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BLADDER AND UROTHELIAL CANCER (A SAGALOWSKY, SECTION EDITOR)

# Recent Advances in Treatment of Advanced Urothelial Carcinoma

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**Abstract** GC (cisplatin and gemcitabine) and MVAC (methotrexate, vinblastine, Adriamycin [doxorubicin], and cisplatin) have been the standard systemic chemotherapy in advanced urothelial carcinoma. These regimens have shown significant response rates in this patient population. Nevertheless, disease does recur with most patients who unfortunately do succumb to the disease. Research efforts are focused in several different areas of therapy, targeted therapy, and immunotherapy. Further efforts include those in improving understanding of the molecular biology of urothelial carcinoma which may lead development of biomarkers that may enhance therapeutic index. This paper reviews recent advances in the treatment and ongoing study of molecular biology of urothelial carcinoma.

**Keywords** Urothelial carcinoma · Chemotherapy · Targeted therapy · Immunotherapy · Biomarker

## Introduction

In the 1980s, platinum chemotherapy showed significant response in metastatic urothelial carcinoma. Since then, research efforts have established platinum-based combination chemotherapy to be the standard front-line systemic therapy for patients with advanced urothelial carcinoma. These combinations are associated with response rates up to 70% in patients, with 10%–20% of patients showing complete response. However, disease ultimately recurs in most patients, and leading to death in majority of these patients not too long after disease recurrence. Second-line chemotherapeutic agents are associated with response rates up to 20% with modest progression-free survival (PFS) ranging from 2–4 months, leaving much to be desired in the treatment of these patients. To this end, recent efforts involve diversified areas of study, which mirror efforts and advances seen in other tumor types. Broadly, these areas involve further investigations in cytotoxic chemotherapy, targeted therapy including the vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) pathways, and lastly, immunotherapy. Most of the studies in the latter 2 categories are in their early stages and will require time to mature for final and more definitive results. Furthermore, efforts are also concentrated in the study of biology of urothelial carcinoma, including biomarker development for prognostic as well as predictive utility.

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## Cytotoxic Chemotherapy

Currently, platinum-based regimens are standard chemotherapy in the treatment of advanced bladder cancer: GC (cisplatin and gemcitabine) and MVAC (methotrexate, vinblastine, Adriamycin [doxorubicin], and cisplatin). Dose-dense MVAC has shown the best response rate among all platinum-based combination therapy, although toxicities of the MVAC regimen have maneuvered many in the oncologic community to GC. Although with significant initial response up to 70% in patients, most patients eventually do relapse after first-line chemotherapy and become in need of second-line chemotherapy. Several single agents that have been tested have demonstrated response rates up to 20% and

PFD benefit of less than 3 months. These agents include vinflunine, pemetrexed, single-agent taxanes, ifosfamide, and oxaliplatin.

Vinflunine is the only agent to have been evaluated in large phase 3 setting [1•]. In this study, 370 previously treated patients with advanced bladder cancer were randomly assigned to vinflunine and best supportive care. Objective response was seen in 9% of patients with a survival benefit of 2.3 months, although it did not reach statistical significance (6.9 vs 4.6 months; HR 0.88; 95% CI, 0.69–1.12). Vinflunine is approved in European community for second-line treatment in bladder cancer. Pemetrexed, an antifolate that has been studied in a phase 2 setting, showed modest response rate of 6% with median survival of 10 months [2, 3]. Pemetrexed also has been studied in combination with gemcitabine without showing improvement in efficacy compared to single-agent gemcitabine [4]. Pemetrexed is currently approved in the second-line setting as a single agent in urothelial carcinoma. Ixabepilone and eribulin also are newer chemotherapeutic agents that have shown some activity in urothelial cancer in second-line setting, although further definitive studies are required to establish their role in the treatment of this patient population.

Abraxane (Celgene Corporation, Summit, NJ), a nanoparticle albumin-bound paclitaxel, also was studied in a phase 2 setting, the results of which were reported at the annual American Society of Clinical Oncology genitourinary cancer symposium in 2011 [5]. This study showed 33% with objective response and 58% of patients having either an objective response or stable disease. Although interesting, for definitive results, larger studies will be needed to evaluate its efficacy compared to standard paclitaxel. Abraxane is also being evaluated in other stage settings such as for intravesical therapy in BCG (Bacillus Calmette-Guérin) refractory cancers.

## Targeted Therapy

### VEGF Inhibitor Therapy

As with other solid tumors, the rationale of tumors necessitating blood vessels for growth and metastasis have shown to be likewise pertinent in the progression of bladder cancer. Preclinical studies as well as retrospective studies have shown significant correlation between degree of microvessel density as well as VEGF expression with prognosis of patients with advanced bladder cancer. Building upon these studies, clinical trials involving antiangiogenic therapy added to a platinum-containing chemotherapy backbone have shown success in phase 2 settings and moving forward [6••, 7]. One such example is bevacizumab, a monoclonal antibody of VEGF. In a phase 2 clinical trial involving 43 patients in metastatic setting in combination with GC, there were complete response in 9 patients (21%) and partial response in 22 (51%), resulting in an overall response rate of 72% [6••]. Furthermore, stable disease was observed in seven patients (16%). With a median followup of 27.2 months, median progression survival was 8.2 months with a median overall survival of 20.4 months. Side-effect profile was not insignificant, however, with grade 3/4 hematologic toxicity including neutropenia in 35%, thrombocytopenia in 12%, anemia in 12%, and neutropenic fever in 2%. Grade 3/4 nonhematologic toxicity included deep vein thrombosis/pulmonary embolism in 21%, hemorrhage in 7%, hypertension in 5%, and proteinuria in 2%. Three treatment-related deaths (one central nervous system hemorrhage, one sudden cardiac death, and one aortic dissection) were observed. A phase 3 intergroup randomized study is underway to further investigate the efficacy as well as side-effect profile of the addition of bevacizumab to GC chemotherapy in chemo-naïve advanced bladder cancer patients [7].

Sunitinib is a multikinase inhibitor (including VEGF), and its effect in bladder cancer patients also has been tested. In a phase 2 study involving 37 cisplatin-ineligible patients, sunitinib monotherapy showed partial remission (PR) in 3 patients (8%), stable disease in 14 patients (53.8%) lasting more than 3 months with clinical benefit rate of 62%, and median PFS of 5.9 months [8•]. A larger study will be needed to confirm its activity, in which case, antiangiogenic agent may prove to be an alternative modality and/or a modality via which systemic chemotherapy can be delayed in "unfit" patients with advanced bladder cancer. Sunitinib also has been tested in relapsed or refractory patients, with activity in these settings with modest PFS of 2 months [9]. Sunitinib also has been combined with GC in the front-line setting in patients with advanced bladder cancer [10] although this combination proved to be intolerable. Another multikinase inhibitor of angiogenesis, sorafenib, in combination with GC was evaluated, unfortunately without significant improvement in efficacy compared to GC backbone [11].

Furthermore, everolimus has been evaluated in second-line setting based on the observation that there is overexpression of activated mammalian target of rapamycin (mTOR) pathway markers including phosphor-S6 and phosphor-4E bP1 in invasive transitional cell carcinoma specimens [12]. The study showed median PFS of 3.3 months, which is comparable with currently available second-line chemotherapy [13].

Results from the mentioned series of phase 2 trials do suggest the relevance of inhibition of angiogenesis blockade in the treatment of advanced bladder cancer, although some agents are better tolerated than others and some are more efficacious than others. Several trials involving other angiogenic factors, including fibroblast growth factor receptor

(FGFR) inhibitor [14•], are ongoing. Importantly, a randomized phase 3 intergroup trial evaluating the efficacy of bevacizumab is currently ongoing accrual as mentioned above and results will shed more definitive light on the efficacy of addition of antiangiogenic therapy to existing systemic chemotherapy backbone.

### EGFR Pathway

Preclinical studies showed that EGFR regulates normal urothelial regeneration [15], and further studies showed that overexpression of EGFR in urothelium elicited urothelial hyperplasia and promoted bladder tumor growth [16]. Furthermore, EGFR was shown to be overexpressed in bladder tumor, and the degree of EGFR expression also has been shown to be associated with poorer prognosis and advanced stage and grade, which provided rationale for targeting the EGFR pathway in the treatment of urothelial carcinoma [17, 18].

Cetuximab is a monoclonal antibody to EGFR and is being studied in combination with existing chemotherapy in advanced bladder cancer. A phase 2 randomized study in second-line setting with or without paclitaxel was conducted. The cetuximab arm closed after 9 of the first 11 patients progressed by 8 weeks. Of the 39 patients enrolled in the study, overall response rate of 28.5% was observed in the combination arm. Four additional patients had unconfirmed PR. Median PFS for the cetuximab–paclitaxel arm was 115 days (16 weeks [95% CI; 58–174 days). Grade 3 adverse events occurring in more than two patients were rash ($n$=5), fatigue ($n$=4), anemia ($n$=4), and low magnesium ($n$=3). This study showed that EGFR inhibition with cetuximab appears to augment the antitumor activity of paclitaxel in patients with previously treated urothelial cancers, warranting further study [19]. Cetuximab is also being examined in front-line setting in combination with GC in a study for which accrual is complete and results are awaited [20].

Gefitinib, an EGFR tyrosine kinase inhibitor (TKI), is also undergoing testing in urothelial cancer in various settings with some disappointing results. In second-line setting, it showed minimal activity in phase 2 trial, with only 1 partial response out of 31 patients and estimated median survival of 3 months [21]. Gefitinib also has been combined with cytotoxic chemotherapy GC in several phase 2 studies, one of which closed prematurely due to excessive toxicity, another showing similar response rate and overall survival compared to placebo arm [22, 23]. Role of gefitinib as a maintenance therapy after optimal response to systemic chemotherapy is also undergoing evaluation. This study has completed accrual and results are not yet available [24].

HER2/neu overexpression provided rationale for the investigation of trastuzumab in urothelial carcinoma in recent years as well. It has been combined with carboplatin, gemcitabine and paclitaxel combination regimen in HER-2/neu overexpressers by immunohistochemistry or fluorescent in situ hybridization [25]. This study showed response rate of 70%, progression survival of 7 months, and overall survival of 14 months, results of which are not dissimilar from the combination chemotherapy without trastuzumab. Another phase 2 study of trastuzumab as a single agent has completed accrual and results are pending [26].

Other agents include erlotinib, a TKI to HER1 and HER2, is also being evaluated in both neoadjuvant and adjuvant setting in muscle-invasive bladder cancer [27]. Furthermore, lapatinib, which blocks both HER2 and EGFR, is also being investigated in front-line as well as second-line setting in combination with standard chemotherapy and results are pending [28].

## Immunotherapy

Immunotherapy has been an attractive modality of therapy in many solid tumors for many decades and currently has gained significant momentum in the treatment of many solid tumors, including prostate cancer (with the first ever vaccine approved for cancer treatment), renal cell cancer, melanoma, and breast cancer among others. Bladder cancer is a tumor type in which immunotherapy has been proven to serve a role. In preclinical studies, BCG induced tumor regression in mice models before transplantation of tumor cells as compared to mice that did not receive BCG, an observation that led to the first ever use of nonspecific immunotherapy: use of BCG in non–muscle-invasive bladder cancer. Built upon that success, immunotherapy has been continually studied in the treatment of bladder cancer including, most recently, vaccines and immune checkpoint inhibitors.

### Vaccines

Several vaccines utilizing tumor cell antigens as relevant targets are undergoing testing in urothelial carcinoma. NY-ESO-1: is a cancer testis (CT) antigen, which has been shown to be expressed in up to 50% of patients with high-grade tumors of the bladder [29]. It is a highly immunogenic member of the CT antigen family, with up to 50% of patients whose tumors express NY-ESO-1 antigen mounting spontaneous humoral and cellular immunity [30]. Furthermore, tumor-infiltrating CD8-expressing T cells have been shown to be predictive of survival in patients, thereby providing further support relevancy of vaccine therapy in urothelial cancer [31, 32]. Based on the retrospective studies and preclinical studies described, NY-EXO-1 vaccine was evaluated in patients whose tumor expressed the NY-ESO-1 antigen in the adjuvant setting [33]. The vaccine was given in combination with intradermal BCG as well as subcutaneous granulocyte–monocyte colony-

stimulating factor and was well tolerated. Of the six patients treated in the adjuvant setting, all developed antigen-specific immune responses, including antibody formation and/or CD8 or CD4 T cell responses.

Another vaccine utilizes the CT antigen melanoma-associated antigen 3 (MAGE-A3) as its target. One approach is using a peptide vaccine loaded onto autologous dendritic cells and another is a recombinant MAGE-A3 protein. Both strategies have shown evidence of immune responses in the treated patients, although at this time its clinical efficacy and utility have not been determined. Lastly, a dendritic cell vaccine in patients whose tumor expresses HER2 is also undergoing clinical testing in the adjuvant setting; accrual is ongoing.

### Immune Checkpoint Inhibitors

The past decade has seen a promising new area of investigation in cancer immunotherapy: targeting of immune modulatory elements of the adaptive immune response. Rationale for the development of these immune modulatory agents (checkpoint inhibitors) are based on the studies that have shown that T cells recognize antigens associated with the major histocompatibility complex as the first signal, but that additional signals via coreceptors are required for optimal T cell recognition and generation of a potent and long-lasting T cell immune response. These additional signals for optimal T cell priming involve agonist coreceptors, such as CD28, 4-1BB, and OX40, and inhibitory coreceptors, such as cytotoxic T-lymphocyte antigen-4 (CTLA-4).

Anti–CTLA-4 antibodies (eg, ipilimumab) have met with success in other solid tumors such as melanoma, gaining regulatory approval by the U.S. Food and Drug Administration in 2011. Currently, overcoming immune-inhibitory effects using immune checkpoint inhibitors are also undergoing testing in urothelial carcinoma. A study involving ipilimumab administration before cystectomy is ongoing, with primary end point of safety and secondary end point of identifying immunologic markers in both the tumor tissue as well as from peripheral blood correlated with drug dosing [34]. Traditionally considered to be an immunogenic tumor with the success shown with BCG, we hope to see further success with specific immune-targeting agents in the future in various settings of urothelial cancer, both in combination with existing cytotoxic chemotherapy as well as combination of immunotherapeutic agents.

## Biomarkers

Aside from efforts in advancing therapeutic options in urothelial cancer, similar efforts are ongoing in discovering patient specific biomarkers in the hopes of maximizing therapeutic index. One novel method of selecting agents is the coexpression extrapolation (COXEN), which evaluates differential gene expression in a set of cell lines with known sensitivity, which is then used to extrapolate the signature of an unknown patient sample [35]. Stemming from its success in predicting sensitivity to either cisplatin or paclitaxel in the past, the interest is high in the bladder cancer community in validating and utilizing the COXEN algorithm in directing selection of therapeutic agents in the treatment of patients with urothelial carcinoma. Further information is available at www.COXEN.org.

Mutation status of *p53* tumor suppressor and its role as a biomarker in urothelial carcinoma remains unclear at this time. Several studies have shown conflicting results: some suggest that *p53* mutation is correlated with poor prognosis and resistance to MVAC chemotherapy, where other studies suggest poor prognosis but increased sensitivity to MVAC or similar combination regimen [36–40]. Other studies did not show any correlation in predicting regimen sensitivity or in prognostication [41•]. Whether *p53* mutation status will serve either as a predictive or prognostic tool remains to be determined. Other potential biomarkers include multidrug resistance p-glycoprotein, multidrug resistance–associated protein, glutathione, metallothioneins, and ERCC-1, but further studies are needed to validate these markers to establish their potential clinical utility [42–47].

## Conclusions

Platinum-based cytotoxic chemotherapy remains the first-line mainstay of treatment of urothelial carcinoma in various settings, including neoadjuvant, adjuvant, and metastatic disease. Several agents have been studied and are under investigation in second-line setting, with modest benefit and much to be desired in making strides against this disease. Recently, many other avenues of therapies are also being explored, including targeted therapy evaluating molecular pathway inhibition, such as the EGFR and VEGFR pathways, in the hopes of improving prognosis in patients with urothelial cancer, although striking results have not yet been seen and additional studies are currently ongoing, with their respective results awaited. Immunotherapy constitutes another area of focus in therapeutic strategy of urothelial carcinoma which poses an intriguing and cautious promise for the future. Lastly, studies have evaluated different molecular markers such as *p53* mutation status and drug resistance–associated proteins as potential predictive and/or prognostic biomarkers, mostly with conflicting results. The most recent addition to potential biomarker is the COXEN model, molecular profiling of patients for unique patient-directed therapy, which may be used as a tool for

maximizing clinical benefit with optimal therapeutic index. Further studies with larger cohorts of patients plus well-designed clinical trials with prospective validation would be needed in establishing their clinical utility.

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## References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance


1. • Bellmunt J, Théodore 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(27):4454. *The only phase 3 trial in a second-line setting that evaluated vinflunine is reported in this paper.*
2. 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.
3. 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.
4. Von der Maase H, Lehmann J, Gravis G, et al. A phase II trial of pemetrexed plus gemcitabine in locally advanced and/or metastatic transitional cell carcinoma of the urothelium. Ann Oncol. 2006;17:1533.
5. 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;suppl 7, abstract 241.
6. •• Hahn NM, Stadler WM, ZOn RT et al. Phase II Trial of Cisplatin, Gemcitabine, and bevacizumab as first line therapy for metastatic urothelial carcinoma: hoosier oncology Group GU 04-75. J Clin Oncol. 2011. *The authors describe a phase 2 study evaluating bevacizumab in combination with chemotherapy. An intergroup trial based on this study is ongoing.*
7. National Institutes of Health Clinical Trials database. http://www.clinicaltrials.gov/. Accessed on September 2011.
8. • Bellmunt J, Gonzalez-Larriba JL, Maroto JP et al. First-line treatment with sunitinib monotherapy in patients with advanced urothelial cancer ineligible for cisplatin-based chemotherapy: Pretreatment levels of IL8 and Hounsfield units as predictors of clinical benefit. J Clin Oncol 28:15s, 2010 (suppl; abstr 4540). *This article delineates the efficacy of VEGFR TKI in urothelial carcinoma.*
9. Gallagher DJ, Millowsky MI, Gerst SR, et al. A phase II study of sunitinib on a continuous dosing schedule in patients with relapsed or refractory urothelial carcinoma (UC). J Clin Oncol. 2009;27:15s. suppl; abstr 5072.
10. Galsky MD, Sonpavde G, Hellerstedt BA, et al. Phase II study of gemcitabine, cisplatin, and sunitinib in patients with advanced urothelial carcinoma. J Clin Oncol. 2010;28:15s. suppl; abstr 4573.
11. Krege S, Rexer H, vom Dorp F, et al. Gemcitabine and cisplatin with or without sorafenib in urothelial carcinoma (AUO-AB 31/05). J Clin Oncol. 2010;28:15s. suppl; abstr 4574.
12. Milowsky MI, Tickoo SK, Gallagher DJ et al. HIF and mTOR pathways in invasive urothelial carcinoma: A study with potential therapeutic implications. J Clin Oncol. 2008. Abstr 292
13. Milowsky MI, Regazzi AM, Garcia-Grossman IR et al. Final results of a phase II study of everolimus (RAD001) in metastatic transitional cell carcinoma (TCC) of the urothelium. J Clin Oncol. 2011;29. (suppl; abstr 4606).
14. • Milowsky MI, Carlson GL, Shi MM et al. A multicenter, open-label phase II trial of dovitinib (TKI1258) in advanced urothelial carcinoma patients with either mutated or wild-type FGFR3. J Clin Oncol. 2011;29 (suppl; abstr TPS186). *This article evaluates another antiangiogenic pathway (FGFR) in treatment of urothelial carcinoma.*
15. Daher A, de Boer WI, El-Marjou A, et al. Epidermal growth factor receptor regulates normal urothelial regeneration. Lab Invest. 2003;83:1333.
16. Cheng J, Huang H, Zhang ZT, et al. Overexpression of epidermal growth factor receptor in urothelium elicits urothelial hyperplasia and promotes bladder tumor growth. Cancer Res. 2002;62:4157.
17. Neal DE, Marsh C, Bennett MK, et al. Epidermal growth factor receptors in human bladder cancer: comparison of invasive and superficial tumors. Lancet. 1985;1:366.
18. Berger MS, Greenfield C, Gullick WJ, et al. Evaluation of epidermal growth factor receptors in bladder tumors. Br J Cancer. 1987;56:533.
19. Wong Y, Litwin S, Plimack ER et al. Effect of EGFR inhibition with cetuximab on the efficacy of paclitaxel in previously treated metastatic urothelial cancer.
20. Clinicaltrials.gov. Study of gemcitabine and cisplatin with or without cetuximab in urothelial cancer (NCT00645593). Available at http://clinicaltrials.gov/ct2/show/NCT00645593. Accessed January 2012.
21. Petrylak DP, Tangen CM, Van Veldhuizen Jr PJ, 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.
22. 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.
23. 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.
24. Clincialtrials.gov. Gefitinib plus combination chemotherapy in treating patients with locally advanced or metastatic bladder cancer. http://clinicaltrials.gov/ct2/show/NCT00041106. Accessed on January 2012.
25. 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.
26. Clinicaltrials.gov. Trastuzumab in treating patients with previously treated, locally advanced, or metastatic cancer of the urothelium (NCT00004856). Available at http://clinicaltrials.gov/ct2/show/NCT00004856. Accessed January 2012.
27. Clinicaltrials.gov. Neoadjuvant Erlotinib (Tarceva) in transitional cell carcinoma (NCT00749892). Available at http://clinicaltrials.gov/ct2/show/NCT00749892. Accessed January 2012.
28. Clincialtrials.gov. Erlotinib before and after surgery in treating patients with muscle-invasive bladder cancer (NCT00380029). Available at http://clinicaltrials.gov/ct2/show/NCT00380029. Accessed January 2012.

29. Sharma P, Shen Y, Wen S, et al. Cancer-testis antigens: expression and correlation with survival in human urothelial carcinoma. Clin Cancer Res. 2006;12:5442.
30. Gnjatic S, Nishikawa H, Jungbluth AA, et al. NY-ESO-1: review of an immunogenic tumor antigen. Adv Cancer Res. 2006;95:1.
31. Sharma P, Gnjatic S, Jungbluth AA, et al. Frequency of NY-ESO-1 and LAGE-1 expression in bladder cancer and evidence of a new NY-ESO-1T cell epitope in a patient with bladder cancer. Cancer Immun. 2003;3:19.
32. Sharma P, Shen Y, Wen S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. Proc Natl Acad Sci USA. 2007;104:3967.
33. Sharma P, Bajorin DF, Jungbluth AA, et al. Immune response detected in urothelial carcinoma patients after vaccination with NY-ESO-1 protein plus BCG and GM-CSF. J Immunother. 2008;31:849.
34. Sun J, Schiffman J, Raghunath A, et al. Concurrent decrease in IL-10 with development of immune-related adverse events in a patient treated with anti-CTLA 4 antibody. Cancer Immun. 2008;8:9.
35. Lee JK, Havaleshko DM, Cho H, et al. A strategy for predicting the chemosensitivity of human cancers and its application to drug discovery. Proc Natl Acad Sci USA. 2007;104:13086.
36. Lorenzo-Romero JG, Salinas-Sánchez AS, et al. Prognostic implications of *p53* gene mutations in bladder tumors. J Urol. 2003;169 (2):492.
37. Kuczyk MA, Bokemeyer C, Serth J, et al. *p53* overexpression as a prognostic factor for advanced stage bladder cancer. Eur J Cancer. 1995;31A(13–14):2243.
38. Edelman MJ, Meyers FJ, Miller TR, et al. Phase I/II study of paclitaxel, carboplatin, and methotrexate in advanced transitional cell carcinoma: a well-tolerated regimen with activity independent of *p53* mutation. Urology. 2000;55(4):521.
39. Koga F, Kitahara S, Arai K, Honda M, Sumi S, Yoshida K. Negative *p53*/positive p21 immunostaining is a predictor of favorable response to chemotherapy in patients with locally advanced bladder cancer. Jpn J Cancer Res. 2000;91(4):416.
40. Sarkis AS, Bajorin DF, Reuter VE, et al. Prognostic value of *p53* nuclear overexpression in patients with invasive bladder cancer treated with neoadjuvant MVAC. J Clin Oncol. 1995;13(6):1384.
41. • Stadler WM, Lerner SP, Groshen S, et al. Randomized trial of *p53* targeted adjuvant therapy for patients with organ-confined node-negative urothelial bladder cancer (abstract #5017). J Clin Oncol. 2009;27:239s. *This article describes p53-directed adjuvant therapy in urothelial carcinoma*.
42. Kim WJ, Kakehi Y, Yoshida O. Multifactorial involvement of multidrug resistance-associated [correction of resistance]protein, DNA topoisomerase II and glutathione/glutathione-S-transferase in non P-glycoprotein-mediated multidrug resistance in human bladder cancer cells. Int J Urol. 1997;4(6):583–90.
43. Kotoh S, Naito S, Yokomizo A, Kohno K, Kuwano M, Kumazawa J. Enhanced expression of gamma-glutamylcysteine synthetase and glutathione S-transferase genes in cisplatin-resistant bladder cancer cells with multidrug resistance phenotype. J Urol. 1997;157 (3):1054.
44. Pendyala L, Velagapudi S, Toth K, et al. Translational studies of glutathione in bladder cancer cell lines and human specimens. Clin Cancer Res. 1997;3(5):793.
45. Petrylak DP, Scher HI, Reuter V, O'Brien JP, Cordon-Cardo C. P-glycoprotein expression in primary and metastatic transitional cell carcinoma of the bladder. Ann Oncol. 1994;5(9):835.
46. Siegsmund MJ, Marx C, Seemann O, Schummer B, et al. Cisplatin-resistant bladder carcinoma cells: enhanced expression of metallothioneins. Urol Res. 1999;27(3):157.
47. Bellmunt J, Paz-Ares L, Cuello M, Cecere FL, et al. Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. Ann Oncol. 2007;18(3):522.