Contemporary best practice in the management of urothelial carcinomas of the renal pelvis and ureter

Maristella Bianconi*, Alessia Cimadamore*, Luca Faloppi, Mario Scartozzi, Matteo Santoni, Antonio Lopez-Beltran, Liang Cheng, Marina Scarpelli and Rodolfo Montironi

Abstract: Upper tract urothelial carcinoma (UTUC) accounts for 5% of urothelial carcinomas (UCs), the estimated annual incidence being 1–2 cases per 100,000 inhabitants. Similarly to bladder UC, divergent differentiations and histologic variants confer an adverse risk factor in comparison with pure UTUC. Molecular and genomic characterization studies on UTUC have shown changes occurring at differing frequencies from bladder cancer, with unique molecular and clinical subtypes, potentially with different responses to treatment. Systemic chemotherapy is the standard approach for patients with inoperable locally advanced or metastatic UCs. Although initial response rates are high, the median survival with combination chemotherapy is about 15 months. In first-line chemotherapy several cisplatin–based regimens have been proposed. For patients with advanced UC who progress to first-line treatment, the only product licensed in Europe is vinflunine, a third-generation, semisynthetic, vinca alkaloid. Better response rates (15–60%), with higher toxicity rates and no overall survival (OS) benefit, are generally achieved in multidrug combinations, which often include taxanes and gemcitabine. The US FDA has recently approved five agents targeting the programmed death-1 and programmed death ligand-1 pathway as a second-line therapy in patients with locally advanced or metastatic UC with disease progression during or following platinum-containing chemotherapy. Potential therapeutic targets are present in 69% of tumours analysed. Many molecular alterations include those involved in the RTK/Ras/PI(3)K, cell-cycle regulation and chromatin-remodeling pathways, many of them have either targeted therapies approved or under investigation. Angiogenic agents, anti-epidermal growth factor receptor therapy, phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) pathway inhibitors and immunotherapeutic drugs are being successfully investigated.

Keywords: antiangiogenic agents, chemotherapy, gene expression profiling, immunotherapy, target therapies, upper urinary tract, urothelial carcinoma

Introduction
Upper tract urothelial carcinoma (UTUC) accounts for 5% of urothelial carcinomas (UCs), the estimated annual incidence being 1–2 cases per 100,000 inhabitants. The pathology of UTUC has been considered to be the same as that of bladder UC (BUC). Such an assumption appears to be reasonable due to the fact that the morphologic features of UTUC and BUC are identical microscopically. However, a growing body of evidence has accumulated suggesting that there are differences between these two neoplasms and that UTUC and BUC are two disparate entities.
Classification and histologic variants
The classification of UTUC and BUC are similar. It distinguishes between noninvasive papillary tumours (i.e. papillary urothelial neoplasia of low malignant potential and low- and high-grade papillary carcinoma), carcinoma in situ (CIS), and invasive carcinoma. Similarly to BUC, divergent differentiations and histologic variants confer an adverse risk factor.

The vast majority of neoplasms of the upper urinary tract are UCs. Even though pure squamous cell carcinoma, adenocarcinoma, and neuroendocrine carcinoma, among others, do occur, more commonly seen are areas of squamous differentiation and, less frequently, glandular differentiation within an otherwise usual UC. Pure UCs can display a host of variant histologies. Recognition of such morphologies is paramount important for proper diagnosis, prognosis and therapy. Histological variants are associated with advanced tumour stage, tumour multifocality, sessile tumour architecture, tumour necrosis, lymphovascular invasion and lymph node metastasis, in comparison with pure UTUC. Outcomes associated with variant histology are worse than with pure UC on univariable analysis. In particular, it is associated with disease recurrence and cancer-specific mortality. However, such an effect does not retain significance on multivariable evaluation.

In patients treated with adjuvant chemotherapy there are no differences in disease recurrence or survival between variant histology and pure UTUC.

Other variants have been described in the bladder but have not yet been fully described in the UTUC, such as the lipid-rich (lipoid) variant, UC with small tubules, and the large nested variant of UC, among others. Any tumour that may be seen in the bladder may also, theoretically, occur in the ureter and pelvis. These should be considered in the differential diagnosis when examining tumours of such locations.

Tumour grade
The 1973 World Health Organization (WHO) classification was the international standard for UC grading. It distinguishes grades G1–G3. The 2004 WHO classification (currently also known as the 2016 WHO classification) distinguishes in the noninvasive papillary tumours, papillary urothelial neoplasia of low malignant potential, and low- and high-grade carcinoma (low grade versus high grade for the invasive forms). The current guidelines are based on the 2016 WHO classifications.

Tumour node metastasis staging
The 8th AJCC and UICC edition of the tumour, node, metastasis (TNM) classification is identical to the previous 7th edition. Concerning the correlation between biopsy tumour stage and resection tumour stage, staging upper urinary tract tumours is fraught with difficulties, mainly due to the small size of the biopsy material submitted for diagnosis. The muscularis propria is rarely seen in the biopsies of the upper tract. Some biopsies may be so small and superficial that only the epithelium is present, thus precluding assessment of invasion entirely. A study by Vashistha and colleagues reported a pT stage concordance of 60% for biopsies. A pT classification was not feasible in 10.6% of cases. Patients with an advanced clinical stage (cT3–4) or with clinically enlarged lymph nodes (cN+) are potential candidates for neoadjuvant chemotherapy (NAC), even though the influence of NAC on OS showed no statistically significant benefit.

Lymph node dissection performed at the time of renal nephroureterectomy (RNU) allows for optimal tumour staging. Its curative role is debated. The regional lymph nodes are the hilar and retroperitoneal nodes, and for the mid and distal ureter, the intrapelvine nodes. The only difference between the 7th and the 8th editions is that, since there are no data to substantiate the three N categories in the 7th edition, the previous category of N3 metastasis in a lymph node greater than 5 cm is now collapsed with N2 in the 8th edition. Lymph node metastases and extranodal extension are predictors of survival outcomes in UTUC.

Lymphovascular invasion (LVI) is seen in about 20% of the UTUCs. Since it is an independent predictor of survival, LVI should be specifically included in the pathology reports of all UTUC specimens.

Positive soft tissue surgical margin (R) after RNU is a factor for disease recurrence. Uropathologists should look for surgical margins and report positive margins at the level of ureteral transection, bladder cuff, and around the tumour if the T stage >2.

Since tumour stage is difficult to assess clinically in UTUC, it is useful to ‘risk stratify’ UTUC into
low- and high-risk neoplasms to identify those patients who are more suitable for renal sparing treatment versus radical extirpative surgery\(^{16}\) (Figure 1).

**Additional pathological features**

The architecture is a strong prognosticator with sessile growth pattern being associated with worse outcome. Concomitant CIS in organ-confined UTUC and a history of bladder CIS are associated with a higher risk for recurrence and cancer-specific mortality.\(^{17}\)

Tumour necrosis (i.e. \(>10\%\) of the tumour area) is present in approximately \(20\%\) of the patients. Its prevalence increases with advancing pathological stage, from \(7\%\) and \(10.6\%\) to \(50\%\) in T1, T2 and T3–4, respectively. In addition, tumour necrosis is associated with features of aggressiveness in UTUC, including a high grade, lymph node metastasis, LVI, a sessile architecture of the tumour and concomitant CIS. Tumour necrosis is not an independent predictor of clinical outcomes.\(^{18}\)

**Predictive tools**

Several predictive tools have been proposed in UTUC, both in the preoperative and postoperative settings. At present, the Yates’ model remains the only one to have been validated in a different population.\(^{19}\) As the current staging system is not useful in the neoplasms of the UTUC prior to RNU, new prognostic factors and tools are needed in the decision-making process. This will help clinicians in the identification of high-risk patients who will benefit from certain types of therapy and those who could be spared from the side effects of an unnecessary therapeutic intervention. In order to select such patients, highly accurate tools estimating individual prognosis are needed. As for the variants, as recently stated by Moschini and colleagues for the urinary bladder, their clinical relevance can manifest at three different levels: ‘diagnostic, as identification is challenging and misinterpretation is not uncommon; prognostic, for patient risk stratification and outcome estimation; and therapeutic, as particular variants could be responsive to specific treatment strategies’.\(^{20}\) The same applies to UTUC and its variants.

**Molecular and genetic markers**

Several studies have investigated the prognostic impact of markers related to cell adhesion, cell differentiation, angiogenesis, cell proliferation (Ki-67), epithelial-mesenchymal transition, mitosis, apoptosis, and vascular invasion, and c-met protein.\(^{21}\) Microsatellite instability (MSI) can help detect germline mutations and hereditary cancers. It is an independent molecular prognostic marker.\(^{22}\) Several molecular markers have been associated with worse outcomes of UTUC. Because of the rarity of UTUC, the main limitations of such studies are their retrospective design and small sample size. None of the markers have fulfilled the criteria necessary to support their introduction in daily clinical decision making. Ki-67 is the only one that has been prospectively evaluated.\(^{23}\)

Targeted next-generation sequencing of 300 cancer-associated genes has been performed in order to define the genomic landscape of UTUC and to compare its results with BUC. The spectrum of genes mutated in UTUC and BUC is similar. However, differences in the prevalence of alterations in several recurrently mutated genes, including FGFR3, HRAS, TP53, and RB1 were seen.\(^{24,25}\) Such data can provide an important reference for the development of multimodal strategies in the management of UTUC. The high prevalence of potentially actionable genetic events in UTUC suggests that routine genomic profiling may speed up the process of developing novel drug targets. In particular, the ‘association of

---

**Figure 1.** Whole mount section of the kidney with urothelial papillary carcinoma involving the pelvis and calyces.
variant histology with specific mutation patterns promises to be helpful in discovering targeted therapeutic approaches based on specific molecular pathways.

Chemotherapy

Systemic chemotherapy is the standard approach for patients with inoperable locally advanced or metastatic urothelial malignancies. Although initial response rates are high, the median survival with combination chemotherapy is about 15 months.

UCs of the upper urinary tract are histologically identical to those originating in the bladder, therefore platinum-based chemotherapy is expected to determine similar results independently of the site of origin. Unfortunately, there are only limited data on the efficacy of chemotherapy in patients with UTUCs. Even though patients with advanced UTUC have often been included in clinical trials for patients with metastatic BUC, they represent only the minority. Therefore, treatment is based upon the much larger experience in patients with advanced BUC. Results of a phase III randomized trial of perioperative chemotherapy versus surveillance in UTUC (CRUK/11/027; ClinicalTrials.gov identifier: NCT01993979) have been recently published. A total of 248 patients post-nephroureterectomy have been randomized (1:1) to treatment with gemcitabine-cisplatin (GC; 125 patients) or surveillance (123 patients). The 2-year disease-free survival was 51% for surveillance and 70% for chemotherapy; metastasis-free survival also supported chemotherapy, with a hazard ratio (HR) of 0.49 [95% confidence interval (CI) 0.30–0.79, \( p = 0.003 \)]. In particular, in this trial (i.e. the POUT trial), both GC and gemcitabine + carboplatin were used, and that benefit with the latter may be less pronounced based on forest plots. According to these results, adjuvant chemotherapy should be considered a new standard of care.

First-line therapy

Currently, a cisplatin-containing combination chemotherapy regimen is considered the ‘gold-standard’ upfront treatment for patients with advanced BUC and urinary tract who are suitable for cisplatin. In this regard it is crucial to stratify the patients into medically ‘fit’ and ‘frail’ populations by using international criteria which can help to define medical frailty. They include the WHO/Eastern Cooperative Oncology Group (ECOG) performance status of 2 or greater or a Karnofsky performance status of 60–70% or less; creatinine clearance less than 60 ml/min, a hearing loss (measured at audiometry) of 25 dB at two contiguous frequencies, grade 2 or greater peripheral neuropathy (i.e. sensory alteration or paresthesia, including tingling, but not interfering with activities of daily living), New York Heart Association class III or greater heart failure.

For medically fit patients with advanced UC, several cisplatin-based chemotherapy regimens have been proposed. The most commonly used regimens include MVAC [methotrexate (30 mg/m² on days 1, 15, 22), vinblastine (3 mg/m² on days 2, 15, 22), doxorubicin (30 mg/m² on day 2), and cisplatin (70 mg/m²), repeated every 28 days for six cycles]; GC [gemcitabine (1000 mg/m² on days 1, 8, 15) plus cisplatin (70 mg/m² on day 2), repeated every 28 days for a maximum of six cycles], and PCG [paclitaxel (80 mg/m² before GC on days 1 and 8), gemcitabine (1000 mg/m² on days 1 and 8), and cisplatin (70 mg/m² on day 1)], repeated every 21 days for a maximum of six cycles.

MVAC is the first combination regimen which demonstrated, based on the results of randomized clinical trials, a clear superiority compared with single-agent cisplatin. MVAC showed a significant improvement in the overall response rate (OR; 39% versus 12%) and progression-free survival (PFS; 10 versus 4 months; OS, 13 versus 8 months). Toxicity is a major concern with the MVAC regimen, particularly since many patients with advanced UC are elderly or have multiple comorbidities and in the case of UTUCs patients often show impaired renal function after radical surgery. Myelosuppression, neutropenic fever, sepsis, mucositis, and nausea and vomiting are common. The use of hematopoietic growth factor support may reduce the rate, grade and length of some of these toxicities, especially myelosuppression and mucositis.

In a phase III randomized trial, MVAC was compared with the combination of GC. In terms of OR (49 versus 36%) and 5-year survival (PFS of both arms, 7 months, OS 14 versus 15 months) the two regimens appeared equivalent. However, toxicity was significantly less in the GC arm with less serious (grade 3/4) neutropenia (71 versus
82%), neutropenic sepsis (2 versus 14%), and mucositis (1 versus 22%). This trial was designed to assess whether GC was superior and was not powered to demonstrate equivalency between the two regimens, but given the similar efficacy and lesser toxicity, GC is widely considered the standard first-line regimen for patients with advanced BUC and upper urinary tract.

In an effort to improve outcomes and lower toxicity with MVAC therapy, the European Organisation for Research and Treatment of Cancer (EORTC) investigated the potential role of high-dose MVAC (hd-MVAC), which consisted of rapid 2-week cycles (instead of the standard 4 weeks). A total of 246 patients were randomized to receive either standard MVAC or hd-MVAC. Although the OR and OS rates were similar, PFS was significantly improved (9.5 months versus 8.1 months, \( p = 0.037 \)) and toxicity was decreased in patients treated with hd-MVAC.\(^{35}\)

In order to improve the outcome obtained with the dual therapy, attempts were made to incorporate a third agent to the doublet of GC. The triplet combination of paclitaxel, gemcitabine, plus cisplatin (PGC) was demonstrated in the EORTC 30987 phase III study, which enrolled 626 patients with advanced UC (81% with primary bladder cancer) and randomly assigned them to treatment with GC or PGC for a maximum of six cycles. Final results after a median follow up of 4.6 years have been reported.\(^{36}\) Compared with GC, PGC resulted in an increase in the OR (56 versus 44%, \( p = 0.003 \)). A trend towards an improvement in PFS (median 8.3 versus 7.6 months, HR for progression 0.87, 95% CI 0.74–1.03), longer OS (median 16 versus 13 months, HR for death 0.85, 95% CI 0.72–1.02) was observed. The trial showed an increased incidence of serious toxicities, including neutropenia (65 versus 51%), fatigue (15 versus 11%), and infections (18 versus 14%), but a lower incidence of serious thrombocytopenia (35 versus 52%). The PGC regimen even if more toxic than the GC regimen may be considered as an alternative option in patients with excellent performance status without relevant comorbidities.

**Carboplatin-based regimens.** A consistent number of patients with advanced UC are not suitable for cisplatin-based regimens due to impaired renal function (glomerular filtration rate <60 but >30 ml/min) or poor performance status (ECOG \( \geq 2 \)). Since monotherapy produces little benefit with responses of short duration and scarce impact on survival, these patients may benefit from carboplatin-based regimens.\(^{40,41}\) In the EORTC 30986 study, 238 chemotherapy-naïve patients unable to receive cisplatin were randomly assigned to treatment with carboplatin and gemcitabine or methotrexate, carboplatin, plus vinblastine (MCAVI).\(^{40}\)

Compared with MCAVI, treatment with carboplatin plus gemcitabine resulted in a higher OR (41 versus 30%, respectively) that did not reach statistical significance. No difference in median OS (9 versus 8 months, HR for death 0.94, 95% CI 0.72–1.22), in median PFS (6 versus 4 months, HR for progression 1.04, 95% CI 0.80–1.35) but less overall serious toxicities (9 versus 21%), including neutropenia (52 versus 63%) and febrile neutropenia (5 versus 15%), were reported. However, it was associated with a higher rate of serious thrombocytopenia (47 versus 18%). The results of this study showed that a carboplatin-gemcitabine regimen has a better toxicity profile compared with MCAVI and support its use in patients with impaired renal function or poor performance status.

**Platinum-free chemotherapy regimens.** The results of several trials investigating the efficacy of nonplatinum combination regimens are reported in the literature. Many combine gemcitabine with a taxane (either paclitaxel or docetaxel) with encouraging results. Paclitaxel plus gemcitabine appears to be more active than docetaxel plus gemcitabine in patients with advanced UC.\(^{42–47}\)

**Second-line chemotherapy**

For patients with advanced UC who progress to first-line treatment, there is no standardized therapy. Most available data come from phase II studies which have too small numbers of patients for carrying out head to head comparisons between different agents or regimens. Patients with advanced bladder cancer who have failed an initial chemotherapy regimen should be encouraged to participate in clinical trials whenever possible.

Currently, the only product licensed for second-line chemotherapy in Europe is vinflunine, a third-generation, semisynthetic, vinca alkaloid. A phase III study comparing vinflunine with best supportive care showed a significant survival benefit in eligible patients (6.9 versus 4.3 months), with an objective response rate (ORR) of 8.6%.\(^{48}\)
The choice of second-line chemotherapy is complex and depends on performance status, first-line treatment used, prior chemosensitivity in terms of duration of response, and the presence of visceral metastases. The most important predictors of poor outcome in the second-line setting are hemoglobin level, presence of liver metastasis, poor performance status and time from prior chemotherapy. For selected patients who progress on first-line cisplatin-based chemotherapy and are still eligible for cisplatin treatment, rechallenge with cisplatin-containing chemotherapy is feasible, depending on their initial response. Several other single-agent chemotherapies including paclitaxel, docetaxel, gemcitabine, ifosfamide, oxaliplatin, pemetrexed and nab-paclitaxel have been tested in second-line therapies with response rates ranging from 5% to 33%, short times to progression and no improvement in OS. Better response rates (15–60%), with higher toxicity rates and no OS benefit, are generally achieved in multidrug combinations, which often include taxanes and gemcitabine.

**Target therapies**

In recent years, also in UC, a better and wider knowledge of the cancer genome has revealed both well-characterized and novel genomic alterations, with extensive work performed in more advanced disease, leading to studies on target therapies.

Potential therapeutic targets were present in 69% of tumours analyzed. Specific molecular alterations included those involved in the RTK/Ras/PI(3)K, cell-cycle regulation and chromatin-remodeling pathways, many of which have either targeted therapies approved or under investigation.

Gene expression profiling by several groups has suggested there are intrinsic subtypes of BUC that may differ in their underlying biology and overall prognosis, potentially having advantage from different therapeutic approaches. This likely reflects a difference in the underlying biology of UTUC, since luminal subtype and fibroblast growth factor receptor (FGFR) mutations have been reported to occur with higher frequency in these patients. In 2015, Sfakianos and colleagues reported the most comprehensive genomic profiling of UTUC to date by sequencing protein-coding exons of 300 genes, including those frequently mutated in the Cancer Genome Atlas (TCGA) analysis of BUC, and comparing the results with those of concomitantly analyzed BUC tumours. High-grade UTUC and high-grade BUC seem to have similar genetic changes, but the frequencies of these alterations significantly differed. Similar to BUC, UTUC harbored alterations in FGFR3, chromatin-modifying genes, HRAS, and TP53. In UTUC tumours, however, HRAS and CDKN2B were more frequently mutated (HRAS: 14 versus 1%, \( p = 0.001 \); CDKN2B: 15 versus 4%, \( p = 0.016 \)), and TP53 and ARID1A were less frequently mutated (TP53: 25 versus 58%, \( p < 0.001 \); ARID1A: 14 versus 28%, \( p = 0.050 \)). A prominently mutated gene in BUC, Rb1, was ubiquitously unaltered in the UTUC tumours analyzed (0% in UTUC versus 19% in BUC; \( p < 0.001 \)), wild-type Rb1 seems a positive predictor of survival.

Conversely, Mullane and Bellmunt profiled 19 UTUC samples, finding variably concordant results. While no alterations in Rb1 were found in 59 high-grade UTUC tumours in the primary study, the smaller study found three Rb1 alterations in 19 samples, supporting the need for independent validation.

Over the last decade, multiple molecular alterations in this pathway have been implicated in many different cancers. A recent analysis of driver cancer genes across various tumour types identified significant alterations in PI3K-AKT-mTOR-signaling components especially PIK3CA in bladder, endometrial, breast, lung and head and neck cancers. Recent genomic analyses of two large cohorts of UC patients showed frequent alterations in PIK3-AKT-mTOR pathway. The largest analysis from the TCGA network, which included a cohort of 131 chemotherapy-naïve high-grade muscle-invasive UC patients (T2–T4a, Nx, Mx), reported mutations and copy number alterations within this pathway in 42% of tumours.

**Antiangiogenic agents**

Angiogenic agents have been tested in UC in different clinical studies, with discordant results. Bevacizumab, a monoclonal antibody directed against the vascular endothelial growth factor (VEGF), showed promising results. In a phase II trial in which bevacizumab was combined with GC as a first-line treatment of metastatic UC, a
72% OR and a median OS of 19.1 months, was reported.\textsuperscript{71} In another phase II study chemo-naive patients with metastatic disease, whose expected survival was approximately 9 months, bevacizumab combined with gemcitabine and carboplatin led to a 63% response rate and OS of 13.9 months.\textsuperscript{72} In both trials an advantage against historical controls was observed. In the neoadjuvant setting, in a phase II trial, bevacizumab was combined with MVAC, resulting in an increased pathologic response, to less than stage T2.\textsuperscript{73}

More disappointing were results with tyrosine kinase inhibitors that target VEGF receptors (VEGFRs). Sunitinib, a multikinase inhibitor, that targets VEGFRs and also c-kit, platelet-derived growth factor receptor (PDGFR)-a and -b, Fms-related tyrosine kinase 3 and RET, was studied as second-line therapy using two different schedules. A partial response was seen in 5% of patients, with OS reported as 6.9 months. The treatment was not well tolerated, 74% of the patients experienced grade 3 and 4 toxicities, most common adverse events were lymphopenia, thrombocytopenia, anemia, fatigue and nausea.\textsuperscript{74} In another study, sunitinib was given as first-line treatment in patients ineligible for cisplatin, due to renal impairment. An 8% response rate and 8.1-month OS were reported. Although the incidence of grade 3 and 4 toxicities was less, 2 of 38 patients died (1 of myocardial infarction and 1 of stroke), possibly related to treatment.\textsuperscript{75}

Finally, sunitinib given as maintenance therapy in a phase II trial to patients who had achieved stable disease, partial or complete response after 4–6 cycles of chemotherapy did not improve 6-month PFS compared with placebo.\textsuperscript{76} Because of these disappointing results, no trials of sunitinib for metastatic UC are ongoing.

Other antiangiogenic agents have been investigated for efficacy against UC.

Sorafenib, which targets VEGFR-2 and -3 and B-Raf, c-Raf, and PDGFR-a and -b, achieved no response as either first-line treatment for cisplatin-ineligible patients or single-agent second-line treatment.\textsuperscript{77,78} These studies led researchers to conclude that sorafenib has little or no activity against UC. Similarly, in a trial of pazopanib, an antiangiogenic agent that targets VEGFR-1, -2, and -3, PDGFR-a and -b, and c-kit, the drug effected no response when given as a single agent in second-line treatment\textsuperscript{79} and led to a 17% response rate in another trial, with a median OS of only 4.7 months.\textsuperscript{80} A trial of pazopanib combined with GC as first-line treatment for cisplatin-ineligible patients (NCT01622660) closed because of hepatotoxicity. A trial of pazopanib combined with vinflunine as second-line treatment was discontinued at the first dose level for safety reasons.\textsuperscript{81}

Although small molecule inhibitors of VEGFR have so far shown limited efficacy, a three-arm randomized phase II study of recently developed monoclonal antibodies targeting VEGFR-1 (icrucumab) and VEGFR-2 (ramucirumab) combined with docetaxel in patients with disease progression after first-line therapy has been recently published. It showed how a combination of docetaxel and ramucirumab improved PFS than docetaxel alone (5.4 versus 2.8 months); icrucumab instead did not experience any PFS improvement.\textsuperscript{82}

**Anti-epidermal growth factor receptor therapy**

Progression of muscle-invasive UC has been linked to epidermal growth factor receptor (EGFR) overexpression.\textsuperscript{83,84} When 20 patients with muscle-invasive disease were treated with erlotinib before cystectomy, 60% were downstaged to pT1 or less, suggesting single-agent activity with EGFR inhibitors.\textsuperscript{85} However, gefitinib administered as a single agent in second-line treatment produced only a 3% response rate and a 3-month OS.\textsuperscript{86} Moreover, gefitinib combined with GC, given either as a standard dose\textsuperscript{87} or at a fixed-dose rate,\textsuperscript{88} did not improve either the response rate or OS for patients with untreated metastatic UC.

Furthermore, the combination of GC chemotherapy and cetuximab, a monoclonal antibody targeting EGFR, was intolerable, and both PFS and median OS proved detrimental.\textsuperscript{89} A randomized phase II trial of another EGFR-specific monoclonal antibody, panitumumab, combined with GC compared with chemotherapy alone as first-line treatment was terminated early because of insufficient recruitment (NCT01374789). Another randomized phase II trial of patients with previously treated metastatic disease reported a 25% response rate for cetuximab combined with paclitaxel, but no activity for
cetuximab as a single agent.\textsuperscript{90} These data support preclinical data showing synergism between anti-EGFR monoclonal antibodies and taxanes.\textsuperscript{91} Overall, it appears that in unselected patients, targeting EGFR has limited efficacy. Therefore, it is imperative to identify biologic markers, most likely to identify patients responding to anti-EGFR treatment. The oral tyrosine kinase inhibitor vandetanib, was studied in the second-line setting combined with docetaxel in a phase II double-blind trial that reported no significantly improved OR, PFS, or OS in patients with advanced UC.\textsuperscript{92} A single-arm phase II study tested the safety and efficacy of the antihuman EGFR-2 (HER2) antibody trastuzumab combined with GC, carboplatin, and paclitaxel in patients with HER2 overexpression found by immunohistochemistry, gene amplification, or elevated serum HER2 levels.\textsuperscript{93}

The patient outcomes exceeded expectations, with an ORR of 70% and an OS of 14.1 months.\textsuperscript{93} Although the incidence of grade 1–3 cardiac toxicity (22.7%) was more frequent than anticipated, these unexpected results prompted additional studies. A randomized phase II trial compared trastuzumab (NCT01828736) with GC or GC and carboplatin \textit{versus} GC or GC and carboplatin alone in HER2-expressing bladder cancer.\textsuperscript{94} That study screened 563 patients with advanced UC over 5 years and found only 75 patients (13.3%) with HER2-positive (HER2\textsuperscript{+}) tumours. No difference was observed in the ORR, PFS, or OS between the chemotherapy-alone arm and the chemotherapy-plus-trastuzumab arm. The investigators did find that the HER2 levels were predictive of PFS regardless of treatment. A study investigating trastuzumab combined with standard GC chemotherapy in the first-line setting closed enrolment early (NCT02006667). Another study investigating trastuzumab as a single agent in the second-line setting (NCT02013765) closed early because of recruitment difficulties.

Lapatinib, an agent that has selective activity against both EGFR and HER2, was hypothesized to have significant clinical activity. Although lapatinib had only a 1.7% response rate as a single agent in unselected patients with platinum-refractory metastatic UC, it led to a marked difference in OS in those with low EGFR/HER2 compared with patients with high EGFR or high HER2.\textsuperscript{95} This subgroup analysis reinforces the concept of selecting appropriate patients for targeted therapies. In a recently completed trial that combined lapatinib with GC (GCL), four cycles of GCL were administered as neoadjuvant therapy for patients with muscle-invasive UC. Although initially designed as a phase II efficacy study with a primary endpoint of pathologic complete response at the time of radical cystectomy, the dose selected for investigation proved excessively toxic.\textsuperscript{96} A completed phase II/III trial comparing maintenance lapatinib with placebo after first-line chemotherapy \textit{[LAMB (maintenance lapatinib \textit{versus} placebo after first-line chemotherapy in patients with locally advanced or metastatic bladder cancer) trial, ClinicalTrials.gov identifier: NCT00949455]} in patients expressing either EGFR or HER2 will shed more light on the role of dual-targeting these growth pathways in UC. Results are awaited.

A phase II trial testing the oral, irreversible inhibitor of the ErbB family, afatinib is currently ongoing (NCT02122172). Preliminary results showed that patients with HER2 or ERBB3 alterations achieved a 3-month PFS \textit{versus} none of patients without alterations. The median time to progression/discontinuation was 1.4 months in patients without alteration compared with 6.6 months in patients with HER2 amplifications or ERBB3 mutations.\textsuperscript{97}

\textbf{PI3K/AKT/mTOR pathway}

Early phase clinical trials of PI3K pathway inhibitors are currently underway in UC. A phase II clinical trial testing buparlisib, a pan-class I selective PI3K inhibitor, in UC is ongoing (NCT01551030). The trial is enrolling metastatic transitional cell UC patients with alterations in the PI3K pathway.\textsuperscript{98} Preliminary results from another phase I study of a PI3K inhibitor GSK2126458 in 170 patients with advanced solid cancer have shown objective responses (1 of 3 UC patients bearing PIK3CA mutations), although responses were also observed in wild-type patients (2 of 15 UC patients).\textsuperscript{99} Further work is required to characterize the relationship between PIK3CA mutation and response to PI3-kinase inhibition in UC.

mTOR plays a central role in metabolism, cell proliferation and growth, and inhibiting this
protein is an important target for therapeutic intervention.\textsuperscript{100} However, the results of two phase II clinical trials of mTOR inhibitors as a single-agent therapy in UC patients have not been promising in unselected patients.\textsuperscript{101,102} In a phase II single-arm, nonrandomized study with everolimus in 45 UC patients who had progressed on cytotoxic agents, 1 patient had near complete response, 1 had a partial response, and several had minor responses. The primary end point of 2-month PFS in more than 70% of patients was not achieved.\textsuperscript{101} Subsequent whole genome sequencing of the patient who showed near complete response to everolimus in the study had nonsynonymous mutations in the tuberous sclerosis complex, lending biomarker credibility to this regulator of mTOR.\textsuperscript{103} Analysis of another phase II study of everolimus in platinum-resistant patients led to the conclusion that intact PTEN activity was required for mTOR inhibitor sensitivity due to feedback through AKT.\textsuperscript{103,104} Recently, a phase I study of an mTOR inhibitor in combination with pazopanib has been reported in which a patient who showed a 14 month complete response to therapy had two activating mutations in mTOR.\textsuperscript{105} Another phase II study of the dual PI3K and mTOR inhibitor BEZ235 in patients with locally advanced or metastatic transitional cell carcinoma showed modest clinical activity and an unfavourable toxicity profile; however, a minority of patients experienced a clinical benefit, suggesting that a complete blockade of the PI3K/mTOR axis could improve the outcome in some specific patients.\textsuperscript{106} These findings highlight the importance of biomarker-enriched trial designs in UC-targeted therapy trials.

**Immunotherapy**

Resistance to cell death and evasion of immune destruction are known cancer hallmarks.\textsuperscript{107} Progress in immunotherapy clinical trials across various tumour types promises to deliver significant benefit to patients with several solid tumour types including UC.\textsuperscript{108}

Newer immunotherapeutics are being investigated in multiple settings including ipilimumab, a monoclonal antibody against CTLA-4 (cytotoxic T lymphocyte antigen 4), which is already approved by the US FDA for the treatment of advanced melanoma. As a monotherapy in the neoadjuvant setting, a trial was performed to evaluate the safety of ipilimumab and immune monitoring in 12 patients with localized UC prior to cystectomy. Overall, two of six patients in the high-dose cohort had to delay surgery due to immune-related adverse events (AEs), though all patients recovered, and toxicity was generally limited to grades 1–2. Immune correlates were associated with OS in a companion study in patients with melanoma.\textsuperscript{109} Another phase II study is evaluating the addition of immunotherapy to chemotherapy by using gemcitabine, cisplatin and ipilimumab as the first-line drugs in the treatment of UC is currently recruiting patients. In this study total of six cycles of chemotherapy are planned with ipilimumab to be added from the third cycle onwards.\textsuperscript{110}

Both programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) antibodies, are an exciting strategy to treat metastatic UC, including UTUC. Several checkpoint inhibitors are in later-stage clinical development as first-line and second-line therapies for metastatic UC.

A phase Ib trial evaluated pembrolizumab, a PD-1 inhibitor, in recurrent, metastatic, or persistent UC whose tumours on immunohistochemistry showed $\geq 1\%$ PD-L1-positive cells.\textsuperscript{111} The ORR was 25%, and in patients with measurable disease, ORR was 38% in PD-L1-positive tumours. At 12 months, 19% continued to maintain a response. Pembrolizumab has been investigated also in chemo-naïve patients ineligible for a cisplatin phase II trial. Preliminary results recently presented showed an ORR of 24%. This trial was interestingly conducted in an elderly population with a median age of 75 years (range 44–94 years), but a 34% of patients older than 80 years. Further data are to be published.\textsuperscript{112} A phase III study comparing pembrolizumab with either paclitaxel or docetaxel or vinflunine in patients with pretreated advanced UC has recently been published (Keynote 045). The end points were OS and PFS, which were assessed among all patients and among patients who had a tumour PD-L1 positivity. The median OS in the total population was higher in the pembrolizumab group, than in the chemotherapy group (10.3 \textit{versus} 7.4 months, HR 0.73; $p = 0.002$). The median OS among patients who PD-L1 expression was 8.0 months in the pembrolizumab group, as compared with 5.2 months in the chemotherapy group (HR 0.57; $p = 0.005$). No significant difference in PFS among groups
in the total population (HR 0.98; \( p = 0.42 \)) or among patients who had a tumour PD-L1 positivity (HR 0.89; \( p = 0.24 \)).\(^{113} \) Moreover, pembrolizumab is being evaluated in several UC clinical trials, either as a single agent or combined with other therapies.

Avelumab is a PD-L1 inhibitor, which demonstrated an ORR of 15.9% in treatment-refractory metastatic UC in a phase Ib study. Durable responses were achieved in 13.6% of patients.\(^{114} \) Among tumours evaluable for PD-L1 expression, ORR was 40% in PD-L1-positive tumours versus 9.1% in PD-L1-negative tumours. An update from the enrolment of another cohort in this trial confirmed the promising results, showing an ORR of 16.5% with 3 complete responses and 15 partial responses; PFS was reported at 6.1 weeks and the PFS rate at 12 weeks was 35.6%.\(^{115} \) Recently, at the 2017 American Society for Clinical Oncology (ASCO) Genitourinary Symposium, pooled data of two phase Ib trials on UC patients progressed to a platinum regimen with avelumab, another PD-L1 inhibitor, were presented. In 153 patients with 6 months or longer follow-up, confirmed ORR was 17.6% with 9 complete responses and 18 partial responses; 24/27 (88.9%) were still receiving the drug at the data cut-off.\(^{116} \)

Rosenberg and colleagues recently published outcomes from a phase II trial evaluating atezolizumab, a PD-L1 inhibitor.\(^{117} \) The trial enrolled 316 patients who were either (1) chemotherapy-naïve and cisplatin-ineligible or (2) cisplatin-refractory. The trial incorporated the evaluation of immune cell (IC) PD-L1 expression (grading IC0–IC3), TCGA cluster subtype, and mutation load. UTUC was the primary site for 21% of all patients and 16% of patients who were IC2/3. The ORR for IC2/3, IC1/2/3 and all patients was 26, 18, and 15%, respectively. The 12-month OS was 48% in the IC2/3 group and 36% in the intention-to-treat group compared with the historical rate of 20% in patients receiving second-line therapy. Median OS for IC2/3, IC1/2/3 and all patients was 11.4, 8.8, and 7.9 months, respectively. Durable responses were achieved in patients with UTUC as well. With a median follow up of 11.7 months, 84% of responders continued to maintain a response. PD-L1 expression, cluster subtype, and mutation load were independently associated with drug response, and elevated PD-L1 expression on immune cells was associated with longer OS. Only 16% of patients experienced grade 3/4 treatment-related AEs and 5% of patients experienced immune-mediated AEs.

Atezolizumab is an effective and well-tolerated treatment for second-line therapy in metastatic UC (including UTUC) and the US FDA granted accelerated approval for atezolizumab in 2016, for the treatment of locally advanced or metastatic UC who have disease progression during or following platinum-based chemotherapy, or whose disease has worsened within 12 months of receiving platinum-based chemotherapy before surgery (neoadjuvant) or after surgery (adjuvant). Recently another phase II trial has been published with atezolizumab in first-line patients ineligible to cisplatin. The ORR was 23%, 11 patients experienced a complete response and 19 of 27 responses were ongoing at the time of analysis. Responses occurred across all PD-L1 subgroups, and instead tumour mutation load correlated with response. Median PFS was 2.7 and median OS was 15.9 months.\(^{118} \)

At last, nivolumab, an anti-PD1, was assessed in a phase II trial (Checkmate 275) in metastatic UC patients who have received prior treatment. In this trial 270 patients were administered with nivolumab as a second-line therapy. The ORR to nivolumab was 19.6%, including seven complete responses; with 16.1% responses in patients with low or no PD-L1 expression. Median duration of response was not reached. Median PFS was reported at 2 months and median OS at 8.74 months. Among 124 patients who had PD-L1 expression in at least 1% of their tumour cells, the ORR was 25%; six of the seven complete responses occurred in this subset. Higher values of the 25-gene interferon-\( \gamma \) signature were associated with a greater proportion of responders to nivolumab and a higher PD-L1 expression. Patients with a high interferon-\( \gamma \) signature were more likely to respond to nivolumab than were those with a low interferon-\( \gamma \) signature (\( p = 0.0003 \)). The strongest interferon-\( \gamma \) signature was noted in patients with a basal 1 subtype. These patients were more likely to have a high interferon-\( \gamma \) signature score than patients with the other subtypes.\(^{119} \) Results from this trial granted accelerated approval also for this drug in February 2017.

Other immune check point inhibitors have been evaluated in UC. Durvalumab, another anti-PD-L1, demonstrated in a phase I/II trial, in the UC cohort in pretreated patients, an ORR of 31%, 46.4% in the PD-L1-positive subgroup and 0% in the PD-L1-negative subgroup. Median duration of response has not yet been reached.\(^{120} \)
Other trials with other target agents and immuno-therapy are summarized in Tables 1 and 2 respectively.

**Conclusion**

Several studies on upper tract carcinomas have shown the diagnostic, prognostic and therapeutic role of standard pathological features, such as tumour stage, grade and lymph node metastasis. Such investigations have also identified novel factors, including lymphovascular invasion, tumour architecture, multifocality, concomitant CIS, variant histology and biomarker status. Based on these, predictive tools have been developed. Molecular and genomic characterization studies on UTUC have shown changes in UTUC occurring at differing frequencies from bladder cancer, with unique molecular and clinical subtypes, potentially with different responses to treatment.
Acknowledgements
MB and RM contributed to the conception and design. AC and LF contributed to drafting of the manuscript. M Scartozzi and M Santoni contributed to the review of the literature. LC, M Scarpelli and AL-B contributed to the critical revision of the manuscript.

Funding
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Conflict of interest statement
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Table 2. Ongoing and closed trials with immunotherapy.

| Trial identifier | Phase | Target | Intervention arm | Control arm | Line | Closed |
|-----------------|-------|--------|------------------|-------------|------|--------|
| NCT02302807     | III   | PDL1   | Atezolizumab     | Vinflunine, paclitaxel or docetaxel | 2nd-line | x |
| NCT02603432     | III   | PDL1   | Avelumab + BSC after 1st-line | BSC after 1st-line | 1st-line | |
| NCT01524991     | II    | CTLA-4 | Ipilimumab + GC  |  | 1st-line | x |
| NCT02553642     | II    | PD1, CTLA-4 | Nivolumab then nivolumab + ipilimumab after progression |  | 2nd-line | |
| NCT02387996     | II    | PD1    | Nivolumab        | Placebo after 1st-line | 1st-line | |
| NCT02500121     | II    | PD1    | Pembrolizumab after 1st-line |  | 1st-line | |
| NCT02335424     | II    | PD1    | Pembrolizumab    |  | 1st-line | |
| NCT02256436     | III   | PD1    | Pembrolizumab    | Vinflunine, paclitaxel or docetaxel | 2nd-line | x |
| NCT02581982     | II    | PD1    | Pembrolizumab + paclitaxel |  | |
| NCT02351739     | II    | PD1, Bruton tyrosine kinase | Pembrolizumab + ACP-196 |  | 2nd-line | x |
| NCT02717156     | II    | PD1    | Recombinant fusion protein sEphB4-HSA + pembrolizumab |  | 2nd-line | |
| NCT02516241     | III   | PD-L1, CTLA-4 | Durvalumab, durvalumab + tremelimumab | Cisplatin, carboplatin, gemcitabine | 1st-line | |
| NCT03549715     | I/II  | PD-L1, CTLA-4 | Durvalumab + ddMVAC; durvalumab + tremelimumab + ddMVAC |  | |

BSC, best supportive care; CTLA-4, cytotoxic T lymphocyte–associated protein 4; ddMVAC, methotrexate, vinblastine, adriamycin, cisplatin; GC, gemcitabine plus cisplatin; NCT, ClinicalTrials.gov identifier; PD-1, programmed death 1; PD-L1, programmed death ligand 1.
References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7–30.

2. Rouprêt M, Babjuk M, Compérat E, et al. European Association of Urology guidelines on upper urinary tract urothelial carcinoma: 2017 update. *Eur Urol* 2018; 73: 111–122.

3. Perez-Montiel D, Wakely PE, Hes O, et al. High-grade urothelial carcinoma of the renal pelvis: clinicopathologic study of 108 cases with emphasis on unusual morphologic variants. *Mod Pathol* 2006; 19: 494–503.

4. Rink M, Robinson BD, Green DA, et al. Impact of histological variants on clinical outcomes of patients with upper urinary tract urothelial carcinoma. *J Urol* 2012; 188: 398–404.

5. Xylinas E, Rink M, Robinson BD, et al. Impact of histological variants on oncological outcomes of patients with urothelial carcinoma of the bladder treated with radical cystectomy. *Eur J Cancer* 2013; 49: 1889–1897.

6. Zhai QF, Black J, Ayala AG, et al. Histologic variants of infiltrating urothelial carcinoma. *Arch Pathol Lab Med* 2007; 131: 1244–1256.

7. Mostofi FK, Sobin LH and Torloni H. *Histological typing of urinary bladder tumours*. Geneva, Switzerland: World Health Organization, 1973.

8. Eble JN, Sauter G, Epstein JI, et al. *World Health Organization classification of tumours. Pathology and genetics of tumours of the urinary system and male genital organs*. Lyon, France: IARC Press, 2004, pp. 89–158.

9. Moch H, Cubilla AL, Humphrey PA, et al. The 2016 WHO classification of tumours of the urinary system and male genital organs—part A: renal, penile, and testicular tumours. *Eur Urol* 2016; 70: 93–105.

10. Paner GP, Stadler WM, Hansel DE, et al. Updates in the eighth edition of the tumor-node-metastasis staging classification for urologic cancers. *Eur Urol* 2018; 73: 560–569.

11. Vashistha V, Shabsigh A and Znyger DL. Utility and diagnostic accuracy of ureteroscopic biopsy in upper tract urothelial carcinoma. *Arch Pathol Lab Med* 2013; 137: 400–407.

12. Kubota Y, Hatakeyama S, Tanaka T, et al. Oncological outcomes of neoadjuvant chemotherapy in patients with locally advanced upper tract urothelial carcinoma: a multicenter study. *Oncotarget* 2017; 8: 101500–101508.

13. Fajkovic H, Cha EK, Jeldres C, et al. Prognostic value of extra nodal extension and other lymph node parameters in patients with upper tract urothelial carcinoma. *J Urol* 2012; 187: 845–851.

14. Lee HY, Li CC, Huang CN, et al. Prognostic significance of lymphovascular invasion in upper urinary tract urothelial carcinoma is influenced by tumor location. *Ann Surg Oncol* 2015; 22: 1392–1400.

15. Zhou H, Ro JY, Truong LD, et al. Intraoperative frozen section evaluation of ureteral and urethral margins: studies of 203 consecutive radical cystoprostatectomy for men with bladder urothelial carcinoma. *Am J Clin Exp Urol* 2014; 2: 156–160.

16. Khene Z-E, Mathieu R, Kammerer-Jacquet S-F, et al. Risk stratification for kidney sparing procedure in upper tract urothelial carcinoma. *Transl Androl Urol* 2016; 5: 711–719.

17. Wheat JC, Weizer AZ, Wolf JS Jr, et al. Concomitant carcinoma in situ is a feature of aggressive disease in patients with organ confined urothelial carcinoma following radical nephroureterectomy. *Urol Oncol* 2012; 30: 252–258.

18. Zigeuner R, Shariat SF, Margulis V, et al. Tumour necrosis is an indicator of aggressive biology in patients with urothelial carcinoma of the upper urinary tract. *Eur Urol* 2010; 57: 575–581.

19. Yates DR, Hupertan V, Colin P, et al. Cancer-specific survival after radical nephroureterectomy for upper urinary tract urothelial carcinoma: proposal and multi-institutional validation of a post-operative nomogram. *Br J Cancer* 2012; 106: 1083–1088.

20. Moschini M, D’Andrea D, Korn S, et al. Characteristics and clinical significance of histological variants of bladder cancer. *Nat Rev Urol* 2017; 14: 651–668.

21. Favaretto RL, Zequi SC, Oliveira RAR, et al. Tissue-based molecular markers in upper tract urothelial carcinoma and their prognostic implications. *Int Braz J Urol* 2018; 44: 22–37.

22. Rouprêt M, Azzouzi AR and Cussenot O. Microsatellite instability and transitional cell
Therapeutic Advances in Urology

23. Krabbe LM, Bagrodia A, Haddad AQ, et al. Multi-institutional validation of the predictive value of Ki-67 in patients with high grade urothelial carcinoma of the upper urinary tract. BJU Int 2005; 96: 489–492.

24. Bagrodia A, Cha EK, Sfakianos JP, et al. Genomic biomarkers for the prediction of stage and prognosis of upper tract urothelial carcinoma. J Urol 2016; 195: 1684–1689.

25. Sfakianos JP, Cha EK, Iyer G, et al. Genomic characterization of upper tract urothelial carcinoma. Eur Urol 2015; 68: 970–977.

26. von der Maase H, Hansen SW, Roberts JT, et al. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study. J Clin Oncol 2000; 18: 3068–3077.

27. von der Maase H, Sengelov L, Roberts JT, et al. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J Clin Oncol 2005; 23: 4602–4608.

28. Birtle AJ, Chester JD, Jones RJ, et al. Results of POUT: a phase III randomized trial of perioperative chemotherapy versus surveillance in upper tract urothelial cancer (UTUC). J Clin Oncol 2018; 36(Suppl. 6): 407–407.

29. Galsky MD, Hahn NM, Rosenberg J, et al. A consensus definition of patients with metastatic urothelial carcinoma who are unfit for cisplatin-based chemotherapy. Lancet Oncol 2011; 12: 211–214.

30. Loehrer PJ Sr, Einhorn LH, Elson PJ, et al. A randomized comparison of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: a cooperative group study. J Clin Oncol 1992; 10: 1066–1073.

31. Bellmunt J, von der Maase H, Mead GM, et al. Randomized phase III study comparing paclitaxel/cisplatin/gemcitabine and gemcitabine/cisplatin in patients with locally advanced or metastatic urothelial cancer without prior systemic therapy EORTC Intergroup Study 30987. Int J Cancer 2012; 30: 1107–1113.

32. Witte RS, Elson P, Bono B, et al. Eastern Cooperative Oncology Group phase II trial of ifosfamide in the treatment of previously treated advanced urothelial carcinoma. J Clin Oncol 1997; 15: 589–593.

33. de Wit R, Kruit WH, Stoter G, et al. Docetaxel (Taxotere): an active agent in metastatic urothelial cancer; results of a phase II study in non-chemotherapy-pretreated patients. Br J Cancer 1998; 78: 1342–1345.

34. McCaffrey JA, Hilton S, Mazumdar M, et al. Phase II trial of docetaxel in patients with advanced or metastatic transitional-cell carcinoma. J Clin Oncol 1997; 15: 1853–1857.

35. De Santis M, Bellmunt J, Mead G, et al. Randomized phase II/III trial assessing gemcitabine/carboplatin and methotrexate/carboplatin/vinblastine in patients with advanced urothelial cancer who are unfit for cisplatin-based chemotherapy: EORTC study 30986. J Clin Oncol 2012; 30: 191–199.

36. Dogliotti L, Carteni G, Siena S, et al. Gemcitabine plus cisplatin versus gemcitabine plus carboplatin as first-line chemotherapy in advanced transitional cell carcinoma of the urothelium: results of a randomized phase 2 trial. Eur Urol 2007; 52: 134–141.
42. Sternberg CN, Calabrò F, Pizzocaro G, et al. Chemotherapy with an every-2-week regimen of gemcitabine and paclitaxel in patients with transitional cell carcinoma who have received prior cisplatin-based therapy. *Cancer* 2001; 92: 2993–2998.

43. Meluch AA, Greco FA, Burris HA 3rd, et al. Paclitaxel and gemcitabine chemotherapy for advanced transitional-cell carcinoma of the urothelial tract: a phase II trial of the Minnie pearl cancer research network. *J Clin Oncol* 2001; 19: 3018–3024.

44. Li J, Juliar B, Yiannoutsos C, et al. Weekly paclitaxel and gemcitabine in advanced transitional-cell carcinoma of the urothelium: a phase II Hoosier Oncology Group study. *J Clin Oncol* 2005; 23: 1185–1191.

45. Calabrò F, Lorusso V, Rosati G, et al. Gemcitabine and paclitaxel every 2 weeks in patients with previously untreated urothelial carcinoma. *Cancer* 2009; 115: 2652–2659.

46. Gitlitz BJ, Baker C, Chapman Y, et al. A phase II study of gemcitabine and docetaxel therapy in patients with advanced urothelial carcinoma. *Cancer* 2003; 98: 1863–1869.

47. Ardavanis A, Tryfonopoulos D, Alexopoulos A, et al. Gemcitabine and docetaxel as first-line treatment for advanced urothelial carcinoma: a phase II study. *Br J Cancer* 2005; 92: 645–650.

48. Bellmunt J, Theodore C, Demkov T, et al. Phase III trial of vinflunine plus best supportive care compared with best supportive care alone after a platinum-containing regimen in patients with advanced transitional cell carcinoma of the urothelial tract. *J Clin Oncol* 2009; 27: 4454–4461.

49. Bellmunt J, Choueiri TK, Fougeray R, et al. Prognostic factors in patients with advanced transitional cell carcinoma of the urothelial tract experiencing treatment failure with platinum-containing regimens. *J Clin Oncol* 2010; 28: 1850–1855.

50. Sonpavde G, Pond GR, Fougeray R, et al. Time from prior chemotherapy enhances prognostic risk grouping in the second-line setting of advanced urothelial carcinoma: a retrospective analysis of pooled, prospective phase 2 trials. *Eur Urol* 2013; 63: 717–723.

51. Han KS, Joung JY, Kim TS, et al. Methotrexate, vinblastine, doxorubicin and cisplatin combination regimen as salvage chemotherapy for patients with advanced or metastatic transitional cell carcinoma after failure of gemcitabine and cisplatin chemotherapy. *Br J Cancer* 2008; 98: 86–90.

52. Edeline J, Loriot Y, Culin S, et al. Accelerated MVAC chemotherapy in patients with advanced bladder cancer previously treated with a platinum-gemcitabine regimen. *Eur J Cancer* 2012; 48: 1141–1146.

53. Vaughn DJ, Broome CM, Hussain M, et al. Phase II trial of weekly paclitaxel in patients with previously treated advanced urothelial cancer. *J Clin Oncol* 2002; 20: 937–940.

54. Pronzato P, Vigani A, Pensa F, et al. Second line chemotherapy with ifosfamide as outpatient treatment for advanced bladder cancer. *Am J Clin Oncol* 1997; 20: 519–521.

55. Galsky MD, Mironov S, Iasonos A, et al. Phase II trial of pemetrexed as second-line therapy in patients with metastatic urothelial carcinoma. *Invest New Drugs* 2007; 25: 265–270.

56. Sweeney CJ, Roth BJ, Kabbinavar FF, et al. Phase II study of pemetrexed for second-line treatment of transitional cell cancer of the urothelium. *J Clin Oncol* 2006; 24: 3451–3457.

57. Sridhar SS, Canil CM, Mukherjee SD, et al. Results of a phase II study of single-agent nab-paclitaxel in platinum-refractory second-line metastatic urothelial carcinoma. *J Clin Oncol* 2011; 29(Suppl. 7): 241–241.

58. Sonpavde G, Pond GR, Choueiri TK, et al. Single-agent taxane versus taxane-containing combination chemotherapy as salvage therapy for advanced urothelial carcinoma. *Eur Urol* 2016; 69: 634–641.

59. Soga N, Onishi T, Arima K, et al. Paclitaxel carboplatin chemotherapy as a second-line chemotherapy for advanced platinum resistant urothelial cancer in Japanese cases. *Int J Urol* 2007; 14: 828–832.

60. Suyama T, Ueda T, Fukasawa S, et al. Combination of gemcitabine and paclitaxel as second-line chemotherapy for advanced urothelial carcinoma. *Jpn J Clin Oncol* 2009; 39(P): 241–241.

61. Albers P, Park SI, Niegisch G, et al. Randomized phase III trial of 2nd line gemcitabine and paclitaxel chemotherapy in patients with advanced bladder cancer: short-term versus prolonged treatment [German Association of Urological Oncology (AUO) trial AB 20/99]. *Ann Oncol* 2011; 22: 288–294.

62. Guo G, Sun X, Chen C, et al. Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved...
75. Bellmunt J, Gonzalez-Larriba JL, Prior C, et al. Phase II study of sunitinib as first-line treatment of urothelial cancer patients ineligible to receive cisplatin-based chemotherapy: baseline interleukin-8 and tumor contrast enhancement as potential predictive factors of activity. Ann Oncol 2011; 22: 2646–2653.

76. Grivas PD, Daignault S, Tagawa ST, et al. Double-blind, randomized, phase 2 trial of maintenance sunitinib versus placebo after response to chemotherapy in patients with advanced urothelial carcinoma. Cancer 2014; 120: 692–701.

77. Sridhar SS, Winquist E, Eisen A, et al. A phase II trial of sorafenib in first-line metastatic urothelial cancer: a study of the PMH Phase II Consortium. Invest New Drugs 2011; 29: 1045–1049.

78. Dreicer R, Li H, Stein M, et al. Phase 2 trial of sorafenib in patients with advanced urothelial cancer: a trial of the Eastern Cooperative Oncology Group. Cancer 2009; 115: 4090–4095.

79. Pili R, Qin R, Flynn PJ, et al. A phase II safety and efficacy study of the vascular endothelial growth factor receptor tyrosine kinase inhibitor pazopanib in patients with metastatic urothelial cancer. Clin Genitourin Cancer 2013; 11: 477–483.

80. Necchi A, Mariani L, Zaffaroni N, et al. Pazopanib in advanced and platinum resistant urothelial cancer: an open-label, single group, phase 2 trial. Lancet Oncol 2012; 13: 810–816.

81. Gerullis H, Eimer C, Ecke TH, et al. Combined treatment with pazopanib and vinflunine in patients with advanced urothelial carcinoma refractory after first-line therapy. Anticancer Drugs 2013; 24: 422–425.

82. Petrylak DP, Tagawa ST, Kohli M, et al. Docetaxel as monotherapy or combined with ramucirumab or irucumab in second-line treatment for locally advanced or metastatic urothelial carcinoma: an open-label, three-arm,
randomized controlled phase II trial. *J Clin Oncol* 2016; 4: 1500–1509.

83. Bellmunt J, Hussain M and Dinney CP. Novel approaches with targeted therapies in bladder cancer: therapy of bladder cancer by blockade of the epidermal growth factor receptor family. *Crit Rev Oncol Hematol* 2003; 46(Suppl.): S85–S104.

84. Nutt JE, Lazarowicz HP, Mellon JK, *et al.* Gefitinib (“Iressa,” ZD1839) inhibits the growth response of bladder tumour cell lines to epidermal growth factor and induces TIMP2. *Br J Cancer* 2004; 90: 1679–1685.

85. Pruthi RS, Nielsen M, Heathcote S, *et al.* A phase II trial of neoadjuvant erlotinib in patients with muscle-invasive bladder cancer undergoing radical cystectomy: clinical and pathological results. *BJU Int* 2010; 106: 349–354.

86. Petrylak DP, Tangen CM, Van Veldhuisen PJ Jr, *et al.* Results of the Southwest Oncology Group phase II evaluation (study S0031) of ZD1839 for advanced transitional cell carcinoma of the urothelium. *BJU Int* 2010; 105: 317–321.

87. Philips GK, Halabi S, Sanford BL, *et al.* A phase II trial of cisplatin (C), gemcitabine (G) and gefitinib for advanced urothelial tract carcinoma: results of Cancer and Leukemia Group B (CALGB) 90102. *Ann Oncol* 2009; 20: 1074–1079.

88. Philips GK, Halabi S, Sanford BL, *et al.* A phase II trial of cisplatin, fixed dose-rate gemcitabine and gefitinib for advanced urothelial tract carcinoma: results of the Cancer and Leukemia Group B 90102. *BJU Int* 2008; 101: 20–25.

89. Hussain M, Daignault S, Agarwal N, *et al.* A randomized controlled phase 2 trial of gemcitabine/cisplatin with or without cetuximab in patients with advanced urothelial carcinoma. *Cancer* 2014; 120: 2684–2693.

90. Wong YN, Litwin S, Vaughn D, *et al.* Phase II trial of cetuximab with or without paclitaxel in patients with advanced urothelial tract carcinoma. *J Clin Oncol* 2012; 30: 3545–3551.

91. Inoue K, Slaton JW, Perrotte P, *et al.* Paclitaxel enhances the effects of the antiepidermal growth factor receptor monoclonal antibody ImClone C225 in mice with metastatic human bladder transitional cell carcinoma. *Clin Cancer Res* 2000; 6: 4874–4884.

92. Choueiri TK, Ross RW, Jacobus S, *et al.* Double-blind, randomized trial of docetaxel plus vandetanib versus docetaxel plus placebo in platinum-pretreated metastatic urothelial cancer. *J Clin Oncol* 2012; 30: 507–512.

93. Hussain MH, MacVicar GR, Petrylak DP, *et al.* Trastuzumab, paclitaxel, carboplatin, and gemcitabine in advanced human epidermal growth factor receptor-2/ neu-positive urothelial carcinoma: results of a multicenter phase II National Cancer Institute trial. *J Clin Oncol* 2007; 25: 2218–2224.

94. Oudard S, Culine S, Viellefond A, *et al.* Multicenter randomized phase 2 trial of gemcitabine-platinum with or without trastuzumab (T) in advanced/metastatic urothelial carcinoma (a/mUC) with HER2 overexpression. *ESMO Congress* 2012; abstract 7860.

95. Wulfing C, Machiels JP, Richel DJ, *et al.* A single-arm, multicenter, open-label phase 2 study of lapatinib as the second-line treatment of patients with locally advanced or metastatic transitional cell carcinoma. *Cancer* 2009; 115: 2881–2890.

96. Narayan V, Mamtani R, Keeffe S, *et al.* Cisplatin, gemcitabine, and lapatinib as neoadjuvant therapy for muscle-invasive bladder cancer. *Cancer Res Treat* 2016; 48: 1084–1091.

97. Choudhury NJ, Campanile A, Antic T, *et al.* Afatinib activity in platinum-refractory metastatic urothelial carcinoma in patients with ERBB alterations. *J Clin Oncol* 2016; 34: 2165–2171.

98. Bajorin D (Principal Investigator). Buparlisib in metastatic transitional cell carcinoma of the urothelium, http://clinicaltrials.gov/show/NCT01551030 (accessed April 2018).

99. Munster PN, van der Noll R, Voest EE, *et al.* Phase I first-in-human study of the PI3 kinase inhibitor GSK2126458 (GSK458) in patients with advanced solid tumors (study P3K112826). *JCO* 2011; 29: 3016.

100. Gomez-Pinillos A and Ferrari AC. mTOR signaling pathway and mTOR inhibitors in cancer therapy. *Hematol Oncol Clin North Am* 2012; 26: 483–505.

101. Milowsky MI, Iyer G, Regazzi AM, *et al.* Phase II study of everolimus in metastatic urothelial cancer. *BJU Int* 2013; 112: 462–470.

102. Seront E, Rottey S, Sautois B, *et al.* Phase II study of everolimus in patients with locally advanced or metastatic transitional cell carcinoma of the urothelial tract: clinical activity, molecular response, and biomarkers. *Ann Oncol* 2012; 23: 2663–2670.
103. Iyer G, Hanrahan AJ, Milowsky MJ, et al. Genome sequencing identifies a basis for everolimus sensitivity. *Science* 2012; 338: 221.

104. Seront E, Pinto A, Bouzin C, et al. PTEN deficiency is associated with reduced sensitivity to mTOR inhibitor in human bladder cancer through the unhampered feedback loop driving PI3K/Akt activation. *Br J Cancer* 2013; 109: 1586–1592.

105. Wagle N, Grabiner BC, Van Allen EM, et al. Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. *Cancer Discov* 2014; 4: 546–553.

106. Seront E, Rottey S, Filleul B, et al. Phase II study of dual phosphoinositol-3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibitor BEZ235 in patients with locally advanced or metastatic transitional cell carcinoma. *BJU Int* 2016; 118: 408–415.

107. Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646–674.

108. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. *Science* 2013; 342: 1432–1433.

109. Carthon BC, Wolchok JD, Yuan J, et al. Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. *Clin Cancer Res* 2010; 16: 2861–2871.

110. Galsky MD, Wang H, Hahn NM, et al. Phase 2 trial of gemcitabine, cisplatin, plus ipilimumab in patients with metastatic urothelial cancer and impact of DNA damage response gene mutations on outcomes. *Eur Urol* 2018; 73: 751–759.

111. Plimack ER, Bellmunt J, Gupta S, et al. (2015) Pembrolizumab (MK-3475) for advanced urothelial cancer: updated results and biomarker analysis from KEYNOTE-012. *ASCO Meet Abstr* 2015; 33(Suppl. 15): 4502.

112. Balar A, Bellmunt J, O’Donnell PH, et al. Pembrolizumab (pembro) as first-line therapy for advanced/unresectable or metastatic urothelial cancer: preliminary results from the phase 2 KEYNOTE-052 study. *Ann Oncol* 2016; 27(Suppl. 6): LBA32_PR.

113. Bellmunt J, de Wit R, Vaughn DJ, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N Engl J Med* 2017; 376: 1015–1026.

114. Apolo AB, Infante JR, Hamid O, et al. Safety, clinical activity, and PD-L1 expression of avelumab (MSB001078C), an anti-PD-L1 antibody, in patients with metastatic urothelial carcinoma from the JAVELIN solid tumor phase Ib trial. *ASCO Meet Abstr* 2016; 34: 367.

115. Patel MR, Ellerton J, Agrawal M, et al. Avelumab (MSB001078C; anti-PD-L1) in patients with metastatic urothelial carcinoma progressed after platinum-based therapy or platinum ineligible. *Ann Oncol* 2016; 27(Suppl. 6): 777PD.

116. Patel MR, Ellerton JA, Infante JR, et al. Avelumab in patients with metastatic urothelial carcinoma: pooled results from two cohorts of the phase 1b JAVELIN Solid Tumor trial. *J Clin Oncol* 2017; 35(Suppl. 6S): abstract 330.

117. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016; 387: 1909–1920.

118. Balar AV, Galsky MD, Rosenberg JE, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017; 389: 67–76.

119. Sharma P, Retz M, Siefker-Radtke A, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. *Lancet Oncol* 2017; 18: 312–322.

120. Massard C, Gordon MS, Sharma S, et al. Safety and efficacy of durvalumab (MEDI4736), an anti-programmed cell death ligand-1 immune checkpoint inhibitor, in patients with advanced urothelial bladder cancer. *J Clin Oncol* 2016; 34: 3119–3125.