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Review

Novel Molecular Targets for the Therapy of Urothelial Cancer

BAGI R.P. JANA and YAN ZHOU

University of Texas Medical Branch, Galveston, TX, U.S.A.

Abstract. *First-line platinum-based chemotherapy combinations are considered standard-of-care in locally advanced and metastatic urothelial cancer. However, long-term outcomes, including disease-specific and overall survival, remain poor. In addition, a number of patients with advanced urothelial carcinoma have co-existing medical issues that preclude the use of conventional chemotherapy. Improvements in our understanding over the molecular mechanisms of urothelial cancer have led to first-generation clinical trials evaluating novel agents targeting molecular pathways that may be relevant, at least in sub-populations. Emerging information regarding outcome with agents targeting novel molecular targets in advanced urothelial cancer is discussed in this review.*

Urothelial cancer (UC) of the bladder is the second most common genitourinary malignancy in the United States and is the most common histological type of bladder cancer which accounts for more than 90% of cases and affects an estimated 76,000 people in the USA each year, causing 16,000 deaths (1). At diagnosis, 30% of UC are muscle-invasive which confers a significant risk of locally advanced or distant metastatic disease. Cisplatin-based chemotherapy regimens for locally advanced or metastatic UC include combinations of methotrexate, vinblastine, doxorubicin and cisplatin (MVAC), dose-dense M-VAC (DD MVAC) and gemcitabine and cisplatin (GC). An average response rate (RR) of approximately 50% and a median overall survival (OS) of 12-14 months are expected (2). Despite more than two decades of studies investigating a number of novel chemotherapeutic agents and combinations, including three-drug regimens (3), cisplatin-based chemotherapy remains the only therapy shown to improve survival in any UC disease state. Major advances are unlikely to be made by adding a third chemotherapeutic agent to therapy for unselected patients (3). In addition, many patients are not candidates for conventional chemotherapy due to co-morbidities (4-6). Development of less toxic, more effective agents is, therefore, crucial. Although new-generation biological and targeted agents have been integrated into the treatment of other cancer types, these therapies have no current use in standard therapy for advanced UC. Recent advances in molecular biology have identified new targets (7). This article provides an overview of ongoing and reported clinical trials using novel molecular targeted agents.

Correspondence to: Bagi RP Jana, MD, Suite 4.164, 301 University Blvd., Galveston, TX, U.S.A. Tel: 409 772 1164, Fax: 409 772 3533, e-mail: Brjana@utmb.edu

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Targeting Angiogenesis

Angiogenesis facilitates progressive tumor growth by providing adequate oxygenation to the tumor through endothelial cell proliferation, motility of endothelial cells through the extracellular matrix toward angiogenic stimuli, and capillary differentiation (8). In the case of cancer, abnormal amounts of angiogenic growth factors, namely vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), interleukin-8 (IL8) and angiopoietins, are produced by cancer cells that cause excessive angiogenesis, overwhelming the effects of natural angiogenesis inhibitors (8-12). A number of studies have suggested a relationship between angiogenesis, tumor behavior, and prognosis in UC (13). Based on these observations, it was hypothesized that anti-angiogenic agents alone or in combination with chemotherapy will improve outcomes in UC. Bevacizumab, is a recombinant humanized monoclonal antibody that works as an angiogenesis inhibitor by inhibiting VEGFA. A phase II study to assess the efficacy and toxicity of bevacizumab in combination with GC as first-line treatment for patients (n=43) with metastatic UC demonstrated a significant antitumor efficacy, with a 72% tumor response rate and a 19% complete response rate (14). A median PFS time of 8.2 months and a median OS of 19.1 months were recorded. Treatment-related hematological toxicities included anemia (49%), thrombocytopenia (26%), neutropenia (51%), and

febrile neutropenia (4%). Bevacizumab-related non-hematological toxicities included deep-vein thrombosis/ Pulmonary embolism (21%), incidence of which was reduced to 8% from 41% after an amendment reduced the dose of gemcitabine from 1250 to 1000 mg/m^2 weekly. Other adverse events associated with bevacizumab included hemorrhage (37%), cardiac toxicity (30%), hypertension (21%), proteinuria (16%), vascular events (2%; aortic dissection), and renal failure (28%). There were three treatment-related deaths (central nervous system hemorrhage, aortic dissection, sudden cardiac death) in the study. Another phase II trial from the Memorial Sloan-Kettering Cancer Center was recently published for the activity of carboplatin, gemcitabine, and bevacizumab in cisplatin-ineligible patients (n=51) with metastatic UC (15). This study showed a 45% tumor response rate and a 6% complete response rate. The median PFS was 6.5 months and median OS was 13.9 months. Twenty patients (39%) experienced grade 3 to 4 toxicity, with a 20% of thromboembolic events (deep venous thrombosis or pulmonary embolism), it is possible that the high rate of thrombosis may be intrinsic to the UC (16).

Bevacizumab has also been tested in the neoadjuvant setting. A phase II study was completed investigating neoadjuvant GC plus bevacizumab followed by adjuvant paclitaxel and bevacizumab in patients with residual invasive tumor at cystectomy. A high postoperative complication rate was recorded, leading to early study closure (17). Another phase II trial at the MD Anderson Cancer Center was to evaluate the activity of neoadjuvant chemotherapy with DD-MVAC and bevacizumab for high-risk UC. of 2-year OS of 75% and pathological down-staging of 45% were reported (18). To better elucidate the role of bevacizumab in the metastatic setting, an ongoing multicenter placebo-controlled phase III trial (CALGB 90601) is comparing GC to GC plus bevacizumab for advanced UC (NCT00941331).

A multi-center, randomized phase II study is ongoing to evaluate the safety and efficacy of Icrucumab (IMC-18F1, VEGFR1 monoclonal antibody) or Ramucirumab (IMC-1121B, VEGFR2 monoclonal antibody) in combination with docetaxel as second-line therapy in patients with locally advanced or metastatic transitional cell carcinoma (TCC) of the bladder (NCT01282463). Aflibercept, a fusion protein that is designed to bind to VEGFA, VEGFB, and placental growth factor had limited single-agent activity in patients with UC previously treated with platinum-containing chemotherapy (19). Trebananib, an angiogenesis-targeting angiopoietin-1/2 antagonist, has showed promising antitumor activity in patients with advanced solid tumors including UC (20, 21). A phase II single-arm study of Trebananib (angiopoietins 1 and 2 inhibitor) is being studied in combination with docetaxel for the treatment of advanced UC after failure of a platinum-containing regimen (NCT01907308).

Sunitinib inhibits cellular signaling by targeting multiple receptor tyrosine kinases including all receptors for PDGF and VEGF. Preclinical studies have demonstrated that sunitinib has antitumor activity against UC as a single agent as well as in combination with cisplatin (22). However, clinical trial results of sunitinib in UC so far have been disappointing. A multicenter phase II trial with sunitinib in the setting of frontline therapy for cisplatin-ineligible patients has revealed modest single-agent activity with clinical benefit (partial response plus stable disease) of 58%, median time to progression of 4.8 months and median OS of 8.1 months (23). A total of 77 patients with advanced, previously treated UC were treated with sunitinib. Clinical regression or stable disease was achieved in 33 out of 77 patients (43%). The PFS (2.4 *vs*. 2.3 months) and OS (7.1 *vs*. 6.0 months) were similar in both cohorts (24). An early report of a phase II study of first-line sunitinib in combination with GC (n=45) yielded activity. But the study required modification with an alternative dosing scheme due to intolerance. The most frequent grade 3-4 toxicities were neutropenia (80%), thrombocytopenia (60%), anemia (20%), and leucopenia (13%) (25). A recent double-blind, randomized, phase II trial evaluating placebo or sunitinib as switch maintenance (n=54) showed that maintenance sunitinib did not appear to improve the 6-month progression rate. The study was limited by premature closure and a small sample size (26). Sunitinib has also led to disappointing results when combined with platinum-based chemotherapy in the neoadjuvant setting. The sample sizes of both trials were small due to early closure secondary to excess toxicity. The response rates were 22% to 49% (27). A phase II study of sunitinib in the first-line, platinum-ineligible setting is ongoing (NCT01118039). Another phase II study of sunitinib in the second-line setting is also ongoing (NCT00578526).

Sorafenib, another multi-targeted small-molecule tyrosine kinase inhibitor targeting VEGF and other pathways did not demonstrate sufficient activity in phase II trials as a single agent, neither in the first- nor second-line setting (28, 29). In a randomized phase II trial (n=132), sorafenib did not improve outcomes in combination with GC in the first-line, advanced setting (30). A phase II study of sorafenib combined with GC in the neoadjuvant setting is ongoing (NCT01222676). Another phase II, multi-center trial of Sorafenib combined with GC in the first-line, and advanced setting is ongoing (NCT00461851). A third small-molecule tyrosine kinase inhibitor with activity against the VEGF pathway, pazopanib, has been investigated in two phase II trials in second-line, metastatic settings that yielded conflicting results. One evaluating pazopanib in the second-line setting reported that 76% of the 41 studied patients derived clinical benefit (17% partial response, 59% stable disease), with a median OS of 4 months (31). In contrast, another showed pazopanib at 800 mg given daily for a

4-week cycle in patients with UC who failed at least one prior chemotherapy produced no response by RECIST criteria in the first 16 evaluable patients during the first four 4-week cycles of treatment. The median PFS was 1.9 months. The trial was recommended to be permanently closed due to futility (32). Recent phase I/II trial of combining pazopanib and vinflunine in the second-line, advanced setting was attempted but was interrupted due to toxicity. The initially planned phase II study was, therefore, not carried out (33). Two phase II studies of pazopanib in the second-line, metastatic (NCT00471536) or relapsed/refractory (NCT01031875) settings have been completed and results are awaited. Another phase II study of pazopanib in combination with weekly paclitaxel for refractory UC is ongoing (NCT01108055). Vandetanib is another tyrosine kinase inhibitor that targets both epidermal growth factor receptor (EGFR) and VEGFR. A randomized double-blind placebo-controlled study compared docetaxel with and without vandetanib in patients with advanced UC with progression after platinum-based chemotherapy. The overall survival and overall response rates were not different between the two arms. The toxicity of vandetanib plus docetaxel was greater than that for vandetanib plus placebo (34). Study of vandetanib in combination with carboplatin and gemcitabine hydrochloride in the first-line setting is ongoing (NCT01191892). So far, disappointing inactivity to these drugs may suggest intrinsic resistance of UC, redundancy of the angiogenic pathways, or tumor dependence on alternate pathways.

EGFR- and HER2-targeting Agents

The human epidermal growth factor receptor (HER) family is composed of four receptor tyrosine kinases. Among them, EGFR (HER1) and HER2/neu have been particularly studied the pathogenesis of invasive UC (35). EGFR is overexpressed in more than 50% of human UCs, and the level of expression correlates with stage, grade, and survival (36). Preclinical studies demonstrated potential cytotoxicity of EGFR inhibitors, making EGFR blockade a rational strategy in UC (37). Pre-clinical activity of cetuximab, a human/murine chimeric monoclonal antibody against EGFR, has led to phase I and II clinical trials in patients with UC. A phase II study was conducted to evaluate efficacy of cetuximab with or without paclitaxel in metastatic patients with previously treated UC who received one line of chemotherapy in the perioperative or metastatic setting. A total of 39 patients were randomly assigned to 4-week cycles of cetuximab at 250 mg/m^2 with or without paclitaxel at 80 mg/m^2 per week. While cetuximab did not demonstrate activity alone, the combination of cetuximab and weekly paclitaxel in the second-line setting yielded responses in 25% of patients. The median PFS and OS were 16.4 and 42 weeks, respectively. The most common treatment-related adverse events were rash, fatigue, and hypomagnesaemia (38). A phase II trial evaluated the effect of GC with or without cetuximab in patients with unresectable, locally recurrent, or metastatic UC (39). Twenty-eight patients were randomly assigned to the standard therapy arm consisting of GC, while 56 patients were randomly assigned to the cetuximab-containing arm (GC plus cetuximab at 500 mg/m^2, on days 1 and 15 of a 28-day cycle then continued on monotherapy with cetuximab after 4 to 6 cycles). Disappointingly, the combination arm led to more adverse events, including more thromboembolism, hypernatremia, rash, and fatigue and two deaths, but it did not improve PFS or median survival rates.

Gefitinib selectively inhibits EGFR tyrosine kinase (40). Pre-clinical study demonstrated the therapeutic potential of gefitinib in pre-clinical models of bladder cancer *via* inhibiting extracellular signal-regulated kinase and protein kinase B (AKT) phosphorylation, as well as EGFR phosphorylation (41). The Southwest Oncology Group studied the role of single-agent gefitinib in the second-line setting in unselected patients treated for metastatic UC (42). Thirty-one patients were enrolled, with only 3% partial response (PR) and the median OS was 3 months. The study was stopped after the first step because disease in the majority of patients (81%) had progressed by the first evaluation. The most commonly reported toxicities were fatigue and rash. Single-agent gefitinib in this setting is thus not recommended based on these negative results. No correlation was seen between expression of EGFR and response. Of the 31 patients in the study, 17 had an evaluable tumor biopsy specimen. Eight of these 17 patients had EGFR 2 or 3+ expression, while three out of 17 had HER2/Neu 2-3+ expression. Two had 2-3+ expression of both HER2/Neu and EGFR. There was amplification of p53 in 70% of specimens. The sole responding patient had EGFR 2+ expression, but no expression of HER2/Neu, and overexpression of p53. Of the 15 patients with progressive disease at first evaluation, four did not express EGFR, and seven had 3+ expression.

Another phase II trial, the Cancer and Leukemia Group B (CALGB) trial 90102, was conducted for the evaluation of gefitinib in combination with GC in the metastatic setting (43). A total of 54 patients were assessed, the overall response rate was 42.6%, the median time to progression was 7.4 months and median OS was 15.1 months. Although the combination appears active, the combination results were not significantly better than historical data for therapy with GC alone. Additional phase II trial of docetaxel compared to docetaxel-plus-gefitinib as maintenance therapy for patients already treated with first-line chemotherapy is ongoing (NCT00479089). Erlotinib is another reversible oral small-molecule EGFR tyrosine kinase inhibitor. A phase II trial showed that when administered in the neoadjuvant setting,

erlotinib can have beneficial effects in terms of postoperative pathology and short-term clinical outcomes in patients undergoing radical cystectomy for invasive bladder cancer (44). Twenty patients were enrolled to receive neoadjuvant erlotinib (150 mg once daily for 4 weeks) before radical cystectomy. The primary endpoint was the rate of pathological complete response and the safety of therapy with erlotinib. At the time of cystectomy, surgical pathology showed 25% of patients with no residual disease, 35% with down-staging to less than pT2 disease, and 75% had organ-confined disease. At 24.8 months of follow-up, 10 patients were disease-free, four with organ-confined disease had disease progression, and nine had died. The treatment was well tolerated, with rash being the most common side-effect. Another phase II trial (NCT00749892) is currently evaluating a similar role for erlotinib in the neoadjuvant setting.

HER2 has become a therapeutic target in multiple solid tumors. Studies have demonstrated that HER2 is frequently expressed in UC (45), and successful targeting of this pathway in other solid tumors makes it an attractive target in this setting. A phase II trial in treatment of naïve advanced UC for 44 patients with HER2-positive tumors were treated with trastuzumab in combination with GC and paclitaxel; 70% responded (five complete responses, 26 partial responses). The median time to progression was 9.3 months and OS was 14.1 months (46). Three patients died due to toxicities and cardiac toxicity was higher than expected (22.7% grade 1-3 events). A phase II trial evaluating single-agent trastuzumab in the second-line setting could not accrue due a low prevalence of HER2-expressing tumors by immunohistochemistry (IHC) (3+) or fluorescence *in situ* hybridization (FISH) (NCT00004856). Trastuzumab is also being evaluated in combination with weekly paclitaxel and radiation therapy for locally advanced HER2-amplified bladder cancer (NCT00238420).

Lapatinib, an oral dual tyrosine kinase inhibits EGFR and HER2, has been shown to have *in vitro* activity against UC cell lines (47). A single-arm, open-label, multi-center phase II study investigated lapatinib monotherapy in patients with metastatic UC that had progressed on first-line platinum-based chemotherapy. It demonstrated a response rate of only 1.7%. The median time to progression was 8.6 weeks, with median OS of 17.9 weeks. Clinical benefit (overall response rate plus stable disease) was associated with EGFR overexpression, and median OS was improved in patients whose tumors overexpressed EGFR and/or HER2/neu (48). Preliminary analysis also suggested that high tumor HER3; high extracellular signal-regulated kinases (pErk) and mutant p53 may predict resistance, while high phospho-AKT and high insulin like growth factor 1 receptor (IGF1R) expression may predict sensitivity to lapatinib. A recent report from a double-blinded randomized study of lapatinib in patients with *HER2*-amplified solid tumors demonstrated that lapatinib monotherapy is associated with modest activity (49). A small study explored the feasibility of a combination of paclitaxel and lapatinib for six patients who were treated after failure of first-line platinum-based chemotherapy (50). An ongoing placebo-controlled randomized phase II trial in the United Kingdom is evaluating lapatinib as a switch maintenance strategy in those with stable or responding disease after first-line chemotherapy and those with EGFR and/or HER 2-expressing tumors (NCT00949455). Another small phase II trial is evaluating lapatinib for ~3 weeks preceding radical cystectomy in order to evaluate tumor tissue for biological activity. An additional phase I study is investigating GC with lapatinib in patients with previously untreated, HER2/neu-overexpressing metastatic or locally advanced UC (NCT00623064).

A novel study is also assessing the affray of autologous cellular immunotherapy against HER2/neu in the adjuvant UC setting. DN24-02, is a autologous cellular immunotherapy product designed to stimulate an immune response against HER2/neu. A randomized, phase II, open-label study is evaluating DN 24-02 as adjuvant therapy in patients with high-risk HER2+ urothelial carcinoma (NCT01353222).

The soy compound genistein is a phytoestrogen and of isoflavones category. Genistein has been observed preclinically to inhibit bladder cancer growth, with one potential mechanism being the inhibition of EGFR phosphorylation. A phase II randomized, placebo-controlled trial investigated whether genistein alters molecular pathways in bladder epithelial tissue when given at 300 or 600 mg/d for 14 to 21 days before surgery for urothelial bladder cancer (51). Genistein displayed a possible bi-modal effect (more effective at the lower dose) on bladder cancer tissue EGFR phosphorylation. Genistein should be further evaluated, possibly in combination with other agents.

Targeting Fibroblast Growth Factor Receptor

The fibroblast growth factor receptor 3 (FGFR3) is a transmembrane receptor tyrosine kinase which harbors activating mutations in UC, indicating a potential role for *FGFR3* in tumorigenesis and offering a possible target for therapy for superficial bladder cancer (52). Indeed, knock-down of mutated *FGFR3*, inhibition with a monoclonal antibody, or with a specific FGFR3 inhibitor in mouse xenografts inhibited tumor growth (53, 54). The treatment of non-invasive bladder tumors requires tolerable agents, given the extended survival and long duration of therapy anticipated for these patients. Agents that block this pathway are under active development. A phase II clinical trial is investigating the efficacy of dovitinib, an oral FGFR3 tyrosine kinase inhibitor (NCT00790426) as second-line therapy, with stratification for *FGFR3* mutation status and this trial has

been completed. In addition to activating point-mutations and amplifications, a recent report describes oncogenic FGFR3 genetic translocations and re-arrangements as an alternative mechanism of pathway activation (55). Ponatinib (AP24534) is an oral multi-targeted tyrosine kinase inhibitor that has also been shown to inhibit the *in vitro* kinase activity of all four FGFRs. A recent study demonstrated that ponatinib is a potent pan-FGFR inhibitor and provided a strong rationale for its evaluation in patients with FGFR-driven cancer (56). Another study investigated a novel inhibitor of FGFR1-3 and RNAi to determine the effects of inhibiting FGFR1 or FGFR3 in a panel of human bladder cancer cell lines. It was demonstrated that FGFR1 and FGFR3 have largely non-overlapping roles in regulating invasion, metastasis and proliferation in distinct 'mesenchymal' and 'epithelial' subsets of human bladder cancer cells. The results suggest that the tumor phenotype will be an important determinant of the biological effects of FGFR inhibitors in patients (57). FGFR holds promise as a therapeutic target, diagnostic and prognostic marker, and for screening tools for early detection and clinical management of UC (58).

Targeting PI3K– AKT-mTOR Pathway

The phoshinositide 3-kinase (PI3K)– serine/threonine kinase (AKT)– mammalian target of rapamycin (mTOR) pathway is an important therapeutic target. Approximately 21% of muscle-invasive UCs have activating *PI3K* mutations, while another 30% demonstrate evidence of PTEN mutations. In addition, 16% of patients were found to have inactivating mutations in tuberous sclerosis 1 (TSC1), an inhibitor of mTOR activation (59). Preclinical studies demonstrated that everolimus has activity against UC (60, 61). Everolimus as a single-agent has been investigated as a second-line agent in a single-arm study. A total of 45 patients with metastatic UC refractory to one to four cytotoxic agents were enrolled in this study. The primary end-points were 2-month PFS rate and the safety of everolimus, with the secondary endpoint being the response rate. In this trial, although everolimus did not meet its primary end-point, one partial response, one near-complete response and 12 minor regressions were reported. Median PFS and OS were 2.6 months (range=1.8-3.5 months) months and 8.3 months (range=5.5-12.1 months), respectively (62). Another phase II study assessed the safety and efficacy of everolimus in advanced TCC after failure of platinum-based therapy. Thirty-seven patients with advanced TCC received everolimus at 10 mg/d until progressive disease or unacceptable toxicity. Two confirmed partial responses and eight cases of stable disease were recorded, with a disease control rate of 27% at 8 weeks. Everolimus was well-tolerated. A higher baseline level of angiopoietin-1 and significant early plasma decrease in angiopoietin-1, endoglin, and PDGFA/B in patients with controlled disease suggested a role of the antiangiogenic properties of everolimus in disease control. *PTEN* loss might be associated with resistance to everolimus (63). Recent pre-clinical study showed that everolimus had a therapeutic effect when used in combination with cisplatin and could therefore be a useful anticancer drug combination for patients with bladder cancer (64). Everolimus is undergoing investigation in combination with paclitaxel in both the first- and second-line settings (NCT01215136 and NCT00933374). The first-line trial enrolls cisplatin-ineligible patients and administers everolimus alone for those with renal dysfunction and poor performance status, and in combination with paclitaxel in those with only one of these conditions. This strategy is based on the finding that combination therapy yielded poor outcomes in those with both renal dysfunction and poor performance status in a phase III trial that compared carboplatin-gemcitabine with methotrexate, carboplatin and vinblastine (65).

Temsirolimus has shown promise against bladder cancer in pre-clinical studies (66) and a phase II study in combination with GC as a first-line therapy (NCT01090466) has been completed. A phase II trial assessed temsirolimus as second-line therapy in patients with metastatic UC after failure of platinum-containing therapy. Fifteen patients were enrolled in this study. The median time to progression was 2.5 months (77 days); median OS was 3.5 months (107 days). Four patients with stable disease were noted. Temsirolimus was well-tolerated. As grade 3-4 adverse events, fatigue (n=2), leukopenia (n=2) and thrombocytopenia (n=2) were observed. Temsirolimus seems to have poor activity in patients with progressive UC after failure of platinum-containing first-line therapy (67).

Immunomodulators

Over the past two decades, intravesical Bacille Calmette Guerin (BCG), a preparation of live attenuated *Mycobacterium bovis*, has remained the most successful therapy for high-grade non-muscle-invasive TCC and carcinoma *in situ* of the bladder (68, 69). The possible mechanism of action of BCG involves an active host defense function against pathogens, including phagocytosis of urothelial cells to degrade antigens and present antigenic proteins on their surface, secrete pro-inflammatory cytokines, and modulate the recruitment and activity of immune cells (70, 71). Intravesical immunotherapy with BCG to prevent recurrence of these tumors has been shown to involve the participation of three different Toll like receptors (TLR), TLR2, TLR4, and TLR9 (72-75). In recent years, alternative immunotherapy involving the induction of innate immune responses *via* TLR activation have been explored, with promising results using *in vitro* and *in vivo* models of bladder cancer. Imidazoquinolines (TLR7 agonist), a class of

synthetic immunomodulating agents have potent direct activity against bladder cancer cells by reducing cell viability and inducing apoptosis and cytokine production (76, 77).

Mycobacterial cell wall DNA complexes (MCC) are complexes of mycobacterial DNA oligonucleotides with components of the cell wall. Intravesical use of MCC showed antineoplastic activity, with reduced toxicity compared with BCG. Mycobacterial DNA was proposed to be the active component in MCC (78, 79). CpG-containing oligonucleotides (TLR9 agonist) (80-83), Helicobacter pylori neutrophil-activating protein (HPNAP) (TLR2 ligand), lipopolysaccharide and their derivatives have been tested as BCG surrogates in the treatment of UC(84, 85).

Cytoxic T Lymphocyte-associated antigen (CTLA-4) is a potent immunoregulatory molecule that suppresses antitumor response by down-regulating T-cell activation. Antibodies directed against CTLA-4 have been reported to enhance T-cell responses that lead to antitumor responses(86). A preoperative clinical trial with ipilimumab was conducted in 12 patients with localized UC (87). In this study, six patients were treated with 3 mg/kg/dose of anti-CTLA-4 and six with 10 mg/kg/dose of this antibody. Both doses were well tolerated. All 12 patients exhibited measurable immunological pharmacodynamic effects as demonstrated by increased frequency of CD4-positive T-cells in tumor tissue and in the systemic circulation.

Beta-human chorionic gonadotropin (βhCG) is another promising target antigen that is expressed in a subset of bladder cancer. CDX-1307 is a protein consisting of a human monoclonal antibody targeting the mannose receptor genetically fused to the entire βhCG. Following binding of fused protein to the mannose receptor, CDX-1307 is internalized, leading to efficient antigen presentation to both CD4-positive and CD8-positive T-cells (88). Furthermore, a greater or more consistent immune response was observed when combined CDX-1307 with three immunoadjuvants, granulocyte–macrophage colony-stimulating factor (GM-CSF) and TLR agonists resiquimod and polyinosinic-polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose (89). This combined multi-agent regimen has been selected for a phase II clinical trial studying the feasibility of the CDX-1307 vaccine regimen when administered neoadjuvantly (with GC regimen) and adjuvant therapy in patients with newly diagnosed, muscle-invasive hCG-β-expressing bladder cancer amenable to resection with curative intent (89).

Lenalidomide, an immunomodulating and anti-angiogenic agent is also being studied in combination with GC as first-line therapy for metastatic UC (NCT01342172). Oportuzumab monatox (OM) is a recombinant fusion protein comprising a humanized single-chain antibody to epithelial cell adhesion molecule EPCAM linked to *Pseudomonas* exotoxin A. Once bound to the cancer cell, OM is internalized and the toxin moiety released into the cytosol where it induces apoptosis. A phase II study was conducted to assess the efficacy and tolerability of intravesical OM in patients with UC *in situ* in second-line setting after failure of BCG treatment. A total of 46 patients received one induction cycle of 6 (cohort 1) or 12 (cohort 2) weekly intravesical OM instillations of 30 mg, followed by up to three maintenance cycles of 3 weekly administrations every 3 months. A complete response to OM was 41% in cohort 1 and 39% in cohort 2 at the 3-month evaluation; 44% achieved a complete response. The median time to recurrence in patients who achieved a complete response was 274 and 408 days in cohorts 1 and 2, respectively. The most common adverse events were mild-to-moderate reversible bladder symptoms. OM was deemed effective and well-tolerated in patients with BCG refractory carcinoma *in situ* of the bladder (90).

Other Novel Agents

Dasatinib, an oral kinase inhibitor targeting the Src family proto-oncogenic tyrosine kinases, ABL, proto-oncogene c-kit, and PDGFR, displayed significant pre-clinical antitumor activity against Src-overexpressing UC (91). A study has been completed to determine feasibility and safety of treatment with dasatinib administered orally once daily for 4 weeks duration prior to radical cystectomy for urothelial carcinoma of the bladder (NCT00706641). Similarly, IGF-IR and MET proto-oncogene are implicated in tumor invasion and metastasis (92, 93). Decorin, a prototype member of the small leucine-rich proteoglycans, may act as a natural antagonist of the IGF-IR in UC (94).

Newer tubulin antagonists have been under evaluation. Nab-paclitaxel, a solvent-free albumin-bound formulation of paclitaxel, is better delivered to tumors *via* molecular pathways involving an endothelial cell surface albumin receptor and an albumin-binding protein expressed by tumor cells and secreted into the tumor interstitium (secreted protein acid rich in cysteine) (95). A phase II study of single-agent nab-paclitaxel as second-line therapy in patients with metastatic UC led to a response rate of 32% and disease control rate of 53%. The median PFS and OS were 6 and 10.8 months, respectively (96).

Eribulin meslylate, a novel synthetic analog of halichondrin B, is a microtubule destabilizing agent shown to improve survival in patients with refractory breast cancer (97). Eribulin was administered as first-line therapy in cisplatin-ineligible patients and yielded a response rate of 37%, a median PFS of 3.9 months and median OS of 9.4 months (98). The agent does not require steroid premedication and appears to have a favorable toxicity profile. A randomized phase II trial is ongoing to evaluate eribulin mesylate in combination with GC for locally advanced or metastatic bladder cancer in the first-line setting (NCT01126749).

A phase II study investigating single-agent bortezomib in patients with previously treated advanced urothelial tract TCC showed that it does not have antitumor activity as second-line therapy in UC(99). Polo-like kinase 1 (PLK1) controls multiple steps of mitosis. Volasertib (BI 6727), a first-in-class selective inhibitor of PLK1 was investigated in patients (n=50) whose disease progressed on chemotherapy. Volasertib showed early signs of benefit, with 14% patients exhibiting a partial response, and 24% had stable disease (100).

Conclusion

In summary, UC is a common and deadly cancer in which platinum-based regimens continue to remain the mainstay of therapy. Efforts to incorporate targeted therapies into UC management are ongoing, but so far without significant success noted for other common solid tumor types. A better understanding of its molecular biology is imperative to make advances. The lack of data supporting new therapies may also have been due to poor study accrual. Targeting angiogenesis, the PI3/AKT pathway and utilizing newer immunotherapy and anti-HER2 therapy seems most promising for future studies. Continued investigation of targeted therapies are needed to improve survival of patients with advanced UC.

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