- and third-line therapy for patients with FGFR-3 mutated and wild-type urothelial carcinoma after it demonstrated activity in preclinical models (Supplementary Table 2) [54]. IGF1R has been demonstrated to promote motility and invasion through AKT- and MAPK-dependent activation of paxillin [55]. Cixutumumab is a monoclonal antibody against IGF1R currently in development [56].

Src & mTOR inhibitors

Dasatinib, a Src-inhibitor that downregulates the AKT pathway, showed antitumor activity *in vitro* against Src-overexpressing transitional cell carcinoma cell lines and was active in combination with cisplatin in a murine xenograft [57]. Based on these preclinical findings, the Hoosier Oncology Group (HOG) is conducting a trial of neoadjuvant dasatinib prior to radical cystectomy for urothelial carcinoma of the bladder (NCT00706641) [57]. In addition to AKT, other active pathways implicated in the pathogenesis of urothelial carcinoma include PI3K and PTEN [58,59]. Downregulation of PTEN is observed in over 20% of muscle-invasive bladder tumors and in up to 40% of tumors in the presence of p53 mutations, and inactivation of this pathways results in loss of control of the mTOR signaling cascade [21]. Everolimus (RAD001), a selective mTOR inhibitor, is currently being assessed in a Phase II trial in second-line therapy for advanced urothelial carcinoma [60] (NCT00805129), as well as in a

Phase II, second-line trial in combination with paclitaxel for advanced disease (NCT00933374), and a Phase II first-line study with or without paclitaxel for patients who are ineligible for cisplatin (NCT01215136).

■ Hormone therapy

Urothelial carcinomas express estrogen receptor (ER), with rates of up to 80% in some series [61]. While the relationship between ER expression and grade of the tumor is inconsistent [62], ER has been shown to mediate estrogen-induced urothelial cell proliferation [63]. A case report in the urological literature describes a patient with metastatic urothelial carcinoma who received tamoxifen, a selective ER mediator, for gynecomastia and whose cancer regressed while on this therapy [64]. Preclinical work with selective ER mediators in a murine model demonstrated inhibition of transitional cell xenografts [65]. Based on these data, and the relatively low toxicity profile, two Phase II trials at Baylor are assessing tamoxifen in the second-line setting for advanced urothelial cell carcinoma (NCT00589017 and NCT00710970).

■ Immune therapy

Stimulation of the immune system with BCG is the standard of care for the treatment of non-muscle-invasive bladder cancer [7]. While significant differences exist between noninvasive and invasive disease, this strategy raises the possibility that the immune system may be potentiated to recognize advanced urothelial carcinoma. Carthon *et al.* recently reported the results of a neoadjuvant study assessing the effects of ipilimumab, an anticytotoxic T-lymphocyte-associated antigen (CTLA)4 antibody, on localized urothelial carcinoma of the bladder [66]. They found that in patients with localized urothelial cancer of the bladder, a brief exposure to anti-CTLA4 with either a 3 or 10 mg/kg/dose for two doses prior to surgery was safe and following therapy with anti-CTLA4 was associated with an increased frequency of $CD4^+ICOS^{hi}$ T cells in systemic circulation and bladder tumor tissue. They then retrospectively correlated the frequency of $CD4^+ICOS^{hi}$ T cells with clinical benefit in a cohort of metastatic melanoma patients and found that increased frequency of $CD4^+ICOS^{hi}$ T cells in tumor tissues and systemic circulation correlated with increased likelihood of overall survival. This result has not yet been reported for urothelial carcinoma.

A vaccine against survivin, an inhibitor of apoptosis protein (IAP) that targets caspases, was recently tested in a Phase I trial that enrolled nine patients, and the survivin-2B80–88 peptide vaccine was demonstrated to be safe without any adverse events reported [67]. While this trial was not designed to assess clinical efficacy, one patient experienced a slight reduction in tumor burden, and five patients had a significant increase in the peptide-specific CTL frequency. Human chorionic gonadotropin (hCG)-β can be produced by urothelial carcinomas and is the target of a dendritic cell vaccine, CDX-1307, in an on-going, randomized, Phase II neoadjuvant study of resectable, muscle-invasive, $hCG\text{-}\beta^+$ bladder cancer [68]. NY-ESO-1, a protein produced by multiple tumor types, is the target of a Phase I/II vaccine study together with immune stimulants resiquimod and poly-ICLC in patients with urothelial carcinomas that express NY-ESO-1 (NCT00948961).

Novel chemotherapy agents

While there has been considerable focus on the testing of targeted therapies in urothelial carcinomas, the lack of efficacy of these agents limits applications in routine clinical practice, and therefore traditional cytotoxic chemotherapy remains the only proven strategy for advanced disease. Urothelial cancer is chemosensitive with response rates of up to 70% for first-line combination gemcitabine and cisplatin or M-VAC. However, nearly 85% of cases relapse, at which point response rates to taxanes, often used as second-line therapy, fall to 10–20% with progression-free survival of 2–3 months and overall survival of 6–9 months [69]. Multiple studies are investigating novel chemotherapy agents and significant efforts are focused on elucidating the mechanism of platinum resistance, currently the most active family of therapeutics against urothelial carcinoma. The one-size-fits-all approach is not applicable in the salvage setting given that individual patients may have residual toxicities from prior regimens, such as peripheral neuropathy secondary to platinum use, and tumor biology and chemosensitivity are altered due to selective pressure following exposure to first-line treatment.

A number of agents with proven activity in other malignancies have been tested against urothelial carcinoma, both as single-agents as well as in combination with gemcitabine and cisplatin, such as oxaliplatin [70–72], docetaxel [73–75], ifosfamide [76,77] and the proteosome inhibitor bortezomib [78,79]. None of these agents demonstrated improved response rates, progression-free survival or overall survival as compared with standard gemcitabine and cisplatin for first-line treatment, and in the second-line setting had only minimal, or no, activity, as in the case of bortezomib. Novel chemotherapy agents recently or currently in testing include *Vinca* alkaloids, microtubule dynamics inhibitors, nanoparticle taxanes, epothilones, antifolates and histone deacetylase inhibitors.

■ Vinflunine

In 2009, vinflunine, the microtubule-inhibiting *Vinca* alkaloid, gained approval by the EMA as the first agent in the treatment of metastatic urothelial carcinoma

after failure of a prior platinum-containing regimen. Preclinical work dating from 2002 demonstrated activity in an orthotopic murine bladder cancer model [80]. A small Phase II trial in 2006 of vinflunine as second-line monotherapy showed a response rate of 18% with a median duration of response of 9.1 months despite the inclusion of patients with relatively poor prognostic factors, including short interval since first-line therapy (19%, <12 months) and visceral involvement (20%) [81]. Vaughn *et al.* conducted a larger Phase II study with 175 patients that demonstrated a response rate of 15% and median duration of response of 6 months [82]. This was followed by a Phase III trial by Bellmunt *et al.* of 370 patients of whom 70% progressed within 6 months of first-line platinum therapy and 80% had visceral disease [83]. In this setting, vinflunine compared with best supportive care produced a significant response rate of 8.6 versus 0% and median progression-free survival of 3.0 vs 1.5 months. While the intention-to-treat analysis showed only a trend towards improved survival with vinflunine compared with best supportive care, multivariate Cox analysis adjusted for prognostic factors confirmed a reduction in the risk of death by 23% and a statistically significant increase in overall survival with vinflunine.

Eribulin

Initially derived from the black Pacific marine sponge *Halichondria okadai* Kadota in 1986, the cytotoxic properties of halichondrin B were recognized by Hirata and Uemura in their initial report [84]. Eribulin (E7389) was subsequently developed as a synthetic analog of halichondrin B, and it inhibits microtubule growth resulting in cell cycle arrest. The results of a Phase II study demonstrated a response rate in patients with neoadjuvant chemotherapy of 34% and median progression-free survival of 3.9 months with median overall survival of 9.4 months [85]. This agent is being studied as first-line therapy in combination with gemcitabine and cisplatin (NCT01126749) and as second-line treatment for patients with renal dysfunction (NCT00365157).

Taxanes

Nab-paclitaxel is an albumin-bound, nanoparticle formulation of paclitaxel that utilizes an albumin receptor on the endothelial cell surface and accumulates in the tumor interstitium [86]. Sridhar *et al.* recently reported the results of a Phase II trial of nab-paclitaxel as second-line therapy for patients with metastatic urothelial carcinoma that demonstrated an overall response rate of 31%, with another 41% of patients experiencing stable disease, for a disease control rate (RR plus stable disease) of 72% [87]. Based on these promising results, the study is accruing an additional 19 patients for a total of 48.

Epothilones

Epothilones are a novel class of antineoplastic agents with activity against cells that have acquired resistance to taxanes through β-tubulin mutation or overexpression by enhanced microtubule stability via tubulin polymerization, which leads to cell cycle arrest at the G2/M transition and ultimately apoptosis [88]. The epothilone analogue ixabepilone (BMS-247550) was found to have very modest activity against urothelial carcinoma with a response rate of 11.9% as second-line therapy in E3800, an Eastern Cooperative Oncology Group (ECOG) study [89]. The combination of gemcitabine and BMS-247550 was assessed in a Phase I study and had high rates of dose-limiting myelosuppression [90].

Antifolates

Methotrexate, an antifolate with known activity against urothelial carcinoma, is administered together with vinblastine, doxorubicin and cisplatin as part of the MVAC regimen. There have been a number of studies of pemetrexed, a multitargeted antifolate, in combination with gemcitabine as first-line therapy [91,92] or as monotherapy in the second-line setting [93,94]. This agent demonstrated a tolerable side-effect profile and moderate activity, although was not superior to single-agent gemcitabine. While there are no current studies of pemetrexed for urothelial carcinoma, its analogue, pralatrexate, is being assessed in a Phase II, second-line trial in combination with folic acid and vitamin B12 (NCT00722553).

Histone deacetylase inhibitors

While its mechanism of action remains unknown, vorinostat (suberoylanilide hydroxamic acid; NSC 701852), a histone deacetylase inhibitor, was licensed by the FDA in 2006 for the treatment of cutaneous T-cell lymphoma. A Phase I trial of this agent in advanced solid malignancies, including bladder cancer, was terminated due to toxicity (NCT 00565227). A Phase II study of single-agent vorinostat in the second-line setting demonstrated a best response of stable disease in three out of 12 patients and was associated with significant toxicity, primarily cytopenias and thrombocytopenic hemorrhage, including five grade 4/5 events, four grade 3, and two early on-study deaths [95].

Biomarkers of cisplatin resistance

In light of the strikingly limited numbers of therapeutic agents with documented activity against urothelial carcinoma, an understanding of the mechanisms of resistance to platinum compounds is essential, and strategies to circumvent this obstacle remain an unmet need. Cisplatin and its less toxic second-generation analogue

carboplatin share the same mechanism of action and cross resistance, in contrast to the analogue oxaliplatin, which has a different mechanism and consequently does not share cross resistance [96]. Platinums bind to DNA and form monoadducts, which usually react to create intra- and inter-strand crosslinks that contort the conformation of the double helix. These DNA lesions ultimately block transcription and replication and activate signaling cascades, including caspases, thus resulting in cytotoxicity via apoptosis.

Human cells have six major DNA-repair pathways: nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), nonhomologous end-joining, base excision repair and translesion synthesis [97]. While defects in any one of these pathways can cause mutations, genomic instability and predispose to the development of malignancy, sensitivity of tumor cells to chemotherapy relies upon the same pathways. Conversely, alteration of DNA repair pathways can lead to chemoresistance. Emerging data suggest that NER, HR and MMR are involved in mediating chemosensitivity and resistance to platinum agents in urothelial carcinomas.

■ NER & ERCC1

Nucleotide excision repair removes platinum adducts that distort the DNA helix. Excision repair cross-complementation group 1 (ERCC1) is required to excise the damaged nucleotide and this allows DNA polymerase to resynthesize the section [98]. Multiple studies have demonstrated that reduced levels of ERCC1 are associated with sensitivity to cisplatin in testicular germ cell tumors [99]. Furthermore, chemosensitivity can be restored in cisplatin-resistant ovarian cancer cell lines with elevated levels of ERCC1 via antisense RNA inhibition of ERCC1 [100]. ERCC1 was shown to predict response to cisplatin-based chemotherapy in advanced bladder cancer patients treated with gemcitabine plus cisplatin; patients with low levels of ERCC1 mRNA expression had a median survival of 25.4 versus 15.4 months in those with high levels of expression [101]. In addition, univariate and multivariate analyses with pretreatment prognostic factors demonstrated that ERCC1 levels were also independently associated with survival. However, it is difficult to determine whether ERCC1 is a predictive or prognostic factor, since it likely mediates response to platinum agents. The same group recently reported that ERCC1 expression by immunohistochemistry was predictive of disease-specific survival in patients with advanced urothelial carcinoma who received cisplatin chemotherapy [102]. Patients with no expression of ERCC1 had a median disease-specific survival of 12.6 months versus 8.6 months for those with high expression.

■ Mismatch repair & p53

Similar to NER, MMR is also initiated by the detection of DNA damage and results in the excision of mismatched nucleotides or insertion/deletion loops, followed by resynthesis of the missing portion by DNA polymerase [98]. Unexpectedly, however, MMR deficiency is associated with cisplatin resistance [103]. It is possible that MMR recognition of DNA damage or the process of excision itself triggers apoptosis [97]. While a predictive biomarker involved in the MMR pathway has not been identified and tested in the setting of urothelial carcinoma, a new report suggests that mutations in mismatch repair genes known to cause Lynch syndrome, which is associated with upper tract transitional cell carcinomas, may also pose an increased risk of bladder cancer [104]. Furthermore, it has been hypothesized that defects in the DNA-repair pathway, including MMR, may mediate the increased risk of bladder cancer secondary to the known carcinogenic effects of tobacco use [105,106]. Therefore, in addition to likely mediating cisplatin resistance in urothelial carcinomas, defects in the MMR pathway may be involved in pathogenesis as well.

The tumor suppressor gene *p53* has long been hypothesized to be a key player in the pathogenesis of invasive urothelial carcinoma, and preclinical studies support the role of *p53* inactivation in urothelial proliferation and invasion [21,107]. While p53 is not directly involved in MMR, loss of both p53 and MMR function results in rapid evolution of cisplatin resistance in a human colon carcinoma cell line treated with cisplatin likely via increased mutagenic translesion synthesis [108]. A prospective trial incorporating p53 status in the selection of adjuvant chemotherapy for patients with muscle-invasive, node-negative urothelial carcinoma following radical cystectomy failed to demonstrate the prognostic or predictive value of p53 immunohistochemistry, although the study was compromised by failure to receive the assigned therapy in many patients and a lower than expected event rate, with a 5-year relapse-free survival of 80% [109]. By contrast, a recent report of over 3000 patients demonstrated that p53 had predictive value in advanced bladder cancer but in not superficial (Ta) disease [110]. As a mechanism to overcome platinum resistance, overexpression of p53 through adenoviral gene transfer has been successful in human bladder cancer cell lines and demonstrated synergy with cisplatin [111]. Adenoviral *p53* gene transfer has also been combined with the use of antisense oligodeoxynucleotide targeting of the antiapoptotic gene clusterin in a bladder cancer model in nude mice, where it resulted in eradication of tumors and lymph node metastases following treatment with cisplatin, suggesting that this strategy may have clinical efficacy [112].

■ Homologous recombination & BRCA1

The tumor suppressor gene *BRCA1* together with proteins mutated in Fanconi's anemia (FA proteins), a rare inherited condition of chromosomal instability, are involved in the DNA-repair pathway of HR, which causes resistance to DNA interstrand crosslinks [113]. Homologous recombination involves the exchange of nucleotide sequences between identical strands of DNA. BRCA1 binds to the protein encoded by *BRCA2*, which was surprisingly found to be the same protein as FANCD1, an FA protein, and this complex is recruited to sites of DNA damage due to interstrand crosslinks [114]. Decreased expression of BRCA1 has been associated with increased cisplatin sensitivity in a number of tumors, including breast and ovarian [115,116], whereas those with elevated BRCA1 levels had better outcomes with taxanes compared with those with low levels in ovarian cancer and non-small-cell lung cancer [115,117].

BRCA1 mRNA expression was recently tested as a predictive marker for response to neoadjuvant cisplatin-based chemotherapy in patients with muscle-invasive, locally advanced bladder cancer [118]. Among patients with low-to-intermediate expression of BRCA1, 66% (24 of 39) achieved a significant pathological response of pT0–1 compared with 22% (four of 18) of patients with high BRCA1 expression. Furthermore, the 5-year survival rate of 64% for patients with low-to-intermediate expression of BRCA1 was significantly improved compared with 12% for those with the highest level of BRCA1 expression. Based on these clinical studies, as well as preclinical data from cell lines that also demonstrate an inverse relationship between BRCA1 levels and resistance to cisplatin/taxane [119], BRCA1 is a candidate predictive marker in selecting chemotherapy for individuals with urothelial carcinoma.

Future perspective

The extraordinary potential of personalized therapy in the fight against cancer has been recognized by the National Cancer Institute through their distribution of nearly $1.3 billion in Recovery Act funds over 2009 and 2010 to programs that support the Personalized Cancer Care/Drug Development Platform, including The Cancer Genome Atlas (TCGA), Cancer Human Biobank (CaHUB), and Accelerating Clinical Trials of Novel Oncologic PathWays (ACTNOW). While money alone will not lead to scientific advancement, it is clear that progress in oncology is dependent upon collaborations across disciplines that will be supported by these funds. In the case of urothelial malignancies, identification of many of the driving genetic aberrations underlying its molecular pathogenesis has not yet translated into progress in the application of clinically useful biomarkers [120] or treatment options for this disease. Findings from the emerging field of miRNAs may result in novel biomarkers and reveal mechanisms of cancer pathogenesis that can be exploited as therapeutic targets.

miRNAs are abundant, small (~20–22 nucleotides) noncoding RNAs that typically dampen gene expression at the post-transcriptional level [121,122] and are mis-expressed in a variety of cancer cells [123]. Early studies of miRNAs in cancer pathogenesis suggest that miRNA expression patterns (termed 'signatures') may be more reliable than mRNA profiles in the identification and/or classification of tumors [124]. Unlike the vast number of mRNAs, there are only ~1000 miRNAs in the human genome, and a modest number of miRNAs may be sufficient to serve as markers to differentiate a specific tumor [125]. Furthermore, miRNAs are distinctly more stable than mRNAs and are easily recovered and detected in paraffin-embedded tissue [125]. The use of miRNA 'signatures' as predictive markers and potential modulators of cancer therapy is being explored for different cancers [126], and results in breast cancer suggest utility of miRNA signatures in predicting sensitivity to both chemotherapy and endocrine therapy [127,128]. Although complementary clinical data are still largely lacking, one example in epithelial ovarian cancer revealed a set of 34 miRNAs that were differentially expressed in patients who responded to platinum-based chemotherapy versus nonresponders [129].

Despite the large numbers of patients affected by urothelial cancers, progress has been impeded by low rates of enrollment in clinical trials. Furthermore, as a disease of older patients, many present with substantial medical comorbidities that limit trial eligibility and treatment options. As discussed by Gonzalez-Angulo *et al.* in their article "Future of personalized medicine in oncology: a systems biology approach", large randomized studies, which are regarded as the highest level of evidence in medicine, inform treatment decisions for the average patient but not individual patients [130]. In an effort to improve predictions of chemosensitivity, Lee *et al.* recently reported on the development and application of a generic algorithm they named 'coexpression extrapolation' (COXEN) that is based on molecular profiling data from the NCI-60 panel, and suggest that this could be used for *in silico* drug discovery and tailoring chemotherapy selection for individual patients [131,132].

Novel clinical trial strategies that incorporate molecular profiling information are needed in order to enrich trials with patients who have the greatest chance of responding to rationally designed therapeutic agents, as well as rigorously test potential biomarkers. In addition, this approach will hopefully reduce toxicity through

personalized drug dosing based on metabolism and molecular crosstalk. The 'brief-duration, biomarker-driven' neoadjuvant strategy could streamline drug development, although results may not predict efficacy in the advanced setting [69].

To date, results of clinical trials of targeted therapies and novel cytotoxics have not improved the outlook for patients with advanced urothelial carcinoma despite the rationale selection of targets and agents. Practice-changing breakthroughs remain dependent upon identification of novel therapeutic targets, improved molecular prognostic markers to predict responses for the available active agents, and clinical trial participation for all eligible patients.

Supplementary data

Supplementary data accompanies this paper and can be found at www.future-science.com/doi/suppl/10.4155/CLI.11.26

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Executive summary

- Personalized medicine has brought significant improvements to the diagnosis and treatment of multiple cancers, but advances in urothelial carcinoma lag behind despite detailed understanding of the molecular pathways driving this disease.
- Platinum-based combination chemotherapy remains the standard, first-line treatment for advanced urothelial carcinoma and is also used for neoadjuvant and adjuvant chemotherapy for locally advanced disease.
- Recent clinical trial results of targeted therapies against urothelial cancers, such as angiogenesis inhibitors and growth factor inhibitors have generally been disappointing.
- Novel chemotherapy agents currently or recently in testing include *Vinca* alkaloids, microtubule dynamics inhibitors, nanoparticle taxanes, epothilones, antifolates and histone deacetylase inhibitors.
- Emerging data suggest that alterations in DNA repair pathways, specifically homologous recombination, nucleotide excision repair, and mismatch repair, may mediate resistance to platinum agents in urothelial carcinoma.
- The field of miRNAs represents a new tool in the search for novel biomarkers and may help to reveal mechanisms of cancer pathogenesis that can be exploited as therapeutic targets.

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■ **Example of how gene expression profiling may be used to improve the prediction of chemosensitivity with potential applications for personalized therapy.**