# Novel Agents in Invasive Urothelial Cancer

Arjun V. Balar, MD, and Matthew I. Milowsky, MD

Dr. Balar is an Oncology Fellow at Memorial Sloan-Kettering Cancer Center, in New York, New York, and an Oncology Fellow at Weill Medical College of Cornell University in New York, New York. Dr. Milowsky is Co-Director of the Multidisciplinary Urologic Oncology Program at the UNC Lineberger Comprehensive Cancer Center, in Chapel Hill, North Carolina.

Address correspondence to: Matthew I. Milowsky, MD 3rd Floor Physicians Office Building 170 Manning Drive Chapel Hill, NC 27599 Phone: 919-843-7718 Fax: 919-966-6735 E-mail: matt milowsky@med.unc.edu

Abstract: Invasive urothelial cancer is an aggressive, biologically heterogeneous disease. Most patients present with non-muscle invasive bladder cancer involving the epithelium as exophytic tumors, in situ carcinoma, or minimally invasive disease involving the lamina propria. Such patients are typically managed with complete transurethral resection with or without intravesical therapy. Muscle invasive urothelial cancer, however, is biologically and clinically distinct. This subtype is characterized by mutations or deletions in tumor suppressor genes, such as TP53, Rb, and PTEN, leading to genomic instability and a more aggressive phenotype. Survival in advanced disease is poor with currently available treatment strategies. Technological advances in the ability to molecularly characterize human cancer have led to the identification of genetic alterations that may be therapeutically exploitable. Novel chemotherapies, such as antifolates and taxanes, have shown promise in urothelial cancer. Agents against novel molecular targets, such as the human epidermal receptor (HER) and vascular endothelial growth factor receptor (VEGFR), are being investigated. This review article focuses on the current status of novel chemotherapeutic and targeted agents as well as immunotherapy currently in clinical development in invasive urothelial cancer.

# Introduction

Urothelial cancer (UC) of the bladder is the second most common genitourinary malignancy in the United States, with 69,250 cases and 14,990 cancer-related deaths estimated for 2011.<sup>1</sup> Approximately 70% of cases will present as non-muscle invasive bladder cancer (NMIBC) involving the epithelium as exophytic tumors (pTa), as in situ carcinoma (pTis), or as minimally invasive disease involving the lamina propria (pT1). NMIBC is typically managed with complete transurethral resection (TUR) with or without intravesical therapy.<sup>2</sup> Muscle invasive UC (pT2 or greater), however, is biologically and clinically distinct. It can present de novo or as the consequence of progression of prior NMIBC, and more

#### Keywords

Urothelial cancer, transitional cell cancer, bladder cancer, targeted therapy, novel therapy

than 50% of patients will progress to metastatic disease despite curative therapy. Low-grade noninvasive papillary tumors are characterized by mutations in HRAS and fibroblast growth factor receptor 3 (FGFR3), implicating an early role for receptor tyrosine kinase-Ras activation in carcinogenesis. Muscle invasive UC is characterized by mutations or deletions in tumor suppressor genes, such as TP53, Rb, and PTEN, leading to genomic instability and a more aggressive phenotype.3 Muscle invasive UC of the bladder is managed with radical cystectomy with bilateral pelvic lymph node dissection for curative intent; however, recurrence-free survival is only 68% and 66% at 5 and 10 years, respectively.<sup>4</sup> Although neoadjuvant cisplatin-based chemotherapy prior to radical cystectomy has been demonstrated to reduce the risk of recurrence and improve survival<sup>5-7</sup> in 2 large randomized trials and a meta-analysis of 11 randomized trials, it remains relatively underutilized.8

Advanced UC is a devastating disease. Outcomes with even the most active chemotherapeutic regimens are unacceptably poor, with an average response rate (RR) of 50% and a median overall survival (OS) of 13–15 months with cisplatin-based therapies.<sup>9</sup> Unfortunately, despite several decades of trials investigating a number of novel chemotherapeutic agents and combinations, including 3 drug regimens,<sup>10</sup> cisplatin-based chemotherapy as a whole remains the only therapy shown to improve survival in any UC disease state.

More recently, technological advancements in the ability to molecularly profile cancer have led to the identification and targeting of driver genetic alterations involved in tumorigenesis and tumor progression. For example, the identification of mutations in the epidermal growth factor receptor (EGFR) gene and translocations resulting in the EML4-ALK fusion oncogene in patients with non-small cell lung cancer (NSCLC) have led to the development of agents that effectively target these genetic defects. Also, recent advancements in the ability to harness the host immune system as an anticancer therapy have also changed the landscape of cancer treatment. A similar effort in UC is under way, and our evolving understanding of UC biology will hopefully lead to the identification of therapeutic targets and the ability to select patients appropriate for targeted therapies. This article will focus on the novel cytotoxic agents (Table 1), as well as the novel targeted agents and immunotherapy (Table 2), currently in development for the treatment of invasive urothelial cancer.

#### **Novel Chemotherapeutic Agents**

## Antifolates

There are several antifolates under investigation in UC that have shown promising results in other cancers.

Pemetrexed (Alimta, Lilly Co) and pralatrexate are in phase II clinical trials.

Pemetrexed is an intravenous multi-Pemetrexed targeted antifolate analog approved by the US Food and Drug Administration for the treatment of NSCLC and malignant mesothelioma. It has demonstrated activity in the second-line treatment of advanced UC. An initial study in 22 untreated patients with advanced bladder cancer showed a promising overall RR of 29% (95% confidence interval [CI], 14-48%).11 However, 2 treatment-related deaths occurred in the initial cohort treated at 600 mg/m<sup>2</sup> every 3 weeks, prompting a dose reduction to 500 mg/m<sup>2</sup>. A subsequent phase II study led by the Hoosier Oncology Group of 47 pretreated patients receiving 500 mg/m<sup>2</sup> every 3 weeks with vitamin  $B_{12}$  and folic acid supplementation showed the regimen was well tolerated and had a 28% overall response rate (ORR), a median time to progression (TTP) of 2.9 months (95% CI, 1.7-4.6 months), and a median OS of 9.6 months (95% CI, 5.1-13.8).12 A smaller study of 13 patients with relapsed disease who received pemetrexed 500 mg/ m<sup>2</sup> every 3 weeks showed an objective response in only 1 patient.13 Two phase II studies evaluated pemetrexed in combination with gemcitabine as first-line treatment in advanced UC and found no added response or survival benefit with the addition of pemetrexed over outcomes expected with gemcitabine alone.14,15 Thus, pemetrexed monotherapy remains an acceptable, well-tolerated option in the second-line treatment of advanced UC.

**Pralatrexate** Pralatrexate is a novel antifolate that is FDA-approved for the treatment of relapsed or refractory peripheral T-cell lymphoma. Although its exact mechanism of action is unknown, preclinical studies suggest it selectively enters and accumulates in cells that express the reduced folate carrier (RFC)-1 cell surface receptor, which is commonly overexpressed on cancer cells. A recent phase II study of pralatrexate in relapsed or refractory metastatic UC after 1 prior platinum-based regimen demonstrated 1 partial response (PR) and 4 unconfirmed PRs in 30 enrolled patients. Median progression-free survival (PFS) and OS were 4.0 months and 9.3 months, respectively.<sup>16</sup>

# Taxanes

**Tesetaxel** Tesetaxel (Genta Inc) is an oral semisynthetic taxane derivative that has been evaluated in early phase clinical trials in metastatic 5-flourouracil-refractory gastric cancer,<sup>17</sup> refractory colon and gastroesophageal junction cancer,<sup>18,19</sup> and platinum-refractory NSCLC<sup>20</sup> with encouraging activity. A multicenter phase II study in metastatic UC after failure of 1 prior regimen is currently enrolling patients (NCT01215877).<sup>21</sup>

Agent	Target/Class	Study Phase/Setting	Regimen	N	RR	Median PFS or TTP (months)	Median OS (months)
Pemetrexed	Antifolate	II/First-line <sup>15</sup>	With gemcitabine	64 (47 E)	28%	3.1 (PFS)	8.1
		II/First-line <sup>14</sup>	With gemcitabine	46	31.8%	5.8 (TTP)	13.4
		II/First-line <sup>11</sup>	Monotherapy (600 mg/m <sup>2</sup> )	22	29%	NR	NR
		II/Second-line <sup>12</sup>	Monotherapy (500 mg/m <sup>2</sup> )	47	27.7%	2.9 (TTP)	9.6
		II/Second-line <sup>13</sup>	Monotherapy (500 mg/m <sup>2</sup> )	13	8%	NR	NR
Pralatrexate	Antifolate	II/Second-line <sup>127</sup>	Monotherapy	30	3%	4.0 (PFS)	9.3
Tesetaxel	Taxane	II/Second-line <sup>21</sup>	Monotherapy	33	Ongoing		
Nab-Paclitaxel	Taxane	I/II/NMIBC <sup>25</sup>	Intravesical NP	47	Ongoing		
		II/Neoadjuvant <sup>23</sup>	GCa + NP	22 of 54 planned	27% (pCR)	NR	NR
		II/Second-line <sup>24</sup>	Monotherapy	48 (47 E)	32%	6.0 (PFS)	10.8
		III/Second-line	Vs weekly paclitaxel	440			
Eribulin	Microtubule Polymerization	II/First-line <sup>28</sup>	Monotherapy	40 (37 E)	38%	3.9 (PFS)	9.4
		II/First-line <sup>31</sup>	GC ± eribulin	95 planned	Suspended		
Amrubicin	Anthracycline	II/Second-line <sup>34</sup>	Monotherapy	35	Ongoing		

Table 1. Novel Cytotoxic Agents

C=cisplatin; Ca=carboplatin; E=evaluable; G=gemcitabine; L=larotaxel; NCIC=National Cancer Institute of Canada; NMIBC=non-muscle–invasive bladder cancer; NP=nab-paclitaxel; NR=not reported; OS=overall survival; pCR=pathologic complete response rate; PFS=progression-free survival; RR=response rate; TTP=time to progression.

Nab-Paclitaxel Nanoparticle albumin-bound (nab)paclitaxel (Abraxane, Celgene Corporation) was developed to enhance the cellular penetration of paclitaxel through the recruitment of gp60-mediated transcytosis and to reduce toxicity by avoiding infusion reactions associated with the use of solvents such as polyethoxylated castor oil (Cremophor, BASF Corp).<sup>22</sup> Studies in metastatic breast cancer led to its approval by the FDA for this indication. Interim results of a phase II study of neoadjuvant gemcitabine and carboplatin plus nab-paclitaxel in locally advanced bladder cancer demonstrated a pathologic complete response rate of 27% and eradication of muscle-invasive disease in 54% of 22 evaluable patients.<sup>23</sup> However, this regimen was associated with significant hematologic toxicity.23 A recently completed phase II study of single-agent nab-paclitaxel in cisplatin-refractory metastatic UC demonstrated a response rate of 32% in 47 evaluable patients, the highest response rate ever reported for any agent in the second-line setting.<sup>24</sup> A phase I/II trial of intravesical nab-paclitaxel in patients with recurrent

superficial bladder cancer is currently enrolling patients (NCT00583349).<sup>25</sup> The National Cancer Institute of Canada (NCIC) is also planning an international phase III trial of nab-paclitaxel versus paclitaxel administered every 3 weeks as second-line therapy in advanced UC and is expected to begin enrollment in early 2013.

**Eribulin** Eribulin (Halaven, Eisai Co) is a synthetic analog of the naturally occurring compound halichondrin B, originally isolated from the marine sponge *Halichondria okadai*, that disrupts microtubule polymerization leading to the accumulation of nonfunctional tubulin aggregates.<sup>26</sup> Preclinical studies in cancer cell lines, including breast and colon cancer, demonstrated its antiproliferative effects,<sup>27</sup> and 2 phase I trials in patients with advanced solid tumors established the recommended phase II dose and schedule as well as an early signal regarding its toxicity profile.<sup>28,29</sup> Neutropenia and fatigue were the most common adverse events; notably, neurotoxicity was relatively uncommon. Eribulin is minimally (<10%) renally cleared

Table 2. Novel 7	argeted Agents
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Agent	Target	Phase/Study Setting	Regimen	N	RR	Median PFS or TTP (months)	Median OS (months)
Trastuzumab	HER2/Neu	II/First-line <sup>41</sup>	With PCaG	44	70%	9.3 (TTP)	14.1
		II/Second-line <sup>44</sup>	Monotherapy	NR	Awaiting results		
Cetuximab	EGFR	II/First-line47	GC ± cetuximab	81	Ongoing		
Gefitinib	EGFR	II/First-line <sup>52</sup>	With GC	54	43%	7.4 (TTP)	15.1
		II/First-line53	GC ± gefitinib	125	Awaiting results		
		II/First-line (maintenance) <sup>54</sup>	D vs D + gefitinib	90	Awaiting results		
		II/Second-line <sup>51</sup>	Monotherapy	31	3%	2 (PFS)	3
Erlotinib	EGFR	II/Neoadjuvant <sup>56</sup>	Monotherapy	20	35% ( <pt2)< td=""><td>NA</td><td>NA</td></pt2)<>	NA	NA
		II/Neoadjuvant <sup>57</sup>	Monotherapy	42	Ongoing		
Lapatinib	EGFR/ HER2	I/First-line <sup>61</sup>	With GC	25	Ongoing		
		II/III/First-line (maintenance) <sup>60</sup>	Lapatinib vs placebo	204	Ongoing		
		II/Platinum- refractory <sup>59</sup>	Monotherapy	59 (34 E)	1.7%	2.1 (TTP)	4.5
Bevacizumab	VEGF	II/Neoadjuvant <sup>82</sup>	With DD-MVAC	44 Bladder 16 Upper Tract	45% ( <pt2) Bladder 75% Upper tract</pt2) 	NR	75% 2 year OS
		II/First-line <sup>76</sup>	With GC	43	72	8.2 (PFS)	19.1
		II/First-line <sup>77</sup>	With GCa	51	42	6.5 (PFS)	13.9
		III/First-line <sup>81</sup>	GC ± Bevacizumab	500	Ongoing		
Aflibercept	VEGF	II/platinum refractory <sup>83</sup>	Monotherapy	22	5%	2.8 (PFS)	NR
Sorafenib	VEGFR -2, -3, PDGFR-β, Raf	II/Neoadjuvant <sup>87</sup>	With GC	45	Ongoing		
		II/First-line <sup>84</sup>	Monotherapy	17	0%	1.9 (TTP)	5.9
		II/Second-line <sup>85</sup>	Monotherapy	27	0%	2.2 (PFS)	6.8
Sunitinib	VEGFR-2, PDGFR-β	II/Neoadjuvant <sup>92</sup>	With GC	9 (8 E)	22%	NR	NR
		II/Neoadjuvant <sup>93</sup>	With GC	18 (15 E)	7%	10 (TTP)	NR
		II/First-line (maintenance) <sup>91</sup>	Sunitinib vs placebo	54	9% vs 7%	5 vs 2.7 (TTP)	NR
		II/Second-line <sup>85</sup>	Monotherapy	27	0%	2.2 (PFS)	6.8
		II/Second-line <sup>89</sup>	Monotherapy (Cohort A: daily continuous; Cohort B: daily 4 of 6 weeks)	77	5% ORR	2.4 vs 2.3 (PFS)	7.1 v 6.0
Pazopanib	VEGFR, PDGFR, c-kit	II/First-line	With gemcitabine	45	Opening soon		

(Table continues on following page)

Agent	Target	Phase/Study Setting	Regimen	N	RR	Median PFS or TTP (months)	Median OS (months)
		II/Refractory <sup>31</sup>	With paclitaxel	32	Ongoing		
		II/Second-line95	Monotherapy	19 (16 E)	0%	1.9 (PFS)	NR
		II/Cisplatin- refractory%	Monotherapy	41	17%	2 (TTP)	4
Vandetanib	VEGFR-2, EGFR, RET	II/First-line <sup>100</sup>	GCa ± vandetanib	122	Ongoing		
		II/Platinum- refractory <sup>99</sup>	D + vandetanib vs D + placebo	142	7% vs 11%	2.6 (PFS) vs 1.6 (PFS)	5.8 vs 7.0
Volasertib	PLK-1	II/Second-line <sup>108</sup>	Monotherapy	50	14%	6.1 weeks (PFS)	NR
TKI258	FGFR, VEGFR, PDGFR–β, Flt-3	II/Treatment- refractory <sup>129</sup>	Monotherapy	20 FGFR WT 20 FGFR Mut	Closed; results not yet reported		
BKM120	PI3K	II/Second-line <sup>116</sup>	Monotherapy	35	Ongoing		
Everolimus	mTOR	II/NMBIC <sup>119</sup>	With intravesical G	35	Ongoing		
		II/First-line <sup>120</sup>	± Paclitaxel	68	Ongoing		
		II/Treatment- refractory <sup>118</sup>	Monotherapy	45 (43 E)	2.7%	3.3 (PFS)	10.5
Ipilimumab	CTLA-4	II/First-line <sup>31</sup>	With GC	36	Ongoing		
DN24-02	HER2/Neu	II/Adjuvant	Versus observation	180	Ongoing		

Table 2.	(Continued)	Novel	Targeted	Agents
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C=cisplatin; Ca=carboplatin; CTLA-4=cytotoxic T lymphocyte antigen-4; D=docetaxel; DD=dose-dense; E=evaluable; EGFR=epidermal growth factor receptor; FGFR=fibroblast growth factor receptor; Flt-3=FMS-like tyrosine kinase 3; HER2=human epidermal growth factor receptor 2; G=gemcitabine; Mut=mutant; mTOR=mammalian target of rapamycin; MVAC=methotrexate, vinblastine, doxorubicin, cisplatin; NMIBC=non-muscle-invasive bladder cancer; NR=not reported; P=paclitaxel; PDGFR=platelet-derived growth factor receptor; PFS=progression-free survival; PI3K=phosphatidylinositol 3kinase; PLK=polo-like kinase; RR=response rate; TTP=time to progression; VEGFR=vascular endothelial growth factor receptor; WT=wild-type.

and, as such, a phase I study in advanced UC patients with renal impairment showed it was safe and well tolerated.<sup>30</sup> Preliminary results of a phase II study in untreated advanced UC patients were presented at the 2010 American Society of Clinical Oncology (ASCO) Annual Meeting. Among the 40 enrolled patients, 72.5% had received prior adjuvant or neoadjuvant chemotherapy. Patients received eribulin at 1.4 mg/m<sup>2</sup> on days 1 and 8 of a 21-day cycle. Analysis of outcomes in 37 evaluable patients demonstrated a response rate of 38%, median PFS of 3.9 months, and median OS of 9.4 months. The most common grade 3/4 adverse event was neutropenia, which occurred in 20 patients, with no episodes of febrile neutropenia. Sensory neuropathy occurred in 19 patients; it was largely limited to grade 1 or 2, with only 1 patient developing grade 3 toxicity. A randomized phase II study of gemcitabine

and cisplatin with or without eribulin in previously untreated patients with advanced bladder cancer was recently suspended, and trial results are not yet available (NCT01126749).<sup>31</sup>

**Amrubicin** Amrubicin (Celgene Corporation), a synthetic 9-amino anthracycline that inhibits topoisomerase II, is approved in Japan for the treatment of small cell lung cancer. Amrubicin is currently being investigated in phase II and III studies in several solid tumor malignancies in the United States. Toxicity appears to be limited largely to myelosuppression, namely neutropenia, with little nonhematologic toxicity, including cardiotoxicity.<sup>32,33</sup> This favorable toxicity profile has led to its investigation in an ongoing phase II study in cisplatinineligible patients with advanced UC in the second-line setting (NCT01331824).<sup>34</sup>

# **Novel Molecularly Targeted Agents**

As our knowledge of the molecular pathways that drive tumorigenesis and tumor progression grows, so too will the ability to selectively target these pathways for therapeutic benefit. This has been borne out in the identification and targeting of activating EGFR mutations and EML4-ALK gene rearrangements in subsets of NSCLC. A similar effort in UC is under way, and several novel targeted agents, as well as other agents previously proven beneficial in other solid tumor malignancies, are in development in UC.

#### Human Epidermal Receptor (HER) Family

The human epidermal receptor (HER) family of receptor tyrosine kinases consists of 4 members—HER1 (EGFR or Erb-B1), HER2 (Neu, Erb-B2), HER3 (Erb-B3), and HER4 (Erb-B4)—that in their inactivated state are typically present in monomers on the cell surface membrane. Receptor activation by its appropriate ligand induces homo- or heterodimerization leading to activation of a number of downstream signals, including the Ras-MAPK signal transduction cascade, which in turn drives cell growth, proliferation, and survival.

A number of preclinical studies have demonstrated the importance of targeting EGFR in urothelial cancer. Gefitinib (Iressa, AstraZeneca), a small molecule inhibitor of the intracellular tyrosine kinase of EGFR, when added to radiation resulted in a significant radiosensitization effect in a study in bladder cancer cell lines.<sup>35</sup> Cetuximab (Erbitux, Bristol-Myers Squibb/Lilly), a monoclonal antibody targeting EGFR, synergizes with photodynamic therapy as well as chemotherapy in mouse xenograft models of bladder cancer.<sup>36</sup> Vandetanib (Caprela, Astra-Zeneca), a dual small molecule inhibitor of EGFR and VEGFR, sensitizes bladder cancer cells to cisplatin in a dose- and sequence-dependent manner.<sup>37</sup> These findings and the successful targeting of the EGFR family in other solid tumor malignancies have led to a number of clinical trials in urothelial cancer.

**Trastuzumab** The humanized monoclonal antibody trastuzumab (Herceptin, Genentech) binds HER2/*Neu* (ERBB2) and has demonstrated clinical benefit in HER2-positive breast cancer in both the adjuvant and metastatic settings.<sup>38</sup> HER2/*Neu* is estimated to be overexpressed in approximately 50% of urothelial cancers and has been associated with higher grade, invasiveness, and increased metastatic potential.<sup>39,40</sup> The multicenter NCI-198 phase II trial evaluated the addition of trastuzumab to carboplatin, paclitaxel, and gemcitabine in patients with HER2-overexpressing metastatic urothelial cancer.<sup>41</sup> Patients were required to have proven HER2 overexpression determined by tissue immunohistochem-

istry (IHC) or fluorescence in-situ hybridization (FISH), or by elevated serum HER2/Neu extracellular domain (ECD) using quantitative enzyme-linked immunosorbent assay (ELISA) to be eligible for the study. Among the 109 patients screened, 57 met HER2 overexpression criteria and 44 were ultimately eligible for the study. The primary endpoint of the study was to assess the rate of cardiac toxicity. Secondary endpoints included RR, time to progression (TTP), and survival. Ten patients (22.7%) developed grade 1–3 cardiac toxicity. The overall RR was 70%, median TTP was 9.3 months, and median OS was 14.1 months. Although the response rate was relatively high, TTP and survival were similar to what has been observed for this regimen without the addition of trastuzumab,<sup>42</sup> suggesting a minimal additional benefit, if any. Post-hoc subgroup analysis demonstrated that patients with the highest HER2 overexpression (3-plus by IHC or FISH-positive) had the highest proportion responding. Furthermore, HER2-overexpressing patients were more likely to have 2 or more sites of metastatic disease (50.9% vs 30.8%; P=.051) and trended toward more lung and liver involvement. Interestingly, only 15 of the 57 patients who met initial HER2 overexpression criteria were found to be FISH-positive, suggesting that, unlike breast cancer, gene amplification is not a major mechanism of HER2 overexpression in UC, which is in agreement with a previous large retrospective study in archival paraffinembedded tissue.43 A Cancer and Leukemia Group B (CALGB) phase II study of trastuzumab monotherapy in previously treated advanced UC patients with HER2 overexpression (3+ by IHC or FISH-positive) recently closed because of poor accrual attributed to a lower than expected rate of HER2 amplification in the screening population (NCT00004856).44 Ultimately, a randomized trial is needed to determine the role of trastuzumab in HER2-overexpressing advanced UC.

**Cetuximab** Cetuximab is a recombinant humanized murine monoclonal antibody targeting EGFR that is FDA approved in the management of metastatic colon cancer and head and neck cancer.<sup>45,46</sup> A multicenter randomized phase II study of gemcitabine and cisplatin with or without cetuximab in patients with locally recurrent or unresectable UC is currently under way (NCT00645593).<sup>47</sup>

**Gefitinib** Gefitinib is an oral small-molecule inhibitor of the EGFR with demonstrated activity in EGFR-mutant NSCLC.<sup>48,49</sup> Preclinical studies of gefitinib in EGFR expressing bladder cancer cells demonstrated suppression of downstream effectors of EGFR signaling and decreased cellular proliferation.<sup>50</sup> A phase II study of second-line gefitinib monotherapy in advanced UC patients showed minimal activity, with only 1 response in 31 enrolled

patients and a median survival of 3 months.<sup>51</sup> The Cancer and Leukemia Group B (CALGB) 90102 phase II study evaluated gemcitabine and cisplatin plus gefitinib as firstline treatment in patients with metastatic UC and demonstrated a 51% response rate and 15-month median overall survival in 54 evaluable patients.<sup>52</sup> These outcomes are similar to what would be expected with gemcitabine and cisplatin alone,9 suggesting little added benefit with the addition of gefitinib. Two additional randomized phase II studies in advanced UC, one a study of gemcitabine and cisplatin with or without gefitinib as first-line therapy (NCT00246974)<sup>53</sup> and the other a study of gefitinib plus docetaxel versus docetaxel alone as maintenance therapy in patients who achieve a major response to first-line treatment (NCT00479089),<sup>54</sup> have completed accrual but have not yet reported results.

**Erlotinib** Erlotinib (Tarceva, Genentech) is an oral EGFR tyrosine kinase inhibitor (TKI) that has demonstrated in vitro activity in UC cell lines.<sup>55</sup> A phase II study of neoadjuvant erlotinib (150 mg orally daily for 4 weeks) prior to radical cystectomy in patients with clinical stage T2 bladder cancer demonstrated a 35% rate of pathologic downstaging (<pT2) and a 25% CR rate.<sup>56</sup> At a median follow-up of 25 months, 10 of the 20 patients treated remained alive without evidence of disease. Erlotinib is also being evaluated in an additional phase II study as neoadjuvant therapy in patients with muscle-invasive or recurrent superficial bladder cancer prior to radical cystectomy (NCT00749892).<sup>57</sup>

Lapatinib Lapatinib (Tykerb, GlaxoSmithKline) is an oral dual EGFR and HER2 TKI that has also demonstrated in vitro activity in UC cell lines with evidence of synergistic anticancer effects with the addition of cytotoxic chemotherapy.58 A multicenter phase II study investigated lapatinib in patients with platinumrefractory UC.59 Among the 59 patients enrolled, 34 were evaluable for the primary endpoint of RR. One PR was observed in the study, with an overall RR of 1.7% (95% CI, 0-9.1%) by intent-to-treat analysis. Stable disease was observed in 18 patients (31%; 95% CI, 19-44%). OS was 17.9 weeks and time to progression was 8.6 weeks. Of note, longer survival was significantly associated with EGFR or HER2 overexpression (P=.0001). 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 (NCT00949455).<sup>60</sup> Eligible patients are those who achieve at least stable disease after first-line chemotherapy and whose tumors overexpress HER2 or HER1 by IHC or FISH. The European Organization for Research and Treatment of Cancer (EORTC) is also

leading a phase I study of gemcitabine and cisplatin plus lapatinib in patients with HER2 or HER1 overexpressing previously untreated metastatic/locally advanced UC (NCT00623064).<sup>61</sup>

*Vascular Endothelial Growth Factor Receptor (VEGF)* More than 30 years ago, Chodak and colleagues first demonstrated proangiogenic substances in the urine of patients with UC.<sup>62</sup> Since then, a steady stream of preclinical and clinical evidence has supported targeting angiogenesis in the management of UC.<sup>63-71</sup> Targeting angiogenesis—specifically, the vascular endothelial growth factor (VEGF) receptor, a major mediator of angiogenesis—has shown benefit in other solid tumor malignancies, which further supported its investigation in UC.

Bevacizumab Bevacizumab (Avastin, Genentech), a recombinant humanized monoclonal antibody that binds all isoforms of human VEGF, has improved survival in advanced colorectal cancer, NSCLC, glioblastoma, and renal cell cancer.72-75 The Hoosier Oncology Group recently reported a phase II study of gemcitabine and cisplatin plus bevacizumab as first-line treatment in advanced UC that demonstrated a 72% RR and 19.1-month median OS, which compares favorably to prior studies of gemcitabine and cisplatin alone.<sup>76</sup> However, this regimen was associated with significant toxicity. Seven (39%) of the first 18 enrolled patients developed venous thromboembolic events (VTEs), prompting a protocol amended dose-reduction in gemcitabine from 1,250 mg/m<sup>2</sup> to 1,000 mg/m<sup>2</sup>. Interestingly, the rate of VTE in the dosereduced cohort was much lower (8%). Three treatmentrelated deaths occurred on study (from central nervous system hemorrhage, sudden cardiac death, and aortic dissection). Another phase II study in cisplatin-ineligible patients conducted at Memorial Sloan-Kettering Cancer Center reported a 42% RR and 13.9-month median OS.<sup>77</sup> The VTE rate in this study was 18%, which is similar to the 17% rate observed in a contemporary cohort of patients treated with gemcitabine and carboplatin alone.78 The findings in this phase II study as well as the study reported by the Hoosier Oncology Group add to the growing controversy regarding the contribution of bevacizumab in the development of VTE.79,80 The CALGB 90601 phase III study of gemcitabine and cisplatin with or without bevacizumab is currently under way to definitively determine bevacizumab's role in the treatment of advanced UC (NCT00942331).81

A phase II study of neoadjuvant dose-dense MVAC plus bevacizumab in invasive urothelial tract cancer conducted at MD Anderson Cancer Center was presented at the 2012 ASCO Genitourinary Cancers Symposium.<sup>82</sup> In patients with invasive bladder cancer, the study demonstrated a 45% rate of pathologic down-staging (<pT2) and 75% and 82% 2-year overall survival and disease-specific survival, respectively. Notably, this study included only patients with high-risk features (clinical T3 disease, lymphovascular invasion, hydronephrosis, and micropapillary histology), which may have led to an underestimation of the rate of pathologic down-staging. Therapy was well tolerated and, notably, less than 10% of patients developed VTE.

**Aflibercept** Aflibercept is a soluble recombinant receptor that binds all isoforms of human VEGF (VEGF-Trap). The California Cancer Consortium led a phase II study of aflibercept in platinum-pretreated patients with metastatic UC. The study enrolled 22 patients and demonstrated limited single-agent activity, with 1 PR and a median PFS of 2.8 months.<sup>83</sup>

**Sorafenib** Sorafenib (Nexavar, Bayer/Onyx) is a multitargeted, small-molecule TKI that inhibits Raf kinase, PDGF receptor (PDGFR)  $\beta$ , and VEGF receptors 2 and 3. A phase II study of sorafenib monotherapy as first-line treatment in advanced UC demonstrated no Response Evaluation Criteria for Solid Tumors (RECIST) responses in 17 evaluable patients and a 1.9-month median TTP and 5.9-month median survival.<sup>84</sup> Another phase II study led by ECOG in the second-line setting also demonstrated no objective responses in 27 evaluable patients.85 This study showed a 2.2-month median PFS and 6.8-month median survival.85 These studies suggest limited activity for sorafenib as a single agent in advanced UC. A randomized phase II study of gemcitabine and cisplatin with or without sorafenib in advanced UC showed no significant added toxicities with sorafenib,<sup>86</sup> but there was no added benefit either. Another combination study with gemcitabine and cisplatin as neoadjuvant treatment in muscle-invasive bladder cancer is ongoing (NCT01222676).87

Sunitinib Sunitinib (Sutent, Pfizer Co) is a first-generation, small-molecule TKI that also inhibits multiple targets, including the VEGF receptor-2. It is a standard of care in the first-line treatment of patients with metastatic renal cell cancer.<sup>88</sup> A phase II study conducted at Memorial Sloan-Kettering Cancer Center of second-line sunitinib in advanced UC enrolled 77 patients to treatment with 1 of 2 dosing schedules: 50 mg orally daily for 4 weeks of a 6-week cycle (Cohort A) and 37.5 mg orally daily continuously (Cohort B).<sup>89</sup> No significant differences in RR, PFS, or overall survival were observed between the 2 dosing schedules. In this study, low levels of hypoxia inducible factor (HIF)-1 $\alpha$  in pretreated tumor tissue and sunitinib-induced hypertension predicted for treatment response, suggesting that these factors may be potential predictive and pharmacodynamic biomarkers for response.<sup>90</sup> The significance of these findings is limited by the sample size. Clinical benefit from sunitinib (defined as PR or SD lasting longer than 3 months) was observed in 22 patients (29%), strengthening the argument that activation of the VEGFR pathway is important in UC progression. This hypothesis was tested in a randomized phase II study of maintenance sunitinib versus placebo in advanced UC patients who achieved at least stable disease after first-line chemotherapy. The study was closed early due to slow accrual, and the outcomes for 54 randomized patients were presented at the 2012 ASCO Genitourinary Cancers Symposium.<sup>91</sup> No difference was observed in the rate of progression at 6 months between sunitinib and placebo-treated patients, and serum VEGF and soluble VEGFR levels did not correlate with median TTP. Two phase II studies have evaluated neoadjuvant gemcitabine and cisplatin plus sunitinib prior to radical cystectomy in muscle-invasive bladder cancer. The first study conducted by the Hoosier Oncology Group closed early due to excessive toxicity in 9 enrolled patients.<sup>92</sup> The other trial was closed early by the study sponsor; treatment did not appear to improve the rate of complete pathologic response in 15 evaluable patients.93

**Pazopanib** Pazopanib (Votrient, GlaxoSmithKline) is an oral second-generation multi-targeted small molecule TKI of VEGF receptors 1, 2, and 3; PDGF receptors  $\alpha$  and  $\beta$ ; and c-kit (CD117).<sup>94</sup> It may be better tolerated than sunitinib.<sup>94</sup> A phase II study led by the Mayo Clinic of pazopanib in advanced UC patients who failed 1 prior therapy was closed early due to futility after no RECIST responses were observed in the first 16 evaluable patients.<sup>95</sup> The final results of a more recent phase II study of pazopanib in cisplatin-refractory advanced UC patients was presented at the 2012 American Association of Cancer Research Annual Meeting.96 A RR of 17% and a median TTP of 2 months was observed in 41 evaluable patients. Stabilization of disease was observed in 59%, and 49% of patients had either densitometric changes on computed tomography (CT) imaging consistent with "necrotic evolution" or decreased positron emission tomography (PET) avidity, which the authors claim is more consistent with-and perhaps the more appropriate measure of-the anti-tumor activity of pazopanib. A phase II study of pazopanib and weekly paclitaxel in refractory advanced UC led by Stanford University (NCT01108055)<sup>31</sup> is currently enrolling patients, and another phase II study of gemcitabine and pazopanib as first-line therapy in cisplatin-ineligible patients at Memorial Sloan-Kettering Cancer Center will be opening soon.

Vandetanib Vandetanib is an oral, small-molecule TKI targeting both the VEGF receptor 2 and the EGF receptor that initially demonstrated significant anti-tumor activity in mouse xenograft models of NSCLC.<sup>97</sup> A phase III study of docetaxel plus vandetanib or placebo in advanced NSCLC demonstrated a significant improvement in PFS in the combination arm over docetaxel and placebo.98 Prompted by these results, a double-blind phase II study in advanced UC evaluated docetaxel and vandetanib versus docetaxel and placebo after progression on prior platinum-based therapy.<sup>99</sup> The primary objective of the study was to determine if docetaxel plus vandetanib provided a 60% improvement in median PFS over docetaxel plus placebo. In 142 randomized patients, there was no significant difference in RR (7% for docetaxel plus vandetanib vs 11% for docetaxel plus placebo), median PFS (2.56 months vs 1.58 months), or median OS (5.85 months vs 7.03 months). A randomized phase II study of gemcitabine and carboplatin with or without vandetanib in cisplatin-ineligible patients with advanced UC is currently ongoing (NCT01191892).<sup>100</sup>

#### Fibroblast Growth Factor Receptor (FGFR)-3

Activating mutations in the gene for fibroblast growth factor receptor-3 (FGFR3), a receptor tyrosine kinase (RTK), have been reported in approximately 70% of low-grade NMIBC and 15-20% of muscle-invasive bladder cancers.<sup>101</sup> Constitutive activation of FGFR3 and subsequent activation of downstream mitogenic signals have been implicated in tumor cell growth, proliferation, and survival.<sup>102</sup> The presence of activating mutations in invasive disease provides rationale for targeting FGFR3, and a number of small-molecule inhibitors and monoclonal antibodies are currently in clinical development.<sup>102</sup> A recently described morphologic pattern in high-grade UC, characterized by a bulky, exophytic component with branching papillary architecture as well as irregular nuclei with a koilocytoid appearance, appears to be sensitive for somatic mutations in FGFR3, and may serve as a rapid screening tool for patient selection in trials of FGFR3 targeted therapy.<sup>103</sup>

**TKI258** TKI258 (Divotinib, Novartis) is an oral multitargeted TKI with activity against FGFR, VEGFR, PDGFR- $\beta$ , and Flt-3 that has been shown to induce apoptosis and inhibit angiogenesis in preclinical cancer models.<sup>104,105</sup> A multicenter, phase II study evaluated the safety and efficacy of TKI258 in patients with refractory advanced UC. Patients were stratified by tumor FGFR3 mutation status. The study had a planned enrollment of 20 patients to each arm with a primary endpoint of response rate. The trial recently closed to accrual, and final results have not yet been reported.

#### Polo-like Kinase

Polo-like kinases (Plk) are important regulators of the cell cycle and are involved in the early formation of the mitotic spindle, leading to mitotic entry and, ultimately, cell division. Polo-like kinase-1 (Plk1) is the best characterized Plk.<sup>106</sup> Its overexpression has been implicated in various solid tumors, including NSCLC and colon cancer, and it may be associated with a poor prognosis.<sup>106</sup> BI6727 (Volasertib, Boehringer Ingelheim) is an intravenously (IV) administered selective inhibitor of all Plks. A first-in-man phase I dose-escalation study in patients with advanced solid tumors established 300 mg administered intravenously every 21 days as the dose for further investigation in phase II studies.<sup>107</sup> Three confirmed PRs were observed in 67 enrolled patients, 1 of whom had advanced UC. Preliminary data presented at the 2011 ASCO Annual Meeting of a phase II study of second-line volasertib in advanced UC demonstrated a RECIST response in 7 (14%) and stable disease in 13 (26%) of 50 evaluable patients.<sup>108</sup> Therapy was well tolerated, and the most common grade 3 or 4 toxicity was hematologic (26% neutropenia and 16% thrombocytopenia).

## PI3K/Akt/mTOR Pathway

RTK activation leads to intracellular signaling through multiple downstream mitogenic pathways. Activation of RTK-bound p85 subunit of phosphatidylinositol 3-kinase (PI3K) leads to phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) and recruits protein kinase B (Akt) to the plasma membrane. Subsequent phosphorylation of Akt by phosphoinositide-dependent kinase-1 (PDK1) leads to multiple downstream consequences, including suppression of tuberous sclerosis complex-2 (TSC2) activity, which normally functions to suppress Ras homolog enriched in brain (Rheb) protein. Loss of TSC2 suppression of Rheb leads to activation of mammalian target of rapamycin (mTOR). Unchecked phosphorylation of Akt as well as activation of mTOR has been implicated in uncontrolled cellular growth and proliferation in human cancer.<sup>109</sup> Inhibition of mTOR signaling has proven beneficial in advanced RCC, breast cancer, and pancreatic neuroectodermal tumors.110-112 Molecular alterations in the genes encoding key proteins of this pathway have been reported in up to 27% of highgrade invasive urothelial cancers and can serve as targets for therapeutic inhibition.<sup>113</sup> Several investigational agents, such as BKM120 (pan-class PI3K inhibitor) and BEZ235 (dual PI3K/mTOR inhibitor), and established agents, such as everolimus (mTOR inhibitor), are currently in clinical development in a variety of advanced solid tumors, including UC.

**BKM120** BKM120 (Novartis) is an oral pan-class PI3K inhibitor with activity against myeloma and NSCLC cell lines.<sup>114,115</sup> It is currently being investigated in a number of disease-specific phase II studies. A phase II study at Memorial Sloan-Kettering Cancer Center of second-line monotherapy in advanced UC is currently under way (NCT01551030).<sup>116</sup>

**Everolimus** Everolimus (Afinitor, Novartis) is a standard of care in the second-line treatment of advanced clear cell RCC after failure of VEGFR TKI therapy.<sup>117</sup> A single-center phase II study in pretreated patients with advanced UC enrolled 45 patients to treatment with everolimus 10 mg orally daily continuously until progression. The primary endpoint of the study was PFS. In 43 evaluable patients, the median PFS and OS were 3.3 months and 10.5 months, respectively.<sup>118</sup> Two patients with lymph node–only disease achieved a PR. Everolimus is also being investigated in combination with intravesical gemcitabine in a phase II study of patients with recurrent high-grade pT1 UC of the bladder (NCT01259063)<sup>119</sup> and in a phase II randomized study with or without paclitaxel in cisplatin-ineligible patients with advanced UC (NCT01215136).<sup>120</sup>

#### Immunotherapy

Early on, bladder cancer was identified as a disease amenable to immunotherapy. Bacillus Calmette-Guerin (BCG) is a live attenuated form of Mycobacterium bovis that, when instilled intravesically, induces a number of localized host immune responses<sup>121</sup> believed to be responsible for the improved risk of recurrence, reduced need for cystectomy, and improved overall survival in patients with high-risk NMIBC.<sup>122</sup> Although early in development, immunotherapy in muscle-invasive or advanced disease may represent a promising new approach to treatment.

Cytotoxic T lymphocyte-associated antigen (CTLA-4) is an immune checkpoint that regulates T-cell activation.<sup>123</sup> Its inhibition by ipilimumab (a monoclonal antibody targeting CTLA-4) has improved overall survival in advanced melanoma.<sup>124</sup> In an effort to better understand the tissue-based immunologic markers of the anti-tumor activity of CTLA-4 blockade, ipilimumab was studied as neoadjuvant therapy in 12 patients with stage T1-T2 bladder cancer prior to radical cystectomy.<sup>125</sup> All 12 patients demonstrated increased infiltration of CD4+ICOShi T cells in tumor tissue as well as peripheral circulation, which has been correlated to increased survival in a small retrospective cohort of patients with advanced melanoma, potentially serving as a basis for further study in UC. The Hoosier Oncology Group is currently leading a phase II study of gemcitabine and cisplatin plus ipilimumab in patients with advanced UC (NCT01524991).<sup>31</sup>

Similarly, the improved overall survival observed with sipuleucel-T (Provenge, Dendreon) in advanced prostate cancer<sup>126</sup> has led to the investigation of autologous cellular immunotherapy in bladder cancer. DN24-02 (Dendreon), an autologous cellular immunotherapy product targeting HER2/Neu, is currently being investigated as adjuvant treatment versus observation in a randomized phase II study in patients with high-risk HER2 expressing bladder cancer (NCT01353222).<sup>31</sup>

# Conclusion

High-grade invasive UC is an aggressive, biologically heterogeneous disease. Outcomes with currently available standard cytotoxic therapies are unacceptably poor. Novel treatment strategies are desperately needed. Recent technological advances in molecular profiling have led to the identification of driver genetic alterations in cancer that can then be therapeutically exploited for clinical benefit. A similar effort in UC will ultimately help identify driver alterations that can then be used as potential predictive biomarkers to prospectively select patients for biologically rational targeted therapy.

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