Novel strategies for treating relapsed/refractory urothelial carcinoma

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Abstract

Advanced urothelial cancer is associated with a poor prognosis and there has been no substantial progress over the past three decades since the development of platinum-based multiagent chemotherapy. Clinical trials evaluating novel agents and combinations including chemotherapeutic drugs, as well as targeted inhibitors, are desperately needed. With a better understanding of the complex molecular alterations that drive urothelial tumorigenesis, new targets for novel therapeutics are being defined. This article will describe the current state of advanced urothelial cancer treatment and provide a comprehensive discussion of novel agents in development.

Keywords

bladder cancer; targeted therapy; transitional cell carcinoma; urothelial cancer

In the USA, bladder cancer is the fourth most common malignancy in men and the eighth most common in women [1]. Approximately 70,980 new cases of bladder cancer were predicted for the year 2009 with an estimated 14,330 deaths [1]. In total, 75% of patients present with non-muscle-invasive (Ta, T1 and Tis) disease, which is treated with a transurethral resection of the bladder tumor (TURBT) with or without intravesical therapy [2,3]. An estimated 70% of these individuals will experience a recurrence and 10–20% of these recurrences will progress to muscle-invasive disease [4]. A minority of patients is diagnosed with either muscle-invasive (20%) or metastatic (5%) disease at the time of presentation. Patients with muscle-invasive disease who are surgical candidates generally undergo a radical cystectomy with pelvic lymph node dissection, with only a minority of these patients receiving perioperative chemotherapy. The mainstay of treatment for those individuals with metastatic disease is systemic chemotherapy alone [5]. In the first-line, meta-static setting, treatment with cisplatin-based chemotherapy results in a modest improvement in survival, with a median survival of approximately 12–15 months [6,7]. Unfortunately, no US FDA-approved therapy exists in the second-line setting, that is, for patients who progress after first-line platinum-based chemotherapy. For these reasons,

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urothelial cancer (UC) is a devastating illness with ongoing research aimed at developing active therapies including novel targeted agents in patients with advanced disease. This article focuses on the current management strategy for patients with relapsed or refractory advanced UC, as well as novel therapies in development including targeted agents.

Standard chemotherapy regimens

Cisplatin-based combination chemotherapy represents the standard of care for first-line treatment of advanced UC. Early trials examined the efficacy of cisplatin administered with methotrexate [8]. Later studies found that the three-drug combination including cisplatin, methotrexate and vinblastine resulted in a response rate (RR) of 56% and a median overall survival (OS) of 8 months [9]. A regimen consisting of methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC) was initially tested in 25 patients with metastatic UC with a 50% rate of complete remission and a 13-month median survival [6]. Based on these promising results, M-VAC was subsequently compared with cisplatin alone in a prospective, randomized trial and was found to be superior with respect to overall RR (ORR; 39 vs 12%; p < 0.0001), progression-free survival (PFS; 10 vs 4.3 months) and OS (12.5 vs 8.2 months) [10]. A 6-year follow-up confirmed superior survival in those patients who received M-VAC, although only 3.7% of these patients were still alive and without evidence of recurrent disease, a finding that underscores the limited durability of response to systemic chemotherapy in advanced bladder cancer [11]. Of note, prognostic factors predicting survival have been derived from five clinical trials examining first-line M-VAC therapy in patients with metastatic or unresectable UC, and have been utilized to stratify patients into risk groups based upon the probability of disease-specific mortality. These factors include Karnofsky performance status (KPS) below 80% and the presence of visceral metastases (lung, liver or bone) [12]. Patients with zero, one or two risk factors have median survival times of 33, 13.4 and 9.3 months, respectively [12].

Gemcitabine and docetaxel were determined to be active single agents in bladder cancer and were subsequently evaluated in combination with cisplatin [13–15]. While the combination of docetaxel and cisplatin was found to be inferior to M-VAC, a Phase III trial comparing gemcitabine plus cisplatin (GC) and M-VAC in locally advanced or metastatic disease revealed similar RRs (46% with GC vs 49% with M-VAC), time to progression (7.4 months in both arms) and median survival (13.8 vs 14.8 months) [7,16]. Patients randomized to M-VAC experienced significantly higher rates of grade 3–4 neutropenia with subsequent complications of fever and sepsis [7]. Based on these data, GC has emerged as a standard of care for the first-line treatment of patients with metastatic UC.

Several triplet chemotherapy regimens have been investigated in Phase II clinical trials including agents such as ifosfamide and paclitaxel [17]. A Phase I/II study evaluated the combination of paclitaxel, cisplatin and gemcitabine (PCG) in 61 patients; the ORR was 78% with Phase I and II median survivals of 24 and 16 months, respectively [18]. A multicenter Phase III study coordinated by the European Organisation for Research and Treatment of Cancer randomized 627 patients with metastatic bladder cancer to receive GC or PCG [19–24]. While the PCG arm demonstrated a superior ORR (57% with PCG vs 46% with GC; p = 0.02), no significant difference was noted in either PFS or OS, although an unplanned subgroup analysis revealed that patients with a bladder primary tumor had an improvement in OS (p = 0.03) [24]. This benefit in OS was only observed in the previously described risk groups 0 or 1. Thrombocytopenia and bleeding were seen more often with GC (12 vs 7%) while febrile neutropenia was more prevalent with PCG (13 vs 4%).

In general, Phase II trials of second-line therapy for metastatic disease have resulted in limited activity with responses seen in 10–20% of patients and a PFS of 2–3 months with a

median survival of approximately 6 months [25]. Uni- and multivariate analyses of 370 patients with metastatic platinum-refractory UC identified the presence of liver metastases, a European Cooperative Oncology Group (ECOG) performance status (PS) greater than 0, and hemoglobin value less than 10 g/dl as pre treatment prognostic factors for patients failing platinum-containing regimens [26]. The median OS for patients with zero, one, two or three factors was 14.2, 7.3, 3.8 and 1.7 months (p < 0.001), respectively [26]. Although no FDA-approved standard of care exists for the treatment of advanced UC in the second-line setting, the current National Comprehensive Cancer Network (NCCN) guidelines suggest the use of a number of chemotherapeutic agents, including a taxane or pemetrexed, while strongly encouraging participation in a clinical trial.

While prognostic markers for survival have been established for advanced UC, biomarkers predicting response to therapy are lacking. Such biomarkers would be especially useful in predicting resistance to platinum agents since cisplatin represents the cornerstone of traditional chemotherapy in UC, so that patients predicted to possess platinum resistance can preferentially be enrolled in clinical trials investigating alternative agents. In one retrospective analysis, mRNA-expression profiling of pretreatment tumors from 57 patients who had received GC or GC plus paclitaxel as part of two clinical trials identified a correlation between expression of the excision repair cross-complementation group 1 (*ERCC1*) gene and OS [27]. Patients with low ERCC1 mRNA levels were found to have a significantly increased OS compared with patients with high transcript levels (25.5 vs 15.4 months; p = 0.03). ERCC1 levels did not correlate with response to chemotherapy; however, since ERCC1 is an integral component of the cellular nucleotide excision repair machinery that removes bulky platinum-induced DNA adducts, high ERCC1 levels may represent heightened DNA repair capability and, therefore, a reduced response to cisplatin. Other molecular mechanisms have been identified that lead to platinum resistance, including reduced intracellular accumulation of drug owing to either increased drug efflux or trapping within intracellular compartments such as endosomes, drug inactivation and the ability to escape apoptosis following DNA damage. The identification of gene signatures predictive of cisplatin resistance is ongoing. A potent method to construct such gene-expression models in bladder cancer involves the identification of candidate biomarker genes that predict response to a compound of interest and that are differentially expressed in resistant and sensitive cell lines within the NCI-60 reference panel of 60 representative cancer cell lines [28]. The candidate genes are then filtered through a co-expression extrapolation (COXEN) algorithm to isolate those genes that are expressed both within the NCI-60 reference panel and within a panel of bladder cancer cell lines following exposure to the compound of interest. These filtered genes are potentially the most relevant for prediction of sensitivity to a given compound and can be utilized to construct a gene-expression signature for that compound. Such gene-expression signatures can be used to design therapeutic clinical trials and to inform clinicians prospectively about the potential utility of specific chemotherapeutic agents for individual patients.

Novel chemotherapy agents

Table 1 summarizes select novel chemotherapy agents in development for advanced UC.

Epothilones

The epothilones are a class of chemotherapeutic agents that stabilize microtubule polymers leading to mitotic arrest and inhibition of cell proliferation [29]. Preclinical data suggested antineoplastic activity for ixabepilone, a semisynthetic analog of the natural myxobacterial product epothilone B, in human cancer xenografts, including paclitaxel-resistant tumors [30]. Ixabepilone was subsequently FDA approved for the treatment of progressive, metastatic or locally advanced breast cancer. Based upon these findings, a Phase II trial was

conducted in which 45 previously treated patients with recurrent UC were administered ixabepilone 40 mg/m^2 every 21 days until disease progression or toxicity [31]. In total, 17 patients (40%) had received prior taxane therapy. The ORR was 11.9%, with five patients achieving an objective partial response (PR). The median PFS was 2.7 months (95% CI: 1.8–4.1 months) with an OS of 8 months (95% CI: 4.9–9.8 months). Moderate toxicity was observed including 12 patients (26%) with grade 4 toxicities. In total, 16 patients experienced grade 3 or 4 granulocytopenia with one treatment-related death related to febrile neutropenia. Three patients developed grade 3 or 4 sensory neuropathy and one patient developed grade 3 renal failure [31]. Based on the toxicity profile and only modest activity, the investigators decided against further investigation of ixabepilone in advanced UC [31].

Pemetrexed

Pemetrexed, a multi-targeted antifolate analog that inhibits the synthesis of nucleotide precursors, is FDA approved for the treatment of both non-small-cell lung cancer (NSCLC) and malignant mesothelioma, and has also been evaluated in the second-line setting in UC. An initial trial by Paz-Ares *et al.* investigated pemetrexed as salvage chemotherapy in UC [32]. Owing to significant toxicity in the first six enrolled patients, including two toxic deaths from sepsis and renal failure, the trial required dose reduction from 600 to 500 mg/m^2. The ORR was 29% (95% CI: 14–48%). In a Phase II study led by the Hoosier Oncology Group (IN, USA), 47 patients received pemetrexed 500 mg/m^2 every 3 weeks with vitamin B12 and folic acid supplementation [33]. This trial resulted in an ORR of 27.7%, including three complete responses (CRs). Median time to disease progression was 2.9 months (95% CI: 1.7–4.6 months) with a median OS of 9.6 months (95% CI: 5.1–13.8 months). In a study performed at the Memorial Sloan–Kettering Cancer Center (MSKCC; NY, USA), 13 patients with relapsed disease received second-line therapy with pemetrexed at 500 mg/m^2 every 21 days, with an objective response seen in only one patient with an ORR of 8% (95% CI: 0–29%) [34]. Pemetrexed is well tolerated and represents an option for the treatment of patients with advanced UC in the second-line setting.

Pralatrexate

Pralatrexate is a novel targeted antifolate that has been FDA approved for the treatment of relapsed or refractory peripheral T-cell lymphoma, and early-phase clinical trials also suggest efficacy and tolerability in NSCLC. The drug binds to the reduced folate carrier (RFC)-1 cell surface receptor, which is overexpressed on cancer cells, and its structure allows for increased intracellular retention once it has been taken up by the target cell. A single-arm Phase II trial is investigating the efficacy of pralatrexate in patients with metastatic UC that has progressed on platinum-based therapy. The primary end point is objective RR and secondary end points include PFS, OS and tolerability. The results of this trial are pending, although pralatrexate was recently provided an orphan drug designation by the FDA for treatment of bladder cancer.

Vinflunine

Vinflunine is a novel fluorinated vinca alkaloid that inhibits the assembly of tubulin monomers, resulting in mitotic arrest and subsequent apoptosis. It has a favorable neurotoxicity profile compared with other vinca alkaloids as a result of reduced binding affinity for tubulin and is also effective in multiple solid tumors, including breast and NSCLC [35]. Based on preclinical activity against the murine bladder cancer cell line MB-49, Vinflunine was first investigated in 57 patients with recurrent disease or progression of disease within 1 year of chemotherapy [36]. The drug was initially dosed at 350 mg/m^2 in six patients; however, a program-wide safety evaluation performed in all Phase II trials of Vinflunine led to a dose reduction to 320 mg/m^2. All six patients treated for a total of 15 cycles at 350 mg/m^2 experienced myelosuppression, with one death related to febrile

neutropenia. Nine out of 51 patients who received Vinflunine at 320 mg/m^2 achieved a PR with an ORR of 18% (95% CI: 8.4–30.9%). Of note, five of the nine PRs occurred at visceral sites of metastases, including the lung and liver. In the patients who experienced a PR, eight out of 34 responses occurred in individuals treated for metastatic disease in the first-line setting. The median PFS was 3 months (95% CI: 2.4–3.8 months), with a median OS of 6.6 months (95% CI: 4.8–7.6 months) and the median response duration was 9.1 months (95% CI: 4.2–15 months). A total of 34 patients (67%) experienced grade 3 or 4 neutropenia, with five (10%) cases of febrile neutropenia. Grade 3 or 4 constipation was observed in four patients (8%). A second Phase II trial investigated Vinflunine in 151 patients with platinum-refractory UC [37]. Patients who were 75 years of age or older, with a history of pelvic irradiation, a KPS of 80–90% or with a creatinine clearance of 60 ml/min or less were administered 280 mg/m^2. All other patients received 320 mg/m^2. The ORR was 14.9%, with a median duration of response of 6.8 months in an interim analysis of 114 patients. Grade 3 or 4 neutropenia, anemia and thrombocytopenia occurred in 59, 17 and 4% of patients, respectively. Grade 3 or 4 constipation was observed in 14% of patients. Given the activity seen in the Phase II studies, a Phase III trial was performed and reported by Bellmunt *et al.* in which 370 patients with previously treated advanced UC were randomized to vinflunine at a dose of 320 mg/m^2 every 3 weeks with best supportive care (BSC) or to BSC alone [38]. Those patients with an ECOG PS of 1 or an ECOG PS of 0 but with a history of prior pelvic irradiation received a dose of 280 mg/m^2 with the initial cycle followed by dose escalation to 320 mg/m^2 in the absence of significant hematologic toxicity. Survival was improved in the Vinflunine arm at 6.9 months compared with 4.6 months with BSC alone, although this was not a significant difference in the intent to treat population with a hazard ratio of 0.88 (95% CI: 0.69–1.12; p = 0.287). A multivariate Cox analysis adjusting for prognostic factors revealed a statistically significant effect of Vinflunine on OS (p = 0.036) with a hazard ratio of 0.77 (95% CI: 0.61–0.98). In the eligible population (n = 357), the median OS was significantly longer for the Vinflunine arm compared with BSC (6.9 vs 4.3 months; p = 0.040). On the Vinflunine arm, toxicities including grade 3 or 4 neutropenia and neutropenic fever occurred in 50 and 6% of patients, respectively. Grade 3 or 4 constipation was observed in 16% of patients, mainly during the initial two cycles of therapy. A low incidence of peripheral neuropathy was noted. There are no ongoing UC clinical trials with Vinflunine.

Larotaxel

The novel taxane larotaxel stabilizes tubulin polymers, preventing disassembly of the microtubules and thereby inhibiting cell division. Preclinical studies in melanoma and breast cancer cell lines suggested improved efficacy over docetaxel in taxane-resistant cancer models. Early-phase clinical trials defined dose-limiting toxicities as grade 3–4 neutropenia and diarrhea. Phase II studies revealed clinical efficacy for larotaxel in taxane-resistant meta-static breast cancer, as well as in the first-line setting in combination with platinum in stage III or IV NSCLC [39]. A Phase III trial was designed in the setting of locally advanced or metastatic UC in which patients were randomized to receive front-line cisplatin plus larotaxel or gemcitabine plus cisplatin; this trial has since been closed to accrual.

Tesetaxel

Another microtubule-stabilizing taxane in early-phase clinical trials is the oral agent tesetaxel. This drug has undergone Phase II testing in metastatic platinum-refractory NSCLC, fluorouracil-refractory advanced gastric cancer and metastatic colon cancer, and has been fast tracked by the FDA for expedited review in gastric cancer [40–42]. A Phase II trial of tesetaxel in advanced UC is planned.

Abraxane®

Albumin-bound paclitaxel (Abraxane®; Celgene Corporation, NJ, USA) has shown efficacy and superior tolerability in metastatic breast cancer. A Phase II two-stage trial of Abraxane in platinum-treated metastatic UC is ongoing [43]. Preliminary results in 14 patients, in which Abraxane was delivered at 260 mg/m^2, revealed a clinical benefit rate of 71%, with ten patients experiencing either PR or stable disease [43]. The second stage of the trial is continuing with an accrual goal of 48 patients.

Eribulin

Eribulin is a synthetic analog of the black Pacific sea sponge macrolide halichondrin B [44]. The drug suppresses microtubule polymerization without affecting depolymerization and results in the formation of nonfunctional intracellular tubulin aggregates [44]. Preclinical studies established the antiproliferative effects of eribulin within multiple cancer cell lines, including prostate and breast, as well as *in vivo* efficacy against paclitaxel-resistant ovarian cell lines. Two Phase I trials defined the dose schedule and toxicity profile of eribulin in patients with advanced solid malignancies, and the most common adverse events included neutropenia and fatigue [45,46]. Neurotoxicity with eribulin was not a predominant toxicity in either trial. Preliminary results from a Phase II trial of eribulin in advanced UC were reported at the American Society of Clinical Oncology (ASCO) 2010 meeting (4–8 June 2010, IL, USA) [47]. In total, 40 patients were enrolled and received eribulin monotherapy at 1.4 mg/m^2 on days 1 and 8 of a 3-week cycle. The ORR in 37 evaluable patients was 38% (95% CI: 23–54%), consisting of one CR and 14 PRs. In those patients who had received prior lines of chemotherapy, a comparable RR of 34% was observed. Median PFS and OS were 3.9 and 9.4 months, respectively, at a median follow-up of 19.8 months. A total of 20 patients experienced grade 3–4 neutropenia. Grade 1–2 sensory neuropathy was observed in 18 patients. In addition, because eribulin is minimally cleared through the kidney (<10%), a Phase I study examined the safety and efficacy of eribulin in patients with UC and depressed renal function [48]; 21 patients with moderate (40–59 ml/min) or severe (20–40 ml/min) renal dysfunction as defined by the Cockgroft–Gault formula were treated with escalating doses of eribulin with a maximum dose of 1.4 mg/m^2. The maximum tolerated dose was defined as the highest dose achieved with no more than one dose-limiting toxicity. RR in 20 evaluable patients was 18% (95% CI: 5–41%) with three PRs. The median PFS and OS were 4.1 and 9.7 months, respectively, at a median follow-up of 11 months. Notably, patients with moderate renal dysfunction tolerated a dose of 1.4 mg/m^2 with no dose-limiting toxicities observed. Among patients with severe renal dysfunction, one out of six patients receiving 1.4 mg/m^2 experienced a dose-limiting toxicity of fatigue and weakness.

Novel targeted agents

As research into the critical molecular pathways that drive bladder tumorigenesis progresses, new targets for selective inhibitor therapy will be identified. With the incorporation of targeted agents into the management of patients with multiple malignancies including NSCLC, breast, colon, glioblastoma and others, the utility of selective inhibitor therapy has been proven, both in combination with chemotherapy and as monotherapy [49–61]. These drugs target aberrantly activated pathways that are typically involved in regulating cellular proliferation, apoptosis and angiogenesis [51]. The major advantage of targeted therapy lies in its specificity and related favorable toxicity profile [50]. Table 2 summarizes trials involving select targeted therapies. Figure 1 depicts the major targets for commonly utilized and novel monoclonal antibodies and small molecule inhibitors.

EGF receptor inhibition

In UC, blockade of either the EGF receptor (EGFR) or its associated tyrosine kinase is under investigation. Overexpression of HER2, a member of the EGFR family, has been observed in 23–80% of bladder cancers, with increased levels of expression correlating with higher grade, increased metastatic potential and poor prognosis [62,63]. HER2 partners with EGFR and forms a heterodimer that binds EGF ligand with high affinity. Ligand binding stimulates the Ras–MAPK signal-transduction cascade, which in turn drives cell proliferation and migration [64]. Controversy exists as to the optimal method for detection of HER2 overexpression in UC, that is, immunohistochemical (IHC) analysis for protein overexpression, FISH to detect *HER 2* gene amplification, or measurement of shed HER2 extracellular domain protein through an ELISA. Furthermore, while HER2 overexpression in breast cancer is characterized by concomitant genetic amplification, HER2-positive bladder tumors typically manifest protein overexpression alone with a much lower incidence of *HER2* genetic amplification. A large retrospective study by Lae *et al.* attempted to define the incidence of both *HER2* gene and protein overexpression by applying the same diagnostic criteria currently utilized in breast cancer to 1005 invasive paraffin-embedded UC samples [65]. In total, 93 tumors (9.2%) revealed either 2+ or 3+ HER2 protein expression by IHC; all samples with 3+ protein overexpression contained gene amplification by FISH compared with none of the samples with 2+ overexpression. By contrast, there is a strong correlation between HER2 protein overexpression by IHC and underlying gene amplification by FISH in breast cancer, suggesting that the majority of *HER 2* gene amplifications in breast cancer result in HER2 protein overexpression. Interestingly, ten out of ten lymph node metastases and matched primary tumors had 3+ HER2 protein overexpression (FISH testing was not performed in these samples), consistent with a previously described association between HER2 overexpression and metastatic potential [66].

Monoclonal antibodies

Trastuzumab—The humanized monoclonal anti-HER2 antibody trastuzumab has demonstrated significant activity in HER2-positive breast cancer, both in the metastatic and adjuvant settings [67]. In a multicenter Phase II NCI-198 trial, 44 patients with advanced UC and overexpression of HER2 by either IHC, FISH or serum HER2 extracellular domain (ECD) testing received chemotherapy consisting of carboplatin, paclitaxel and gemcitabine in combination with trastuzumab [68]. Metastatic disease was present in all treated patients and 32.6% of patients had received prior chemotherapy. The primary end point of this study was cardiac toxicity rate while secondary end points included RR, time to disease progression and survival. Ten patients (22.7%) experienced grades 1–3 cardiac toxicities. The RR was 70% (31 patients), with five CRs and 26 PRs. The median time to progression was 9.3 months while median survival was 14.1 months (95% CI: 11.5–17.1 months); median survival was 15.8 months (95% CI: 11.4–24.3 months) for patients with HER2 overexpression. Interestingly, 75% of patients with 3+ HER2 over expression by IHC exhibited a response, compared with 67% in those with 2+ IHC overexpression; furthermore, 82% of patients with FISH-positive overexpression experienced a response versus 67% of FISH-negative patients. These results suggest that RRs may be highest in those patients with the greatest degree of HER2 overexpression, similar to findings in HER2-positive breast cancers [69]. Of note, patients with HER2 overexpression also had a higher median number of metastatic sites (two sites vs one site; p = 0.014), a trend toward increased visceral and bony metastases, and the presence of two or more metastatic sites was more common (51 vs 31%; p = 0.051), thus comprising a high-risk baseline population. Prospective measurement of HER2 expression using IHC, FISH or detection of serum HER2 ECD levels revealed that 52% of patients displayed HER2 overexpression. In total, 93% of

patients with HER2 overexpression were detected by IHC, while only a subset of these patients were positive by FISH or serum testing (26 and 23%, respectively). Again, in contrast to the concordant *HER2* gene amplification found in breast cancer, these results concur with the finding that the majority of HER2 overexpression in UC occurs without underlying genetic amplification [70].

Cetuximab—Cetuximab is a recombinant humanized murine monoclonal antibody that targets the extracellular domain of the EGFR (HER1), leading to inhibition of downstream mitogenic signal transduction pathways typically stimulated by ligand binding. Its efficacy in metastatic colorectal cancer, as well as squamous cell carcinoma of the head and neck, has led to its FDA approval for these diseases [71,72]. Cetuximab possesses antiproliferative activity at least partly through an antiangiogenic mechanism in mouse models of UC, and this effect is synergistic with paclitaxel [73,74]. A randomized Phase II trial coordinated by the University of Michigan (MI, USA) is currently accruing in which patients with metastatic, locally recurrent or unresectable UC are administered GC either alone or in combination with cetuximab.

Tyrosine kinase inhibitors

Gefitinib—Gefitinib, an oral inhibitor of the tyrosine kinase activity of EGFR, has shown significant clinical activity in EGFR mutant NSCLC [75]. Gefitinib is also effective at inhibiting cell proliferation in EGFR-expressing human bladder cancer cell lines with addition of drug leading to suppression of both Akt and ERK phosphorylation [76]. A Phase II Cancer and Leukemia Group B (CALGB) trial investigated the efficacy of gefitinib in combination with gemcitabine and cisplatin in patients with advanced UC [77]. This combination of agents had been previously shown to have synergistic effects compared with chemotherapy alone with GC in mouse xenograft models. Such synergy was thought to be related to blockade of EGFR signal transduction with the concomitant antiproliferative effects of chemotherapeutic agents. The initial cohort of 25 patients were administered a fixed dose rate infusion of gemcitabine 10 mg/m^2/min with cisplatin plus gefitinib. Significant grade 4 nonhematologic toxicities, including hyponatremia, hyperuricemia, dyspnea, and venous thromboembolism, as well as death from internal carotid artery thrombosis and urosepsis occurred, leading to accrual discontinuation and subsequent modification of the gemcitabine administration to a 30-min infusion. A total of 54 patients were evaluated with an ORR of 42.6% (95% CI: 29.2–56.8%) consisting of seven CRs and 16 PRs. Median OS was 15.1 months (95% CI: 11.1–21.7 months) and median time to progression was 7.4 months (95% CI: 5.6–9.2 months). A follow-up analysis at 39.5 months revealed that 51 patients had died. The OS and time to progression with the addition of geftinib was not significantly different compared with historical gemcitabine and cisplatin alone [7]. There are no ongoing clinical trials evaluating geftinib in the metastatic setting.

Erlotinib & lapatinib—The oral EGFR tyrosine kinase inhibitor (TKI) erlotinib has been shown to inhibit the proliferation of UC cells *in vitro*[78]. This drug is currently being investigated in the neoadjuvant setting in patients with locally advanced UC. Lapatinib, a dual EGFR and HER2 TKI, has shown preclinical anti-tumor activity in multiple bladder cancer cell lines [79]. A single-arm Phase II trial investigated the efficacy of lapatinib in patients with platinum-refractory UC. Out of 59 enrolled patients, 34 were evaluable for response. An ORR of greater than 10% was seen in 1.7% of patients (95% CI: 0–9.1%). Stable disease was observed in 18 patients (31%; 95% CI: 19–44%). OS was 17.9 weeks and time to progression 8.6 weeks. Of note, OS was significantly increased with EGFR or HER2 overexpression (p = 0.0001) [80]. A Phase II/III trial is currently examining the role of maintenance lapatinib in patients with HER1 and/or HER2-positive advanced UC following chemotherapy. Eligibility criteria include an objective response or stable disease by

Response Evaluation Criteria in Solid Tumors (RECIST) after administration of first-line chemotherapy, as well as evidence of HER1 and/or HER2 overexpression by FISH or IHC. Patients are randomized to receive lapatinib 1500 mg daily or placebo until disease progression.

Antiangiogenesis agents

Angiogenesis plays a crucial role in tumor development and maintenance, as well as metastasis. In metastatic bladder cancer, the presence of high serum VEGF levels corresponds to decreased disease-free survival [81]. Microvessel density, a surrogate marker of angiogenesis in UC, is also associated with a poor prognosis, and both microvessel density and VEGF mRNA levels were defined as independent predictors of metastasis and disease recurrence in 51 patients who were treated with neoadjuvant M-VAC chemotherapy and cystectomy [82,83]. Targeting tumor dependence on angiogenesis with antiangiogenic therapies in the form of monoclonal antibodies and small-molecule inhibitors has significantly expanded treatment options for patients with advanced solid tumor malignancies.

Monoclonal antibodies

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal antibody targeting all isoforms of circulating VEGF. It has led to an improvement in outcome in advanced colorectal cancer, glioblastoma, NSCLC, breast cancer and renal cell cancer [84–87]. In a Phase II trial led by the Hoosier Oncology Group, bevacizumab was administered concomitantly with GC as first-line therapy in 43 patients with metastatic UC [88]. Patients initially received gemcitabine at a dose of 1250 mg/m^2. However, after seven thromboembolic events occurred in the first 17 accrued patients, the gemcitabine dose was reduced to 1000 mg/m^2. Preliminary results presented at the ASCO Annual Meeting 2009 revealed an ORR of 67% (95% CI: 51–82%), with six CRs and 18 PRs. In total, 47% of patients experienced grade 3–4 hematologic toxicity (thrombocytopenia and neutropenic fever) while grade 3–4 nonhematologic toxicity was present in 77% of patients (including deep vein thrombosis/pulmonary embolism, CNS hemorrhage, proteinuria and hypertension). These results suggest the potential for an improvement in response with the addition of bevacizumab complicated by significant treatment-related toxicities. A CALGB Phase III randomized trial of GC with or without bevacizumab in the first-line treatment of metastatic disease is ongoing. Additional trials currently accruing include a Phase II study of first-line gemcitabine, carboplatin and bevacizumab in patients with advanced UC who are not candidates for cisplatin (MSKCC), as well as a Phase II trial of dose-dense M-VAC combined with bevacizumab in the neoadjuvant setting (MD Anderson Cancer Center, TX, USA) [89].

Tyrosine kinase inhibitors

The multikinase inhibitors sorafenib, sunitinib and, more recently, pazopanib have revolutionized the treatment of meta-static renal cell carcinoma (RCC) and are also under investigation in advanced UC [90–92].

Sorafenib

Sorafenib inhibits Raf kinase, PDGF receptor (PDGFR)-β, VEGF receptor (VEGFR)-2, and VEGFR-3, while sunitinib blocks the activity of VEGFR-2 and PDGFR-β. The efficacy of first-line sorafenib in advanced bladder cancer was tested in a Phase II multicenter study through the Princess Margaret Hospital (ON, Canada) and the National Cancer Institute of

Canada for which preliminary results suggested no significant response to therapy in 14 evaluable patients [93]. A similar Phase II ECOG trial examined sorafenib monotherapy in patients with advanced UC who had progressed on platinum-based chemotherapy and also revealed minimal activity [94].

Sunitinib

Sunitinib possesses antiproliferative activity against human bladder cancer cell lines and this activity is synergistic with the addition of cisplatin in murine xenografts [95]. Based upon these pre-clinical data, a Phase II study was performed at MSKCC in which sunitinib was administered to 77 patients with advanced UC in the second-line setting. Two drug-administration schedules were tested in separate cohorts: the initial cohort A received 50 mg once daily for 4 consecutive weeks out of a 6-week cycle, while cohort B received 37.5 mg once daily continuously. The primary end points were RR and safety while secondary end points included time to progression and OS. Three patients (7%) displayed a PR and 11 had stable disease in cohort A by RECIST criteria; in cohort B, one PR (3%) was observed and 15 patients had stable disease. Overall, a reduction in measurable disease was observed in 29 patients, with six patients experiencing greater than 20% reduction [96]. Clinical benefit from sunitinib (defined as PR or standard deviation lasting longer than 3 months) was observed in 22 patients (29%). Although response was modest, the significant number of patients with stable disease underscores the potential for targeted inhibition of the VEGF pathway in prior platinum-treated UC. A randomized double-blind, placebo-controlled Phase II trial is investigating the efficacy of maintenance sunitinib following 4–6 cycles of standard chemotherapy to determine if sunitinib decreases progression rates in patients with advanced UC who have obtained stable disease or better after standard chemotherapy [97].

Pazopanib

Pazopanib inhibits VEGFR-1, -2, -3, c-kit and PDGFR [98]. In a Phase III randomized controlled study of pazopanib versus placebo in metastatic RCC, a significant prolongation of PFS and RR was observed in patients receiving pazopanib [92]. As such, its antiangiogenic effects in metastatic UC are currently being evaluated in an ongoing Phase II trial. The primary end point is ORR while secondary end points include time to progression, OS, and frequency and type of adverse events.

Vandetanib

Vandetanib is an oral TKI that blocks the activity of VEGFR-2 and EGFR, as well as RET [99,100]. This agent has been shown to inhibit the growth of preclinical models of NSCLC [101]. Furthermore, a large Phase III trial in locally advanced or meta-static NSCLC demonstrated the superiority of vandetanib plus docetaxel in extending PFS compared with docetaxel alone [102]. A Phase II trial is currently accruing patients with advanced UC in which individuals are randomized to receive docetaxel alone or docetaxel plus vandetanib. The primary outcome of the study is PFS and secondary outcomes include OS, ORR, and incidence of adverse events and toxicities grade 3 or higher.

Vascular-disrupting agents

Representing a distinct class of anti-tumor drugs, the vascular-disrupting agents (VDAs) destroy existing tumor-associated vasculature, leading to significant tumor necrosis and cell death [103,104]. Preclinical findings suggest that exposure to VDAs leads to selective endothelial cell apoptosis through intracellular cytoskeletal disruption [103,105]. These agents differ from anti angiogenic agents, which target new blood vessel growth, thereby inhibiting further tumor growth and metastasis.

ASA404

ASA404, a novel VDA, demonstrated promising preclinical activity against multiple tumor cell lines as well as synergy when administered with taxanes [106,107]. Unfortunately, a randomized Phase III trial investigating this compound in the first-line setting for advanced NSCLC (ATTRACT-1) was recently discontinued following a planned interim analysis that showed no benefit with the addition of the drug to standard platinum-based therapy [201]. However, a Phase II trial of ASA404 in combination with docetaxel in patients with metastatic UC in the second-line setting is currently accruing.

Epigenetic-modulating agents

Histone deacetylase inhibitors

Tumor suppressors such as p53 and Rb serve as checkpoints to neoplastic transformation by halting cell proliferation or inducing apoptosis in the face of irreparable genetic damage. Inactivation of tumor suppressors with subsequent tumor development can occur through multiple mechanisms, including gene deletion, loss of heterozygosity, or epigenetic transcriptional silencing via aberrant promoter methylation or histone deacetylation. This last process is mediated by histone deacetylase enzymes (HDACs) and leads to a tighter association between packaging histones and DNA, preventing access to transcription-promoting sequences. The HDAC inhibitors vorinostat and romidepsin have been approved for the treatment of cutaneous T-cell lymphoma.

Vorinostat—Preclinical data with suberoylanilide hydroxamic acid (SAHA), another HDAC inhibitor, has demonstrated inhibition of proliferation of the T24 human bladder cancer cell line [108]. A Phase II trial of second-line SAHA in 14 patients with locally recurrent or metastatic UC was performed through the California Cancer Consortium [109]. SAHA was administered orally at a dose of 200 mg twice daily until disease progression or toxicity. Three patients achieved stable disease while eight experienced progression. The median disease-free survival was 1.1 months and OS was 4.3 months. Two on-study deaths occurred, five patients experienced either grade 4 or 5 toxicity, and four patients experienced grade 3 toxicity. The study was closed to further accrual, with the conclusion that SAHA did not display significant efficacy in the second-line setting and was associated with toxicity.

Demethylating agents

Decitabine

Demethylating agents are also being studied in the treatment of UC. 5-aza-2′-deoxycytidine, or decitabine, is a DNA methyltransferase inhibitor that has shown preclinical synergistic activity in combination with cisplatin against five human bladder cancer cell lines [110]. Early-phase clinical trials will measure the efficacy of decitabine in patients with UC.

Proteasome inhibitors

Bortezomib

Bortezomib is an inhibitor of the 26S proteasome complex that degrades ubiquitinated proteins. Proteasome blockade sub sequently leads to cell cycle arrest and apoptosis. Exposure of UC cell lines to combination bortezomib and gemcitabine resulted in inhibition of proliferation as well as stimulation of apoptotic pathways [111,112]. Bortezomib has been tested in two Phase II trials in patients with advanced UC. A total of 20 eligible patients were enrolled through the Princess Margaret Hospital and the University of Chicago (IL, USA) Phase II consortia and were treated twice weekly for 2 out of 3 weeks with infusional bortezomib [113]. No objective responses were detected, the median time to progression

was 8.1 weeks (95% CI: 6.4–9), and median OS was approximately 15 weeks (95% CI: 3.6–NA). A total of 15 patients experienced either grade 3 or 4 toxicities. In a sub sequent trial conducted by the CALGB, 25 patients with advanced UC refractory to prior chemotherapy were administered bortezomib [114]. No objective responses were noted, the median time to progression was 1.4 months (95% CI: 1.1–2 months) and median survival time was 5.7 months (95% CI: 3.6–8.4 months). Based on these studies, bortezomib does not appear to possess significant activity against UC in the second-line setting.

Other TKIs

Fibroblast growth factor receptor-3 (FGFR3) is a receptor tyrosine kinase that is mutated in up to 75% of low-grade, non- muscle-invasive bladder tumors and approximately 20% of muscle-invasive lesions [115,116]. Hotspot mutations within exons 7, 10 and 15 account for the majority of alterations and characteristically lead to constitutive dimerization and subsequent activation of the kinase [117]. FGFR3 is thought to transduce extracellular mitogenic signals using both the MAPK and PI3K–Akt pathways, resulting in increased cell growth, proliferation and survival [118]. Interestingly, mutations within both *FGFR3* and *TP53* rarely coexist, suggesting that urothelial tumors possessing *FGFR3* mutations may be uniquely dependent on the activated FGFR3 for maintenance of a neoplastic phenotype [117]. The relatively high frequency of *FGFR3* mutations in UC has generated substantial interest in the development of targeted inhibitors against the activated enzyme.

Tyrosine kinase inhibitor 258

Tyrosine kinase inhibitor 258 is a multi-targeted TKI that blocks the tyrosine kinase activity of FGFR, VEGFR, PDGFR-β and Flt-3, and has been shown in preclinical studies to have both direct cytotoxic and antiangiogenic activity [119]. Phase I studies have proven the safety and defined the maximum tolerated dose of this drug [120]. It will be investigated in an upcoming Phase II two-stage clinical trial in patients with advanced platinum-refractory UC who are stratified based upon the FGFR3 mutation status of their tumors. A total of 20 patients in each arm will receive 500 mg once daily of drug on a 5 days on and 2 days off schedule. The primary end point of the study is ORR with secondary end points including PFS and safety.

PI3K–Akt pathway inhibitors

A wide spectrum of PI3K–Akt pathway mutations has been reported in UC. PI3K is a heterodimeric lipid kinase that, upon activation by receptor tyrosine kinases, catalyzes the conversion of phosphotidylinositol-4,5-bisphosphate (PIP_2) to phosphotidylino-sitol-3,4,5-triphosphate (PIP_3) [121]. PIP recruits multiple proteins to the plasma membrane including PDK1 and Akt. Akt is activated by phosphorylation at threonine 308 and stimulates numerous downstream pathways that upregulate cell growth and proliferation and suppress apoptosis [122]. For example, activated Akt phosphorylates and inhibits the GTPase-activating protein tuberous sclerosis complex-2 (TSC2), a molecule that constitutively represses the Ras homolog enriched in brain (Rheb) protein. Subsequent activation of Rheb results in stimulation of mTOR, a key promoter of translation in response to nutrient levels [122,123]. mTOR exerts its effects through phosphorylation and activation of S6 kinase and the eukaryotic initiation factor 4E-binding protein (4EBP1) [123].

A number of alterations of this pathway can result in unchecked cell growth and proliferation in UC. These include mutations within the *PIK3CA* gene that encodes for the p110α catalytic subunit of PI3K (seen in up to 27% of some low-grade tumor series) as well as loss of function mutations of PTEN, the lipid phosphatase that negatively regulates PI3K-mediated conversion of PIP_2 to PIP_3[124]. Homozygous deletions as well as downregulation

of *PTEN* expression through copy number loss are frequently found in invasive UC concomitant with *TP53* mutations [124]. Mutations of the *PIK3CA* and *AKT* genes can lead to increased activation of the pathway [118]. Inactivation of TSC1 leads to constitutive activation of mTOR, resulting in uncontrolled cell growth and proliferation, and up to 14.5% of UC tumors contain alterations within TSC1 [124]. *Ras* mutations can also lead to in appropriate PI3K–Akt pathway stimulation since Ras is known to initiate PI3K-mediated signaling [125].

While a plethora of possible aberrations exist that can lead to inappropriate activation of the PI3K–Akt pathway, these aberrations may also serve as targets for inhibition. A number of PI3K pathway inhibitors are currently in development and include PX-866 and BKM120, which block the activity of PI3K alone, as well as BEZ235, a dual PI3K/mTOR inhibitor currently being tested in a Phase I/II trial [126]. Two major classes of PI3K inhibitor exist, namely the non-isoform-specific and the isoform-specific drugs. Whether pan-PI3K inhibition leads to an increased incidence of toxicities is unknown at this time but this issue will need be evaluated in the ongoing Phase 1 trials of these agents. Preliminary data suggest that pathway inhibition, regardless of the class of drug utilized, results mainly in cytostasis as opposed to cytotoxicity [126–128]. Figure 1 depicts inhibitors of the PI3K–Akt pathway that are currently being investigated.

mTOR inhibitors

Mammalian target of rapamycin inhibition has been shown to be highly effective in RCC, and the rapalog temsirolimus is now FDA approved in the management of this disease [129]. More recently, everolimus gained FDA approval for use in the second-line setting following failure of sorafenib or sunitinib in RC [130]. The efficacy of everolimus in advanced UC is currently being investigated through a Phase II trial in which patients with progressive, metastatic disease receive 10 mg of drug orally daily until progression. The primary end point of this study is 2-month PFS rate as well as characterization of toxicities associated with everolimus. Secondary outcome measures include RR and assessment of mTOR activation through IHC detection of markers of mTOR activation, namely phosphorylated S6 and 4EBP1, in pretreatment tissue. Prior data in the form of a tissue microarray comprising 92 muscle-invasive UC specimens revealed overexpression of both phospho-S6 and 4EBP1 with significant correlation of expression within the mTOR pathway, suggesting that this pathway is active in subsets of UC patients and may be an effective target for small-molecule inhibition [131].

Expert commentary

Systemic chemotherapy consisting of a platinum-based regimen remains the standard of care for patients with advanced UC. However, with a median survival in the metastatic setting of approximately 12–15 months and RRs for single-agent therapy in the second-line setting ranging from 10 to 20%, novel therapies are desperately needed in this disease.

Five-year view

While significant discoveries have been made regarding the molecular pathogenesis of UC, no biologic agents have yet been approved either as monotherapy or in combination with traditional chemotherapy in the treatment of metastatic or recurrent UC, although numerous clinical trials evaluating these agents, as well as new cytotoxic therapies with improved tolerance, are underway. A more complete understanding of the complex biology underlying urothelial carcinogenesis will most certainly lead to the development of more effective rational therapies in this disease.

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Key issues

- Systemic chemotherapy with a platinum-based regimen is the current standard of care for advanced urothelial cancer (UC); however, approximately half of patients with advanced disease are not candidates for cisplatin-based treatment regimens and represent a true unmet medical need.

- While both the methotrexate, vinblastine, doxorubicin and cisplatin, and gemcitabine plus cisplatin regimens possess similar efficacy in patients with advanced UC, gemcitabine plus cisplatin is better tolerated with fewer side effects and has become a standard of care for the first-line management of patients with metastatic UC who are candidates for cisplatin-based chemotherapy.

- Second-line chemotherapy agents and regimens have shown only modest activity in advanced UC and there is no available US FDA-approved agent.

- A two-variable prognostic model that correlates with shortened median survival in the metastatic setting includes Karnofsky performance status of 80% or less and the presence of visceral metastases. The proportion of patients in the associated risk categories may have a significant impact on the results of clinical trials.

- Biomarkers that predict response to both standard cytotoxic agents and novel targeted therapeutics are desperately needed.

- Identification of the key signaling pathways driving bladder tumorigenesis will open new avenues for the use of targeted therapies with the potential for improved toxicity profiles compared with standard chemotherapy.

- Ongoing clinical trials evaluating the efficacy of selective kinase inhibitors and other targeted agents in UC patients whose tumors possess the genetic profile predicted to be most likely to respond will ultimately lead to the development of rational combination strategies for patients in whom combinations of mutations drive malignant transformation.

- Numerous clinical trials are underway to investigate the efficacy of novel cytotoxic agents and targeted therapies in advanced UC, and clinicians must enroll patients in these trials whenever possible.
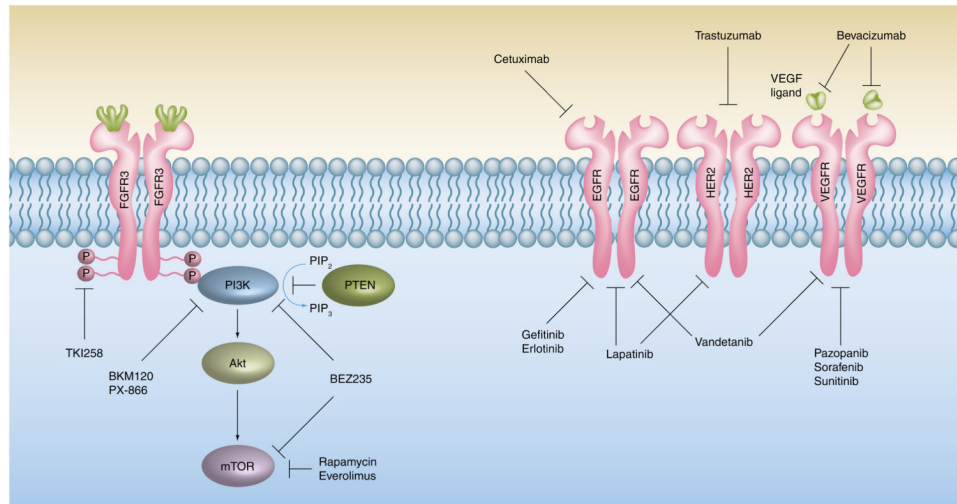
Figure 1. The PI3K–Akt pathway and select tyrosine kinase receptors with relevant pathway and receptor inhibitors
EGFR: EGF receptor; FGFR: FGF receptor; P: Phosphate; PIP_2: Phosphotidylinositol-4,5-bisphosphate; PIP_3: Phosphotidylinositol-3,4,5-triphosphate; VEGFR: VEGF receptor.

Table 1

Novel chemotherapeutic agents in advanced urothelial Cancer.

Agent	Phase/n	RR (%)	OS (months)	Ref.
Ixabepilone	II/45	12	8	[31]
Pemetrexed	II/47	27.7	9.6	[33]
	II/13	8	NA	[34]
Pralatrexate	II/41	Ongoing	Ongoing	
Vinflunine	II/57	18	6.6	[36]
	II/151	15	8.2	[37]
	III/370	8.6	6.9	[38]
Larotaxel	III/900	Closed to accrual	Closed to accrual	
Tesetaxel	Planned			
Albumin-bound paclitaxel	II/48	Ongoing	Ongoing	[43]
Eribulin	II/40	38	9.4	[47]

NA: Not applicable; OS: Overall survival; RR: Response rate.

Table 2

Novel targeted agents in advanced urothelial Cancer.

Author/study	Agent	Target	Regimen	Phase/n	RR (%) (95% CI)	Median OS (months)	Ref.
Philips *et al.*	Gefitinib	EGFR	With GC	II/54	42.6 (29.2–56.8)	15.1	[77]
MDACC (TX, USA)	Erlotinib	EGFR	Once daily	II/42	Ongoing	Ongoing	
Dana–Farber Cancer Institute (MA, USA)	Vandetanib	EGFR, VEGFR-2, RET	Docetaxel ± vandetanib	II/140	Ongoing	Ongoing	
University of Michigan (MI, USA)	Cetuximab	EGFR	GC ± cetuximab	II/81	Ongoing	Ongoing	
Hussain *et al.*	Trastuzumab	HER2/neu	With G + carboplatin + paclitaxel	II/44	70 (55–83)	14.1	[68]
Hahn *et al.*	Bevacizumab	VEGF	With GC	II/45	67 (51–82)	NR	[88]
CALGB 90601			With GC	III/500	Ongoing	Ongoing	
MSKCC (NY, USA)			With G + carboplatin	II/47	Ongoing	Ongoing	
MDACC			With dose-dense M-VAC	II/60	Ongoing	Ongoing	
Sridhar *et al.*	Sorafenib	VEGFR-2, -3, PDGFR-β, Raf	Twice daily	II/17	None observed	5.9	[93]
Dreicer *et al.*			Twice daily	II/27	None observed	6.8	[94]
Gallagher *et al.*	Sunitinib	VEGFR-2, PDGFR-β	Cohort A: daily for 4 out of 6 weeks Cohort B: daily	II/77	29	A: 7.1 vs B: 6 (p = 0.4)	[96]
Mayo Clinic (MN, USA)	Pazopanib	VEGFR-1,-2,-3, PDGFR, c-kit	Once daily	II/32	Ongoing	Ongoing	
Hoosier Oncology Group (IN, USA)	ASA404	Endothelial cytoskeleton	With docetaxel	II/40	Ongoing	Ongoing	
Cheung *et al.*	Vorinostat	HDAC	Twice daily	II/14	None observed	4.3	[109]
Gomez-Abuin *et al.*	Bortezomib	26S proteosome complex	Monotherapy	II/20	None observed	15 weeks	[113]
Rosenberg *et al.*			Monotherapy	II/25	None observed	5.7	[114]

CALGB: Cancer and Leukemia Group B; EGFR: EGF receptor; G: Gemcitabine; GC: Gemcitabine plus cisplatin; HDAC: Histone deacetylase enzyme; MDACC: MD Anderson Cancer Center; MSKCC: Memorial Sloan–Kettering Cancer Center; M-VAC: Methotrexate, vinblastine, doxorubicin and cisplatin; NR: Not reported; OS: Overall survival; PDGFR: PDGF receptor; RR: Response rate; VEGFR: VEGF receptor.