

New therapeutic targets in the management of urothelial carcinoma of the bladder

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Abstract: Urothelial carcinoma of the bladder, despite the myriad of treatment approaches and our progressively increasing knowledge into its disease processes, remains one of the most clinically challenging problems in modern urological clinical practice. New therapies target biomolecular pathways and cellular mediators responsible for regulating cell growth and metabolism, both of which are frequently overexpressed in malignant urothelial cells, with the intent of inducing cell death by limiting cellular metabolism and growth, creating an immune response, or selectively delivering or activating a cytotoxic agent. These new and novel therapies may offer a potential for reduced toxicity and an encouraging hope for better treatment outcomes, particularly for a disease often refractory or not amenable to the current therapeutic approaches.

Keywords: targeted therapy, intravesical agents, systemic therapies

Introduction to the management issues – current and emerging treatment options

Urothelial carcinoma (UC) of the bladder (UCB) is the ninth most common cancer worldwide, affecting approximately four times more men than women, with a global mortality rate of 4 per 100,000 amongst men, compared with 1.1 per 100,000 amongst women.¹ Approximately 70% of newly diagnosed patients have non-muscle-invasive tumors. Recurrence of non-muscle-invasive UCB is common despite treatment; low and high grade tumors have recurrence rates of 50%–70% and more than 80%, respectively.^{2–4} Stage progression occurs in 15%–20% of non-muscle-invasive cases.^{3,4} Muscle-invasive UCB entails a foreboding outlook in terms of progression and survival. Patients with muscle-invasive UCB have a significantly high risk for progression to regional and systemic disease, and regardless of treatment, the 5-year overall survival (OS) rate of patients with muscle-invasive UCB is around 50%.^{5–7}

The task of treating UCB is further constrained by the fact that it is primarily a disease of the elderly. In the United States, the median age of diagnosis is 73 years, which is the highest age of diagnosis compared with all other cancers.⁸ Age-related factors such as impaired renal function, higher frequency of comorbidities, and lower performance status can significantly affect treatment choices and the ability to treat in a timely fashion. The impact of such factors can be seen more clearly in patients with muscle-invasive, advanced, and metastatic UCB. Age-related factors render an estimated 30%–50% of patients unfit for cisplatin, and around 23%–50% of patients over the age of 65 receive either nonaggressive treatment or no treatment for muscle-invasive UCB.^{9–11}



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Through improved standards of current care, there has been a modest reduction in the number of deaths due to UCB. However, given the stark outcomes of UCB, we continually search for other potential avenues of treatment to bolster the current standards of care and offer an efficacious alternative for those patients deemed unfit for standard treatment. Additionally, there is an unmet need in those patients with metastatic disease who fail first-line systemic therapy. Ongoing improvements in our understanding of the biological pathophysiology of UCB have made targeted therapy an intensely investigated field for all stages of UCB given its impact on other cancers.

Overview of current treatment options

Non-muscle-invasive bladder tumors

Once a tumor or suspicious lesion has been identified, a transurethral resection of bladder tumor (TURBT) is the

standard first-line treatment. TURBT serves a dual purpose; it is diagnostic for staging and therapeutic for noninvasive disease, except for carcinoma in situ (CIS). Most guidelines recommend a repeat TURBT if high grade disease is detected, since the risk of understaging is substantial and residual tumor can be found up to 50% of the time, particularly with T1 disease where the muscularis propria is not present in the surgical specimen^{12–16} (Table 1).

The next step in management involves the usage of intravesical therapy as either a prophylactic or adjuvant treatment. The intravesical agents are generally classified into 2 types: immunotherapy and chemotherapy.

The most common and widely used intravesical immunotherapy is bacillus Calmette-Guérin (BCG). It is considered to be the first-line treatment for CIS and the preferred adjuvant therapy for high grade and T1 disease. Tumor recurrence rates have been demonstrated to be reduced by up to 30%, and

Table 1 Guideline treatment recommendations for non-muscle-invasive bladder cancer^{14–16}

	NCCN	AUA	EAU
Low risk treatment	Low grade Ta tumor TURBT. A single, postoperative instillation of intravesical chemotherapy should be considered. If bulky or multifocal, induction chemotherapy should be considered	Low grade Ta tumor TURBT. A single postoperative instillation of intravesical chemotherapy is recommended	EORTC-GU score 0 for both recurrence and progression TURBT. A single postoperative instillation of intravesical chemotherapy should be given
Intermediate risk treatment	High grade Ta tumor TURBT. Repeat resection if no muscularis propria is found in the initial TURBT specimen. Intravesical therapy, preferably BCG, is optional	Bulky or multifocal low grade Ta tumor TURBT. Induction course of either BCG or MMC is recommended. Maintenance therapy is optional	Recurrence score 1–17, progression score 2–6 TURBT. A single postoperative instillation of intravesical chemotherapy should be given, followed by induction BCG with maintenance for 1 year or further intravesical chemotherapy for 6–12 months
High risk treatment	All grades T1 and Tis TURBT and repeat resection. BCG therapy 3–4 weeks after repeat resection is highly recommended. Patients with pTis should receive an induction course of BCG and at least 1 year of maintenance BCG	High grade Ta, all grades T1 and Tis TURBT. A repeat resection should be done if no muscularis propria is found in the initial TURBT specimen. BCG induction with maintenance is recommended	Progression score 7–23 TURBT and repeat resection 2–6 weeks from the initial TURBT. BCG induction and maintenance for at least 1 year. Patients with pTis should receive BCG induction and at least 1 year of maintenance
Indications for cystectomy	High grade T1 with residual disease or any recurrent disease refractory to intravesical therapy	Recurrent high risk disease and BCG refractory disease	Multifocal recurrent high grade tumors, high grade tumors with concomitant CIS, BCG refractory disease

Abbreviations: NCCN, National Comprehensive Cancer Network; AUA, American Urological Association; EAU, European Association of Urology; TURBT, transurethral resection of bladder tumor; EORTC-GU, European Organization for Research and Treatment of Cancer Genito-Urinary; BCG, bacillus Calmette-Guérin; MMC, mitomycin-c; CIS, carcinoma in situ.

a complete response (CR) has been achieved in 55%–60% of cases with small papillary tumors and up to 75% of CIS cases.¹⁷ Clinical trials conducted by the Southwest Oncology Group found that with additional maintenance therapy, the recurrence-free survival was noted to be twice as long compared with patients who had only received induction BCG therapy.¹⁸ BCG with maintenance therapy also has some impact on disease progression, with a 27% reduction in the odds of progression.¹⁹

The limitations of BCG are largely due to toxicity, with patients experiencing a high frequency of side effects that are mostly local in nature. In trials conducted by the European Organization for Research and Treatment of Cancer (EORTC), they found that 75% of patients experienced local side effects, most notably cystitis, and that toxicity caused treatment discontinuation in 20% and delay in 18% of patients.²⁰ Given its origins as a mycobacterium, BCG has the potential to cause a life-threatening sepsis, making it absolutely contraindicated in immunocompromised patients.²¹ Also, BCG treatment eventually fails in 30%–40% of patients, with another 30%–40% of patients who had a favorable initial response developing a recurrence within 5 years.²² These patients may be given a second induction course, with CR rates of 10%–20%.²³ Consecutive courses of BCG beyond a second induction were found to have a negative impact on outcomes by being associated with a 20%–50% risk of tumor progression.²⁴ Despite the greater morbidity associated with BCG, the higher efficacy reported in numerous trials have made BCG the preferred intravesical therapy for superficial UCB (particularly high grade) among consensus panels of leading experts.

Interferon- α is another intravesical immunotherapy that has found some limited usage as an adjuvant after TURBT. Its usage is primarily hampered by high cost and inferior effectiveness as monotherapy compared with BCG and intravesical chemotherapy. However, interferon- α does have a favorable toxicity profile, rarely causing cystitis and occasionally resulting in brief flu-like symptoms. It has been investigated in a salvage setting for BCG failure in combination with low dose BCG, where a 2-year disease-free rate of 45%–53% was observed.^{25,26}

Among the intravesical chemotherapy agents, mitomycin-c (MMC) is the most widely utilized. MMC reduces tumor recurrence rates by 15%, and demonstrates a 50% CR rate in patients with CIS.^{27,28} Despite the advantages of BCG in terms of treatment efficacy, MMC is a highly suitable option for low risk disease, due to its better toxicity profile compared with BCG. MMC can also be given immediately

postoperatively, since there is no risk of sepsis from the drug itself. A meta-analysis conducted by the EORTC found that a single, postoperative dose of intravesical chemotherapy reduces the rate of recurrence by 11.7%.²⁹ However, based on the meta-analysis, the patients who were most likely to benefit from a single, postoperative instillation had small, solitary, low grade tumors.

Other chemotherapeutic agents which have been used for intravesical treatment include doxorubicin, epirubicin, valrubicin, and thiotepa. Valrubicin has a particular niche because it is approved by the Food and Drug Administration (FDA) in the United States for patients who have BCG refractory CIS disease and are not suitable candidates for extirpative surgery. A clinical trial conducted by Steinberg et al demonstrated a 21% CR rate, with a median follow-up of 30 months for patients with BCG refractory CIS of the bladder.³⁰

Muscle-invasive bladder tumors

Radical cystectomy (RC) with extended pelvic lymph node dissection (PLND) and urinary diversion is the gold standard treatment for muscle-invasive UCB. This can be performed either open, laparoscopically, or more recently, robotic assisted. Surgical, intermediate-term oncologic, and functional outcomes for robotic-assisted RC are promising, but long-term outcomes are awaited. The PLND is important for staging and curative purposes. The extent of the lymph node dissection is controversial, and at least two randomized controlled trials are currently addressing this important question.^{31,32} Male urethrectomy (if the urethra is involved by tumor) is either performed at the time of or staged following RC depending on surgeon's preference.³³ Either a continent or noncontinent urinary diversion is created, with the decision being made based on several factors including margin status, previous or expected pelvic radiation, the patient's ability to perform clean intermittent catheterizations, and surgeon/patient preference. An orthotopic neobladder is commonly used as a bladder substitute, but if contraindicated, catheterizable pouches can be created with the continence mechanism based on an anatomic sphincteric valve (the ileocecal valve is a classic example). An ileal conduit is the most commonly used noncontinent diversion.

Other treatment options for muscle-invasive tumors include partial cystectomy in selected cases (a single small tumor situated away from the bladder base and ureteral orifices) and bladder sparing protocols which usually entail a multimodal approach of concurrent radiotherapy and sensitizing systemic chemotherapy following a maximal TURBT. Bladder preservation is an acceptable alternative for patients

who are unfit for surgery due to extensive comorbidities or who refuse RC despite counseling. The 5-year OS rates for patients treated with bladder sparing protocols have been reported to be 48%–64%.^{7,34–36}

Neoadjuvant systemic chemotherapy is recommended for patients with either muscle-invasive UCB exhibiting adverse clinicopathologic features or locally advanced UCB by both the National Comprehensive Cancer Network (NCCN) and the European Association of Urology (EAU), with the premise of treating micrometastatic disease prior to surgery^{14,37} (Table 2). Several randomized trials and meta-analyses show a 5% OS benefit in patient cohorts who receive neoadjuvant chemotherapy.^{5,38,39} The equally efficacious platinum-based regimens of methotrexate, vinblastine, adriamycin, and cisplatin (MVAC) and gemcitabine and cisplatin (GC) are considered to be first-line systemic chemotherapy in UCB. Although MVAC is the historical standard in treating metastatic UCB, GC is more preferred given its favorable toxicity profile.⁴⁰ For patients with impaired renal function, both the EAU and NCCN guidelines find that substituting carboplatin for cisplatin is acceptable. However, the NCCN recommends that those with borderline or minimal renal dysfunction should be considered for a split dose of cisplatin rather than carboplatin substitution¹⁴ (Table 2).

In patients with pT3-4 and/or node-positive disease, adjuvant systemic chemotherapy has been shown to improve survival in a few small randomized trials, with the advanced

bladder cancer meta-analysis collaboration suggesting a 9% improvement in absolute survival at 3 years.⁴¹ However, these trials have been criticized for their small numbers and irregular treatment protocols. Given the paucity of larger studies on adjuvant chemotherapy, the EAU does not implicitly recommend its use after RC.³⁷ On the other hand, the NCCN believes it should be considered where the patient was not given chemotherapy neoadjuvantly.

Metastatic UCB

Unfortunately, surgery alone has no curative role in metastatic UCB, where platinum-based systemic chemotherapy is the mainstay of treatment for these patients. The NCCN additionally recommends that clinically abnormal lymph nodes should be biopsied if technically feasible.¹⁴ Also, the NCCN finds that offering radiotherapy with chemotherapy is acceptable except in cases of disseminated metastatic disease. The EAU makes two additional recommendations pertaining to systemic chemotherapy that are not approved for clinical use in North America: dose-dense (DD) MVAC in a first-line setting and vinflunine as a preferred second-line therapy. DD-MVAC is however currently being investigated in clinical trials across multiple NCCN-affiliated institutions. The EAU additionally recommends that either standard or DD-MVAC should be accompanied with granulocyte colony stimulating factor (GCSF), given the increased incidence of major neutropenic toxicities

Table 2 Guideline recommendations for systemic chemotherapy for urothelial carcinoma of the bladder^{14,37}

	NCCN	EAU
Indications for neoadjuvant chemotherapy	cT2 or cT3 (strongly consider) disease	cT2 or cT3 disease
Indications for adjuvant chemotherapy	Consider for pT3-4 disease or positive nodes, if no neoadjuvant therapy was given	Not indicated after cystectomy. Should be given in a clinical trial setting. Should not be given to patients who cannot tolerate cisplatin
Indications for up-front chemotherapy	Clinically positive nodes, cT4, and metastatic disease	Clinically positive nodes, cT4, and metastatic disease
First-line regimens	GC (preferred), MVAC	GC, MVAC (preferably with GCSF), or DD-MVAC with GCSF
Patients unfit for cisplatin	If renal function is borderline or minimal dysfunction, consider a split dose administration (ie, 35 mg/m ² on days 1 and 2 or days 1 and 8). Otherwise consider carboplatin and taxane-based regimens as alternative first-line regimens or single-agent therapy	Carboplatin-containing combination chemotherapy, preferably with gemcitabine-carboplatin as first-line treatment
Second-line agents	No standard, but patients should seek participation of a clinical trial at this juncture	Vinflunine (approved in Europe) or participation in a clinical trial. Zoledronic acid or denosumab are recommended for bone metastasis

Abbreviations: NCCN, National Comprehensive Cancer Network; EAU, European Association of Urology; GC, gemcitabine + cisplatin; MVAC, methotrexate + vinblastine + doxorubicin (Adriamycin) + cisplatin; GCSF, granulocyte colony stimulating factor; DD, dose-dense.

associated with the regimen.⁴⁰ Although GCSF is commonly administered in the clinical setting, the NCCN does not explicitly include its use as a part of the guidelines.³⁷

New and emerging candidate agents

Researchers have become more interested in developing novel targeted therapies and are currently exploring with agents that have proven useful in different organ sites (Table 3).

Agents for muscle-invasive and advanced UCB

Angiogenesis inhibitors

Vascular endothelial growth factor (VEGF) receptor inhibitors act by blocking angiogenesis, resulting in tumor hypoxia with subsequent ischemia and cell death. Sunitinib is a multiple tyrosine kinase inhibitor (TKI) which has been utilized for metastatic renal cell carcinoma (RCC), with a reported survival benefit.⁴² Bellmunt et al conducted a small Phase II study which included 38 patients with metastatic UCB who were unable to receive cisplatin and were treated with sunitinib 50 mg daily in a 4 week on and 2 week off regimen. The reported median progression-free survival (PFS) and OS was 4.3 and 8.1 months, respectively. A partial response (PR) was noted in 8% of patients, while 50% had stable disease (SD) for more than 3 months.⁴³ A Phase II study examined sunitinib as a second-line agent for 77 previously treated patients with metastatic UCB. Patients were divided into two cohorts; 45 patients received 50 mg of sunitinib per day for 4 weeks on and 2 weeks off (cohort A), while 32 received 37.5 mg per day continuously (cohort B). A PR was noted in 7% of cohort A as compared with 3% in cohort B. Forty-three percent had SD, with the PFS and OS being similar in both cohorts, 2.4 versus 2.3 months and 7.1 versus 6.0 months, respectively.⁴⁴ A clinical trial further investigating the impact of sunitinib on advanced UCB is the SUCCINCT trial (available at <http://www.cancerresearchuk.org/cancer-help/trials/trials-search/trial-cisplatin-gemcitabine-sunitinib-advanced-transitional-cell-cancer-urinary-system-SUCCINCT>). It is a Phase II study in the UK that aims to recruit 63 patients to investigate the efficacy of sunitinib in combination with GC as a first-line treatment (Table 4).

Sorafenib, another TKI including VEGF receptors 2 and 3, has been investigated in at least two Phase II studies. Sridhar and colleagues have reported their outcomes on 17 patients with metastatic disease who received sorafenib as a first-line therapy. No objective response was noted, and the median

time to progression (TTP) and OS were 1.9 and 5.9 months, respectively.⁴⁵ Dreicer et al conducted a Phase II trial with sorafenib in a second-line setting for metastatic UCB. They also reported no objective response amongst the 22 eligible patients treated and determined the median PFS and OS of their cohort to be 2.2 and 6.8 months, respectively.⁴⁶ Both investigators concluded that sorafenib as single agent therapy was not clinically meaningful in the first- and second-line settings. However, given the cytostatic nature of sorafenib in other cancers, the investigators believe that it can be investigated as maintenance therapy for those patients who respond to first-line chemotherapy. A Phase II trial is currently active in Italy investigating sorafenib in combination with GC in a neoadjuvant setting with a recruitment goal of 45 patients to assess pathologic response to the investigational regimen.

Pazopanib, a TKI including VEGF receptors 1–3 and platelet-derived growth factor receptor has been observed to have antineoplastic activity against UCB in preclinical studies.⁴⁷ A recently completed Phase II trial utilizing pazopanib conducted by Necchi et al treated 41 patients with advanced UC (26 with primary UCB) who had failed platinum-based chemotherapy. Seven (17.1%) had partial response and 14 (34.2%) had confirmed SD, with a median PFS and OS of 2.6 and 4.7 months, respectively.⁴⁸ Active Phase II studies are examining pazopanib in combination with gemcitabine in a first-line setting for patients with advanced UCB who are unfit for cisplatin (Table 4).

Dovitinib (TKI258) is another example of a TKI which also has shown activity against fibroblast growth factor receptor–dependent UCB growth within laboratory studies.⁴⁹ A Phase II trial with 44 patients with advanced UCB with disease progression despite first-line therapy has recently completed with results pending (Table 4).

Bevacizumab is a monoclonal antibody of VEGF and also blocks tumor angiogenesis. It is a well established RCC agent that was examined in a Phase II study with 43 patients with metastatic UCB given in combination with GC. The study demonstrated an overall response rate (RR) of 72%, with 19% having a CR and 53% a PR. The median PFS was 8.2 months and median OS was 19.1 months.⁵⁰ A multicenter Phase III randomized double-blind study is active using bevacizumab with GC in a first-line setting for patients with advanced or metastatic UCB. This study aims to enroll 500 patients, with the primary objective of assessing OS of patients who received GC with bevacizumab versus those who received GS with placebo.

Aflibercept is a human recombinant fusion protein that binds VEGF with greater affinity than monoclonal antibodies,

Table 3 Targeted therapy for advanced urothelial carcinoma of the bladder^{a3-46,48,50,51,54,57,60,64-66}

Drug/regimen n = number of patients	Dose	Prior chemotherapy	PR (%)	CR (%)	SD (%)	PD (%)	TTP (months)	PFS (months)	OS (months)	Trial features/notes
Sunitinib n = 45/32	50 mg daily in 4/2 week schedule or 37.5 mg daily	Yes	7/3	0	43	60/41	2.4/2.3	2.4/2.3	7.1/6.0	Two cohorts with different dosing schedule Sunitinib used as first-line agent
Sunitinib n = 38	50 mg daily in 4/2 week schedule	No	8	0	50	32	4.8	4.3	8.1	
Lapatinib n = 59	1250 mg daily	Yes	2	0	31	25	2.0	-	4.1	
Sorafenib n = 17	400 mg twice per day	No	0	0	24	36	1.9	-	5.9	
Sorafenib n = 22	400 mg twice per day	Yes	0	0	14	41	-	2.2	6.8	4-month PFS rate was 11.0%
Pazopanib n = 41	800 mg daily	Yes	17.1	0	34.1	48.8	-	2.6	4.7	21 patients received pazopanib in the third-line or beyond setting
Bevacizumab with GC n = 43	G: 1250 mg on days 1 and 8 C: 70 mg/m ² on day 1 B: 15 mg/kg on day 1 of each 21-day treatment cycle	Not within 1 year	53	19	9	14	-	8.2	19.1	
Aflibercept n = 22	4 mg/kg/60 min on day 1 of a 14-day cycle	Yes	4.5	-	-	63	-	2.8	-	Trial terminated due to inadequate response
Trastuzumab with PacGCa n = 44	T: loading dose 4 mg/kg/90 min, then 2 mg/kg/30 min for subsequent doses Pac: 200 mg/m ² on day 1 G: 800 mg/m ² on days 1 and 8 Ca: AUC 5/15 min on day 1	Yes	59	11	11	25	9.3	-	14.1	
Cetuximab with Pac n = 28	Cetuximab: 400 mg/m ² loading dose and weekly doses of 250 mg/m ²	Yes	14.3	10.7	39	7.1	-	3.8	9.7	Single-agent cetuximab arm was closed after 9 of 11 patients progressed by 8 weeks
Everolimus n = 37	Pac: 80 mg/m ² weekly 10 mg daily	Yes	5.4	0	21.6	73	-	2.0	3.3	
Everolimus n = 45	10 mg daily	Yes	-	-	-	-	-	3.3	10.5	Two partial responses in nodal metastases
Temsirolimus n = 14	25 mg daily for 8 weeks	Yes	-	-	29	-	2.5	-	3.5	Early stop due to insufficient response

Abbreviations: AUC, area under the curve; PR, partial response; CR, complete response; SD, stable disease; PD, progression of disease; TTP, time to progression; PFS, progression-free survival; OS, overall survival; G, gemcitabine; C, cisplatin; B, bevacizumab; T, trastuzumab; Pac, paclitaxel; Ca, carboplatin.

and neutralizes multiple VEGF isoforms. In a Phase II trial, Twardowski and colleagues treated 22 patients with advanced UC who had failed platinum-based regimen, with 4 mg/kg intravenously every 2 weeks and reported one PR (4.5%) and median PFS of 2.79 months. The drug was well tolerated.⁵¹

Tumorigenesis inhibitors

Epidermal growth factor receptor (EGFR) and human epidermal receptor 2 (HER2/neu) are often overexpressed in malignant urothelial cells. Overexpression of HER2/neu, which occurs in about 28% of primary urothelial tumors and more than 50% of metastatic tumors, has been associated with higher tumor grade, invasiveness, and increased metastatic potential.^{52,53} Lapatinib, is a TKI that blocks both of these receptors, with subsequent decrease in cell growth. Wulfing et al reported their outcomes with lapatinib from a multicenter Phase II study of 59 patients with locally

advanced or metastatic UCB refractory to first-line systemic chemotherapy. An objective RR of less than 10% was noted in 1.7% of patients. However, 31% achieved SD, with a median TTP and OS of 8.6 weeks and 17.9 weeks, respectively. They also noted that patients experiencing clinical response overexpressed EGFR and/or HER2/neu.⁵⁴ Lapatinib is currently being investigated in patients with advanced UCB who have responded to chemotherapy and express EGFR or HER2/neu receptors (Table 4).

Trastuzumab, a well recognized HER2/neu monoclonal antibody used in the treatment of breast cancer, has been studied in advanced UCB. Pilot studies show that monotherapy with trastuzumab has limited activity against UCB, but when combined with cytotoxic chemotherapy, has more modest results.^{55,56} Hussain et al conducted a Phase II study with trastuzumab in combination with paclitaxel, gemcitabine, and carboplatin in patients with advanced UC, overexpressing HER2/neu. They

Table 4 Ongoing and planned clinical trials of novel agents/regimens for muscle-invasive and advanced urothelial carcinoma of the bladder

Drug/regimen	Study number/ name	Institution	Trial phase	Drug/regimen setting	Enrollment
GC-sunitinib	SUCCINCT	Multicenter (UK)	Phase II	First-line	63
GC-sunitinib	NCT00847015	MSKCC	Phase II	Neoadjuvant	18
Sunitinib	NCT01042795	U Michigan	Phase II	Adjuvant	33
GC-sorafenib	NCT01222676	Italy	Phase II	Neoadjuvant	45
Lapatinib	LaMB	Multicenter (UK)	Phase II	First-line consolidation	204
GC/GCa-dovitinib	NCT01496534	Mount Sinai	Phase I	First-line	24
Dovitinib (TKI258)	NCT00790426	Multicenter (International)	Phase II	Second-line	44
GCa-vandetanib	TOUCAN	Multicenter (UK)	Phase II	First-line	122
Dasatanib	NCT00706641	Baylor-HOG	Phase 0	Neoadjuvant	25
G-pazopanib	NCT01622660	MSKCC	Phase II	First-line, renal dys	45
Pac-pazopanib	NCT01108055	Stanford/Karmanos	Phase II	Salvage	32
Docetaxel-gefitinib	NCT00479089	MDACC	Phase II	Second-line consolidation	90
Cabozantinib	NCT01688999	NCI	Phase II	Salvage	55
Erlotinib	NCT00380029	U North Carolina	Phase II	Neoadjuvant	27
Erlotinib	NCT00749892	MDACC	Phase II	Neoadjuvant	42
GC-bevacizumab	NCT00942331	NCI/Multicenter	Phase III	First-line	500
GC-cetuximab	NCT00645593	U Michigan/multicenter	Phase II	First-line	89
Cetuximab-Pac	NCT00350025	Fox Chase	Phase II	Second-line	34
Cetuximab-(C or MMC-5FU) + RT	TUXEDO	Multicenter (UK)	Phase I/II	Second-line consolidation	60
Pac-RT-trastuzumab	NCT00238420	Multicenter/NCI	Phase I/II	First-line, bladder sparing	88
Everolimus	NCT00805129	MSKCC	Phase II	Second-line	46
G-split dose C-everolimus	NCT01182168	MSKCC	Phase I	First-line	30
Everolimus-Pac	NCT01215136	HOG	Phase II	First-line, cisplatin ineligible	68
GC-temsirolimus	NCT01090466	Multicenter (UK)	Phase I/II	First-line	99
BKM120	NCT01551030	MSKCC	Phase II	Second-line	35
DN24-02	NCT01353222	Multicenter	Phase II	Adjuvant	180
GC-OGX-427	NCT01454089	Multicenter	Phase II	First-line	180
GC-ALT-801	NCT01326871	Multicenter	Phase I/II	First-line	76
GC-Ipilimumab	NCT01524991	HOG	Phase II	First-line	36

Abbreviations: G, gemcitabine; C, cisplatin; Ca, carboplatin; Pac, paclitaxel; MMC, mitomycin-c; 5-FU, 5-fluorouracil; RT, radiation therapy.

treated 44 with this regimen and reported a 70% RR, with a median TTP and OS of 9.3 and 14.1 months, respectively.⁵⁷

Cetuximab is a monoclonal antibody to the EGFR pathway which has previously shown effect to head and neck tumors and is now being studied in UCB. The EGFR pathway is important in cell regeneration and has been shown to be overexpressed in UCB and associated with a worse prognosis^{58,59} and therefore constitutes a potential target for EGFR inhibitors such as cetuximab and gefitinib, another TKI which inhibits EGFR. Wong et al performed a randomized Phase II trial comparing cetuximab with or without paclitaxel, with 39 patients with metastatic UCB who progressed despite receiving first-line therapy. They noted that cetuximab had limited activity as a single agent but appeared to augment the anti-tumor activity of paclitaxel.⁶⁰ Currently, there is a trial in the UK examining cetuximab in combination with radiotherapy and different types of chemotherapy for muscle-invasive UCB (TUXEDO trial, available at <http://www.cancerresearchuk.org/cancer-help/trials/a-trial-of-cetuximab-with-chemotherapy-and-radiotherapy-for-muscle-invasive-bladder-cancer-tuxedo>). Gefitinib has not shown any significant survival benefit but is currently being investigated with docetaxel as second-line consolidation therapy (Table 4).

Other TKIs and monoclonal antibodies (dasatinib, vandetanib, pazopanib, erlotinib, and trastuzumab) are currently being investigated as well (Table 4).

Inhibitors of the PI3K-Akt-mTOR pathway

The mammalian target of rapamycin (mTOR) is a part of an intracellular signaling pathway that governs over cell growth and proliferation. In a variety of different malignancies, the activation of the mTOR pathway is left unchecked, resulting in uncontrolled cell proliferation. Everolimus inhibits the mTOR pathway and has been used for refractory advanced RCC, with a reported improvement in PFS.⁶¹ The clinical implications of this pathway have been shown for muscle-invasive urothelial tumors as well, and its urothelial antitumor activity has been demonstrated in both in-vitro and in-vivo models.^{62,63} Seront et al performed a Phase II trial using everolimus in a second-line setting among 37 patients with advanced UCB who had disease progression after first-line chemotherapy, with cancer control rates at 8 weeks being the primary endpoint. Disease control rate was defined as SD, PR, or CR. At 8 weeks, 27% had reached the primary endpoint (two patients had a PR and eight had SD).⁶⁴ Milowsky et al further examined everolimus in a Phase II trial on 45 patients with advanced UC, who had progressed on chemotherapy.

The drug was reasonably well tolerated with some grade 3/4 toxicity reported, and the median PFS and OS were 3.3 and 10.5 months, respectively.⁶⁵ A Phase II study is underway studying everolimus with and without paclitaxel in the first-line setting for patients with advanced and metastatic UCB who are unfit for cisplatin. This study hopes to recruit 68 patients, with measuring RR at 4 months as the primary objective.

Gerullis and associates reported modest response rates to temsirolimus in patients who relapsed after first-line chemotherapy for metastatic UCB. SD was found in 4 of the 14 patients who participated in the study. The median TTP and OS were 2.5 months and 3.5 months, respectively.⁶⁶ Unfortunately, they had stopped the trial early since the median OS did not reach the primary endpoint of an OS of 6 months. A multicenter Phase I/II study in the UK is investigating temsirolimus in combination with GC in the first-line setting for advanced and metastatic UCB. The study aims to enroll 99 patients, with the primary endpoint of assessing PFS at 6 months.

The phosphatidylinositol 3-kinases (PI3K) are lipid kinases whose products participate in the PI3K-Akt-mTOR signaling cascades that regulate cell survival, proliferation, motility, and differentiation. Uncontrolled activation of class IA PI3K has been discovered to be intimately involved in the pathogenesis of cancer including UCB.⁶⁷⁻⁶⁹ BKM120 is a pan-class I PI3K inhibitor that is currently being studied in a Phase II trial for patients with metastatic UCB who have failed first-line chemotherapy. The study plans to enroll 35 patients, with the primary endpoint being PFS at 2 months (Table 4).

Immunotherapy

Immunotherapy has been studied in many solid tumor types, with BCG, sipuleucel-T, IL-2, and interferon- α being good examples of successful agents used for bladder, prostate, and kidney cancers. Vaccines are now being developed against tumor cells, with promising preliminary results reported. The NY-ESO-1 recombinant protein vaccine was designed to recognize NY-ESO-1 antigen, which is predominantly expressed in high grade urothelial tumors.⁷⁰ This vaccine was tested in a Phase I trial on six patients who had undergone RC or nephroureterectomy and were not candidates for adjuvant systemic chemotherapy. It was administered in combination with intradermal BCG and subcutaneous GCSF to further boost a vaccine-induced immune response. The vaccine was well tolerated, and all of the patients showed immune responses.⁷¹ Another study conducted by Zhang et al

demonstrated that dendritic cell vaccine modified by the Ag85A gene enhanced antitumor immunity against bladder cancer, with a significant inhibitory effect on tumor growth in murine models.⁷²

Survivin, an anti-apoptosis protein, is expressed by many tumors, including UCB.^{73,74} Honma et al vaccinated nine patients with advanced UCB against survivin-2B80-88 peptide to assess safety and efficacy. They had previously demonstrated how this peptide in conjunction with human leukocyte antigen class I stimulates a CD8⁺ cytotoxic T-lymphocyte (CTL) response.^{74,75} Measurable increases in CTL against the survivin peptide were detected in five patients, and one patient had slight reduction in tumor volume.⁷⁶

Ipilimumab is a fully humanized monoclonal antibody directed against cytotoxic T lymphocyte associated antigen (CTLA-4), a regulatory molecule found on the surface of activated T cells and subsets of regulatory T cells. It has been recently approved by the FDA for the treatment of metastatic melanoma. Ipilimumab has been demonstrated in a preclinical trial to generate an immune response in patients with organ-confined UCB.⁷⁷ The primary marker chosen to measure response is inducible costimulator (ICOS)-expressing CD4 T cells (CD4⁺ICOS^{hi}), as it is thought to be a marker of T-cell activation and, interestingly, can recognize NY-ESO-1 antigen.⁷⁸ Persistent increases in CD4⁺ICOS^{hi} have been associated with improved survival in a small retrospective cohort of patients with metastatic melanoma who received ipilimumab.⁷⁷ A Phase II trial is currently

active assessing GC with ipilimumab in a first-line setting for patients with metastatic UCB (Table 4). This study plans to enroll 36 patients, with the primary objective of determining 1-year OS in this cohort.

Agents for non-muscle-invasive UCB

Some of the new candidate drugs' mechanism of action is to deliver traditional therapies in a more effective manner. Apaziquone, a synthetic derivative of MMC, relies on deoxythymidine-diphosphorase, a cellular reductase enzyme that has been found to be highly active in 40% of bladder tumors, to make it more cytotoxically active.⁷⁹ This activation property is particularly important because traditional intravesical therapies' most common side effect is chemical cystitis, which is one of the primary reasons why patients cannot tolerate repeated drug delivery and subsequently leads to altered and/or inadequate treatment courses. Phase II studies of apaziquone have reported that the incidence of bacterial and chemical cystitis range between 11% and 34%, compared with BCG, doxorubicin, and MMC which have incidence rates of cystitis up to 80%, 56%, and 41%; respectively.⁸⁰ Apaziquone shows promise in a Phase II trial done in the Netherlands using it as an adjuvant therapy after TURBT for high risk non-muscle-invasive tumors, yielding recurrence rates of 34.7% at 12 months and 44.9% at 18 months.⁸⁰ Currently, Phase III multicenter trials with apaziquone as an adjuvant treatment for non-muscle-invasive UCB are ongoing (Table 5).

Table 5 Ongoing and planned clinical trials of novel agents/regimens for non-muscle-invasive urothelial carcinoma of the bladder

Drug/regimen	Study number/ name	Institution	Trial phase	Drug/regimen setting	Enrollment
Allopurinol	RAPOR	UK	Phase II	Low grade chemorefractory	64
Postop MMC + celecoxib- (MMC/BCG)	BOXIT	Multicenter (UK)	Phase III	Adjuvant	475
DEPDCI-9-294, MPHOSPHI- 9-278 peptide vaccines	NCT00633204	Iwate Univ (Japan)	Phase II	Prevention after TURBT	150
INSTILADRIN (rAd-IFN with Syn3)	NCT01687244	Multicenter	Phase II	BCG refractory	40
Valproate	NCT01738815	SUNY-Upstate	Phase 0	Before TURBT	50
DTA-HI19/PEI	NCT00595088	Multicenter (Israel)	Phase II	Refractory, second-line	39
ALT-801	NCT01625260	MDACC Orlando	Phase I/II	Refractory, second-line	52
Lenalidomide-BCG	NCT01373294	HLMCC	Phase II	Adjuvant	70
Dovitinib	NCT01732107	HOG	Phase II	BCG refractory	50
EO9 (Apaziquone)	NCT01475266	Multicenter (Korea + Japan)	Phase II/III	Immediate postoperative adjuvant	120
Apaziquone	NCT01469221	Multicenter (International)	Phase III	Adjuvant	658
Everolimus-intravesical gemcitabine	NCT01259063	MSKCC	Phase I/II	BCG refractory CIS	45

Abbreviations: BCG, bacillus Calmette-Guérin; MMC, mitomycin-c; IFN, interferon- α ; TURBT, transurethral resection of bladder tumor; CIS, carcinoma in situ.

Another emerging candidate for intravesical use is oportuzumab monatox (OM), a recombinant fusion protein consisting of humanized anti-epithelial cell adhesion molecule (EpCAM) antibody linked to *Pseudomonas* exotoxin A.⁸¹ Though EpCAM can be overexpressed in many different cancers, in UCB, the level of EpCAM overexpression was found to be associated with the grade of the disease; high grade tumors express more EpCAM compared with low grade tumors, making it clinically relevant for targeted therapy.⁸² Phase I and II trials have been conducted investigating OM for non-muscle-invasive UCB among patients who were BCG refractory or could not tolerate prior BCG therapy. The Phase I trials observed an overall CR rate of 39%.⁸¹ In Phase II trials, Kowalski et al explored the efficacy of OM, specifically for BCG-refractory CIS disease. They achieved a CR rate of 27% at 6 months, with 16% remaining disease free at 1 year.⁸³ In comparison, valrubicin produced a CR in 21%, with 8% remaining disease free at a median follow-up of 30 months.³⁰ Adverse events related to OM were reported in 31% and 65% of patients for the Phase I and II studies, respectively, with mild dysuria being the most commonly reported side effect in both studies.⁸³

Conventional drugs commonly used in general practice are being investigated as possible preventative agents for superficial UCB. The Bladder COX Inhibition Trial (BOXIT) was designed to reduce the risk of recurrent superficial bladder cancer, with standard therapy and celecoxib (a nonsteroidal anti-inflammatory agent (COX-2 inhibitor)). The COX-2 inflammatory response mediator has been observed to be overexpressed in UCB.⁸⁴ BOXIT is a Phase III randomized controlled study, with a recruitment goal of 475 patients among newly diagnosed or recurrent superficial UCB who underwent TURBT and a single postoperative MMC instillation followed by an induction course of MMC or BCG with either oral celecoxib or a placebo drug over a 2-year period. Recruitment ended in August 2012, with the results pending. Allopurinol, an inhibitor of xanthine oxidase attributed to gout, is also being investigated as a preventative agent, on the basis that xanthine oxidase has been reported to have increased activity in patients with papillary UCB⁸⁵ (Table 5).

Conclusion

UCB is a complex disease which is often difficult to manage given the chemorefractory and high recurrence rates of these tumor types. With an increasing understanding of cancer biology, new agents have been proposed and evaluated with variable reported results for both noninvasive and

more advanced disease. Intravesical agents have been used to reduce the risk of recurrence and progression of UC, with several new drugs such as apaziquone and OM having shown promising results in Phase II trials. Platinum-based cytotoxic systemic chemotherapy is the first-line treatment option for both organ-confined muscle-invasive and advanced UCB (in the neoadjuvant, adjuvant, and/or metastatic settings) but with a limited survival benefit, paving the way for alternative systemic approaches. Targeted agents aimed at different molecular pathways (VEGF, EGFR, mTOR) and immunotherapy have been developed and preliminarily investigated for other tumor types, with favorable initial results within the different clinical presentation spectrums of UCB. A select few of these targeted agents have shown modest survival benefits as second-line single agents in advanced UCB, but unfortunately most of these studies are small and nonrandomized. Instead, the results from trials indicate the need for more unique strategies to treat the complex nature of UCB. Few Phase III trials are currently being conducted using targeted agents in the second-line setting, but nevertheless at the present time, we recommend that clinicians treating UCB adhere to accepted treatment guidelines but keep abreast of these exciting developments in our evolving therapeutic approaches to this often highly aggressive tumor phenotype.

Disclosure

The authors report no conflicts of interest in this work.

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