Accelerated Methotrexate, Vinblastine, Doxorubicin, and Cisplatin Is Safe, Effective, and Efficient Neoadjuvant Treatment for Muscle-Invasive Bladder Cancer: Results of a Multicenter Phase II Study With Molecular Correlates of Response and Toxicity

Elizabeth R. Plimack, Jean H. Hoffman-Censits, Rosalia Viterbo, Edouard J. Trabulsi, Eric A. Ross, Richard E. Greenberg, David Y.T. Chen, Costas D. Lallas, Yu-Ning Wong, Jianqing Lin, Alexander Kutikov, Efrat Dotan, Timothy A. Brennan, Norma Palma, Essel Dulaimi, Reza Mehrazin, Stephen A. Boorjian, William Kevin Kelly, Robert G. Uzzo, and Gary R. Hudes

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Elizabeth R. Plimack, Rosalia Viterbo, Eric A. Ross, Richard E. Greenberg, David Y.T. Chen, Yu-Ning Wong, Alexander Kutikov, Efrat Dotan, Essel Dulaimi, Reza Mehrazin, Robert G. Uzzo, and Gary R. Hudes, Fox Chase Cancer Center, Temple Health; Jean H. Hoffman-Censits, Edouard J. Trabulsi, Costas D. Lallas, Jianqing Lin, and William Kevin Kelly, Thomas Jefferson University Hospital, Philadelphia, PA; Timothy A. Brennan and Norma Palma, Foundation Medicine, Cambridge, MA; and Stephen A. Boorjian, Mayo Clinic, Rochester, MN.

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Corresponding author: Elizabeth R. Plimack, MD, MS, Fox Chase Cancer Center, Temple Health, 333 Cottman Ave, Philadelphia, PA 19111-2497; e-mail: elizabeth.plimack@fccc.edu.

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ABSTRACT

Purpose

Neoadjuvant cisplatin-based chemotherapy is standard of care for muscle-invasive bladder cancer (MIBC); however, it is infrequently adopted in practice because of concerns regarding toxicity and delay to cystectomy. We hypothesized that three cycles of neoadjuvant accelerated methotrexate, vinblastine, doxorubicin, and cisplatin (AMVAC) would be safe, shorten the time to surgery, and yield similar pathologic complete response (pT0) rates compared with historical controls.

Patients and Methods

Patients with cT2-T4a and N0-N1 MIBC were eligible and received three cycles of AMVAC with pegfilgrastim followed by radical cystectomy with lymph node dissection. The primary end point was pT0 rate. Telomere length (TL) and *p53* mutation status were correlated with response and toxicity.

Results

Forty-four patients were accrued; 60% had stage III to IV disease; median age was 64 years. Forty patients were evaluable for response, with 15 (38%; 95% CI, 23% to 53%) showing pT0 at cystectomy, meeting the primary end point of the study. Another six patients (14%) were downstaged to non-muscle invasive disease. Most (82%) experienced only grade 1 to 2 treatment-related toxicities. There were no grade 3 or 4 renal toxicities and no treatment-related deaths. One patient developed metastases and thus did not undergo cystectomy; all others (n = 43) proceeded to cystectomy within 8 weeks after last chemotherapy administration. Median time from start of chemotherapy to cystectomy was 9.7 weeks. TL and *p53* mutation did not predict response or toxicity.

Conclusion

AMVAC is well tolerated and results in similar pT0 rates with 6 weeks of treatment compared with standard 12-week regimens. Further analysis is ongoing to ascertain whether molecular alterations in tumor samples can predict response to chemotherapy.

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INTRODUCTION

Before 2003, the standard of care for clinically localized, muscle-invasive urothelial carcinoma of the bladder was upfront cystectomy with curative intent. In 1987, the Southwest Oncology Group (SWOG) initiated a prospective, randomized controlled trial comparing three cycles of neoadjuvant chemotherapy followed by cystectomy with cystectomy alone, using a standard regimen of metho-

trexate, vinblastine, doxorubicin, and cisplatin (MVAC).¹ The study, published in 2003, showed a significant improvement in the rate of pathologic complete response (38% v 15%; $P < .001$) and a 2.6-year median overall survival (OS) benefit (6.4 v 3.8 years; $P = .05$) in the chemotherapy arm as compared with cystectomy alone. A subsequent meta-analysis of this and other studies of neoadjuvant cisplatin-based chemotherapy showed a 9% disease-specific survival benefit at 5 years.²⁻⁴ As a

result, neoadjuvant chemotherapy has been recommended by consensus guidelines in both the United States and Europe.^{5,6}

Despite this evidence, guideline concordance is low.⁷ A recent survey showed that only 11.3% of patients received perioperative chemotherapy for bladder cancer, and of this group, only a minority received it preoperatively.⁸ Reasons cited for failure to recommend neoadjuvant chemotherapy include the perception that it is morbid and toxic and concern regarding the delay of definitive surgery to accommodate chemotherapy.⁹ Lack of clarity regarding optimal choice of regimen may also contribute to underuse. Standard MVAC, used in the SWOG neoadjuvant trial, was developed in the 1980s, before the advent of antiemetics and growth factors, and carried with it significant toxicity, primarily neutropenia, nausea, vomiting, and stomatitis; it is now rarely used.¹⁰ In 2000, von der Masse et al¹¹ reported similar efficacy but improved toxicity with gemcitabine and cisplatin (GC) compared with standard MVAC in patients with metastatic bladder cancer. This experience in the advanced disease setting has been extrapolated to the neoadjuvant setting; thus, a 12-week regimen consisting of three to four cycles of GC is commonly used, although it has never been prospectively evaluated.¹²

To capitalize on the potential for dose-dense chemotherapy to improve outcomes, the standard MVAC regimen has been modified to what is now synonymously termed high-dose-intensity, dose-dense, or accelerated MVAC (AMVAC). AMVAC was prospectively compared with standard MVAC in the metastatic setting in a randomized phase III trial.¹³ The results demonstrated improvement in response rate, progression-free survival, and OS with AMVAC, with lower rates of neutropenia, neutropenic fever, and mucositis. In the neoadjuvant setting in particular, AMVAC provides another advantage over standard MVAC, in that all three cycles can be completed within 6 weeks, thus minimizing the time to cystectomy.

In this prospective phase II multicenter study, we set out to test the hypothesis that three cycles of AMVAC in the neoadjuvant setting would be safe and efficient, yield similar pathologic response rates compared with historical controls, and shorten the time to cystectomy. In addition, we sought to analyze the molecular alterations present in baseline tumor samples, seeking biomarkers predictive of response. We also analyzed telomere length (TL) in genomic DNA to assess for correlation with toxicity and tolerability of chemotherapy, because such correlations have previously been reported.^{14,15}

PATIENTS AND METHODS

Patients

Patients were required to have pathologically confirmed clinical stage T2-T4a M0 urothelial carcinoma. Patients with a solitary lymph node measuring < 2 cm were included. According to current American Joint Committee on Cancer (AJCC) staging (seventh edition), these patients would now be considered to have N1 disease.¹⁶ This group of patients was included to be consistent with SWOG-8710, where patients with lymph nodes < 2 cm in diameter were included but considered to have nonmetastatic disease using a prior AJCC edition.¹⁷ Creatinine clearance > 50 mL per minute, as estimated using the Cockcroft-Gault formula or measured with 24-hour urine collection, was required. Patients with left ventricular ejection fraction < 50% were excluded as a precaution, given the potential cardiotoxic effects of doxorubicin. This study enrolled patients with bladder or upper tract urothelial cancer but was powered to meet its end point in the bladder cohort only. The upper tract cohort was considered exploratory, and as such, results will be analyzed and reported separately. The

study was approved by institutional review boards at all participating sites. All patients provided written informed consent.

Treatment

Enrolled patients were assigned to receive three cycles of AMVAC (methotrexate 30 mg/m², vinblastine 3 mg/m², doxorubicin 30 mg/m², and cisplatin 70 mg/m²), administered on day 1 with a minimum of 2 L intravenous hydration. Split-dose cisplatin (35 mg/m² on days 1 and 2) could be substituted at physician discretion for creatinine clearance < 60 mL per minute. Peg-filgrastim was administered 24 to 48 hours after completion of chemotherapy. Antiemetics typically consisted of aprepitant, ondansetron, and dexamethasone but were not specified. Radical cystectomy with bilateral lymphadenectomy was performed within 4 to 8 weeks after the last cycle of chemotherapy. Procedures could be open, laparoscopic, or robotic, with type of diversion left to the discretion of the surgeon and patient.

Objectives and Statistical Plan

We hypothesized that three cycles of AMVAC administered over 6 weeks would yield a pathologic complete response (pT0) rate comparable to the currently used 12-week regimens. A two-stage Simon design was used to test the [null hypothesis](#) that the pathologic complete response (pT0) proportion among evaluable patients would be at most 23% versus the alternative hypothesis that the true proportion would be 43%. If there were ≥ three pT0s among the first 15 evaluable patients, up to an additional 27 patients would be accrued, for a total of 42. Otherwise, the therapy would be declared ineffective, and the trial would stop. The trial would be declared a success if ≥ 15 pT0s were observed. Our null hypothesis and success thresholds were based on historical controls for 12-week GC and standard MVAC, respectively.^{1,18} Patients were evaluable for efficacy analysis as predefined per protocol if they completed all treatment as defined or discontinued treatment early for protocol-defined toxicity or progression. The design had 4% type I error and 86% power. Early stopping rules for toxicity were included. Secondary objectives were to characterize the toxicity profile of AMVAC in the neoadjuvant setting, quantify the number of patients able to complete three cycles, and assess OS and relapse-free survival (RFS) rates. Kaplan-Meier methods and log-rank tests were used to characterize and compare OS and RFS between pT0 and non-pT0 evaluable patients. OS and RFS were defined as time from start of treatment to death and interval from treatment initiation to relapse (radiographic or clinical) or death, respectively. χ^2 tests were used to evaluate the relationship between p53 mutation status and pathologic complete response. Wilcoxon rank sum tests were used to assess the association between TL, TL percentile, and incidence of grade ≥ 3 adverse events. Secondary analyses were two sided, with a 5% level of significance.

Molecular Analysis of Tumor Samples

Genomic DNA was extracted from 40 μ m of macrodissected tumor tissue. Extracted DNA (200 ng) was sheared to approximately 100 to 400 bp by sonication, followed by end repair, dA addition, and ligation of indexed Illumina (San Diego, CA) sequencing adaptors.¹⁹ Sequencing libraries were hybridization captured using a pool of 5'-biotinylated DNA oligonucleotides (Integrated DNA Technologies, Coralville, IA) designed to target all coding exons of 287 cancer-related genes and selected introns of 19 frequently rearranged genes. DNA sequencing was performed using the HiSeq instrument (Illumina) with 49 × 49 paired-end reads. Sequence data were analyzed for all classes of genomic alterations, including base substitutions, indels, copy number alterations, and selected rearrangements. We report here the findings related to p53.

TL Measurement

DNA (10 ng) was extracted from peripheral-blood mononuclear cells collected at baseline and assayed by SpectraCell Laboratories (Houston, TX) for TL as previously described.²⁰ This assay determines a relative TL by measuring the factor by which the sample differs from a reference DNA sample in its ratio of telomere repeat copy number to single gene copy number (36B4 gene), which is proportional to the average TL. The results were reported as a telomere score that approximates the average amount of kilobase length per telomere and a telomere percentile that places the patient's TL relative to other

Table 1. Patient Demographic and Clinical Characteristics (N = 44)

Characteristic	No.	%
Age, years		
Median	64	
Range	44-83	
Sex		
Male	30	68
Female	14	32
Race/ethnicity		
White	40	91
African American	3	7
Asian	1	2
ECOG PS		
0	37	84
1	7	16
Clinical stage		
T2 N0 M0	16	36
T3 N0 M0	19	43
T4a N0 M0	6	14
T any N1 M0	3	7

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

patients of the same age. All samples were run in duplicate, with one negative control and two positive controls.

RESULTS

Patient Characteristics

Between December 2009 and February 2012, 44 patients with bladder cancer were accrued. Patient characteristics are described in Table 1. Sixty percent had clinical stage III or IV disease at baseline. Median age was 64 years (range, 44 to 83 years), with 32% age > 70 years. Median calculated creatinine clearance was 67 mL per minute (range, 33 to 161 mL per minute), with 13 patients (30%) documenting a calculated clearance < 60 mL per minute. Fourteen patients had 24-hour urine creatinine clearance measurements performed. Calculated creatinine clearance underestimated measured clearance in this group for all but one patient, with a

median underestimation of 33 mL per minute (range, 4 to 61 mL per minute). Four patients were not evaluable because of early discontinuation of study therapy at investigator or patient discretion in the absence of grade > 2 toxicity or disease progression. Cited reasons were anxiety with respect to surgical delay (n = 1), grade 2 constipation (n = 1), grade 2 bradycardia (n = 1), and noncompliance (n = 1).

Efficacy

Forty patients were evaluable for response as predefined in the protocol. Fifteen (38%; 95% CI, 23% to 53%) had no residual cancer found in their surgical specimens at the time of cystectomy, meeting the primary end point of the study early, obviating the need to extend accrual to the target of 42 evaluable patients. Another six (14%) were downstaged to non-muscle invasive disease (pT1/Tis/Ta and N0M0) for a total rate of downstaging to non-muscle invasive disease of 53% (95% CI, 37% to 68%). In addition, each patient's baseline clinical stage was compared with his or her final pathologic stage, demonstrating that 65% (95% CI, 50% to 80%) of evaluable patients were downstaged to a lower pathologic stage at cystectomy. These results are summarized in Figure 1. Within the evaluable cohort, 37 patients (93%) completed all three cycles of chemotherapy. Two discontinued chemotherapy early because of protocol-defined toxicity, and one because of early development of metastases. With a median follow-up of 20 months, nine patients have recurred, eight of whom had advanced disease at or before surgery (pT3, pT4, pN+, or M1). Five of these recurrences had resulted in cancer-related death as of data cutoff. Two additional patients died before recurrence. Kaplan-Meier RFS and OS estimates are shown in Figure 2.

Safety

All 44 enrolled patients with bladder cancer were evaluable for toxicity. Chemotherapy was generally well tolerated, with 82% of patients reporting only mild (grade 1 to 2) adverse effects. Eight patients (12%) experienced grade 3 or 4 treatment-related adverse events, which are summarized in Table 2. Toxicity was consistent with prior published experience with AMVAC, with myelosuppression and fatigue being the most common.²¹ There were no grade 3 or 4 renal toxicities and no treatment-related deaths. One patient developed

Fig 1. Pathologic response and downstaging. (A) Pathologic response and (B) pathologic compared with clinical stage.

Fig 2. (A) Relapse-free (RFS) and (B) overall survival (OS) by pathologic complete response (pT0) rate; (C) RFS and (D) OS for patients downstaged to non-muscle invasive disease.

metastases and therefore did not undergo cystectomy; all other patients ($n = 43$) proceeded to cystectomy within 8 weeks of their last chemotherapy infusion. Median time from start of chemotherapy to cystectomy was 9.7 weeks (range, 4.6 to 13 weeks). Approach to cystectomy was at the surgeon's discretion, with 44% performed robotically and 23% involving continent diversion. Median number of lymph nodes retrieved at surgery was 30 (range, nine to 62). Postoperative complications were captured using the Clavian-Dindo scale and are summarized in Table 3.²² No substantial difference in the incidence or severity of postoperative complications was noted in this series compared with historic controls.^{23,24}

Identification of p53 Mutations

Baseline tumor tissue obtained before chemotherapy was available for 39 of 40 evaluable patients, of whom 19 had alterations in p53. Complete pathologic responses were evenly divided between the group with altered p53 (seven of 19) and the group with no detectable alterations in p53 (seven of 20), demonstrating no correlation between altered p53 and response to chemotherapy. Mutations were then subdivided into those specifically suspected to be deleterious ($n = 7$)

versus missense mutations of uncertain significance ($n = 12$). No significant difference was seen between the group with deleterious mutations (three responses) and the rest of the cohort with respect to response.

TL Measurement

We measured TL in 43 patients for whom baseline peripheral blood samples were available. Median telomere score was 8.34 (equivalent to 8.34 KB in length; range, 5.19 to 12.98). Most patients had a TL in the ≥ 75 percentile range in comparison with their age group (34 patients; 79%), with only two patients < 50 th percentile. No correlation was found between patients' TL percentile and incidence of grade ≥ 3 adverse events while receiving treatment.

DISCUSSION

This phase II prospective multicenter study demonstrates that AMVAC is safe, is well tolerated, and yields pathologic response rates with three infusions over 6 weeks that are comparable to the more

Table 2. Grade 3 and 4 Chemotherapy-Related Toxicities

Toxicity	Grade 3		Grade 4	
	No.	%	No.	%
Anemia	3	7		
Fatigue	3	7		
Lymphocytopenia	2	5	1	2
Neutropenia			2	5
Dehydration			1	2
Sepsis			1	2
Proteinuria			1	2
Thrombocytopenia			1	2
Pharyngitis			1	2
Mucositis			1	2
Anorexia	1	2		
Hyponatremia	1	2		
Hypokalemia	1	2		
Lymphopenia	1	2		
Hyperkalemia	1	2		

NOTE. Worst grade per patient.

commonly used 12-week MVAC and GC regimens. Moreover, the higher rates of toxicity noted in the 1990s with standard MVAC were not seen using this accelerated schedule, which incorporates modern antiemetics and growth factor support. Surgical complication rates did not seem higher than historical controls, suggesting that AMVAC did not increase surgical morbidity. Given the low rate of adoption of neoadjuvant chemotherapy in both community and academic practices, the improved efficiency (patients proceeded to surgery at median of 9.7 weeks from day 1 of chemotherapy) combined with favorable overall toxicity profile of AMVAC may help improve adoption of this guideline-recommended approach to MIBC.

Several questions remain as to how to optimally administer AMVAC in the neoadjuvant setting. It is unclear how many cycles of AMVAC are ideal. A Japanese study of neoadjuvant standard MVAC, although terminated early because of slow accrual, showed a pT0 rate of 34% with just two cycles.²⁵ Another study using four cycles of AMVAC reported a pT0 rate of 28% in a group of patients, nearly half of whom had clinically node-positive disease at baseline, with a total of 47% downstaged to non-muscle invasive disease.²⁶ A third phase II trial of four cycles of neoadjuvant AMVAC plus bevacizumab in high-risk urothelial cancer showed a pT0 rate of 38%, with 54% downstaged to non-muscle invasive disease—rates comparable to those seen with AMVAC alone, thus leading the authors to conclude that bevacizumab did not add benefit.²⁷

Table 3. Postoperative Complications at 30 Days

Grade	Definition	%
I	Oral medications or bedside intervention	39
II	Intravenous medications, total parenteral nutrition, or blood transfusion required	42
III	Interventional radiology, endoscopy, intubation, angiography, or surgery required	17
IV	Disability requiring rehabilitation or organ resection	2
V	Death	0

NOTE. According to Clavien-Dindo classification²²; worst grade per patient.

Debate is ongoing as to which patients with bladder cancer are appropriate candidates for cisplatin-based chemotherapy. A calculated creatinine clearance threshold of 60 mL per minute is typically cited as a requirement for cisplatin, although several ongoing bladder cancer trials have liberalized this to 40 to 50 mL per minute (NCT00942331 and NCT01812369).²⁸ We and others have shown that calculated creatinine clearance often dramatically underestimates true clearance in this population.²⁹ In this trial, we considered measured 24-hour urine creatinine clearance to be the most accurate measure of renal function. A clearance of 50 mL per minute (measured or calculated) was required for study entry, and chemotherapy was administered with aggressive hydration. With this approach, no grade 3 to 4 renal events were observed. Additionally, many are hesitant in practice to treat patients age > 70 years, as evidenced by the lower-than-expected number of patients in this age group included in the retrospective neoadjuvant literature.^{12,30} Approximately one third of the patients treated in this study were age > 70 years, consistent with the demographics of this disease. We assessed TL as a potential biomarker of chemotherapy-related toxicity based on reports in the literature showing a correlation between shorter telomeres and decreased chemotherapy tolerance specifically among older patients.^{14,15} Our study showed no correlation between TL and toxicity, possibly because of the low rate of grade 3 to 4 adverse events noted on this study and overall long TLs seen among our patient cohort.

Rapid improvements in molecular analytic technology impart hope that we might identify molecular markers that may predict response to specific therapies. Recently, several groups have reported molecular alterations present in banked bladder cancer samples collected across multiple stages, grades, and treatment settings.³¹⁻³⁴ These studies reveal multiple potentially actionable alterations, fueling interest in testing targeted therapies for this disease. However, because the samples are not reliably linked to response data, these studies do not help ascertain which molecular alterations predict response to therapy—targeted or conventional. Identification of biomarkers predictive of response and toxicity would help select the patients most likely to benefit from and tolerate neoadjuvant chemotherapy. Overexpression of *p53* as measured by immunohistochemistry has been correlated with a poor response to neoadjuvant MVAC in earlier studies.^{35,36} However, we saw no difference in response patterns between patients with or without short-variant molecular alterations in *p53*. A phase III clinical trial investigating the efficacy of MVAC in the adjuvant setting also failed to show a significant difference when patients were stratified to treatment assignment based on *p53* expression by immunohistochemistry, although this study was limited by a low overall event rate and failure of randomization, leading it to be underpowered.³⁷ Although our study does not definitively rule out *p53* alterations as predictive, it is likely that alterations in > one gene are necessary to affect response to cytotoxic chemotherapy. We are performing ongoing analysis of our data set to investigate this further.

Although our study is limited by small sample size, the accumulated prospective and retrospective experiences with neoadjuvant AMVAC consistently show low rates of toxicity and high rates of pathologic downstaging to non-muscle invasive disease on the order 49% to 65%.^{26,27,30} This compares favorably with our retrospective series showing that patients taken to surgery without neoadjuvant chemotherapy are upstaged at cystectomy 73.2% of the time.³⁸ Given the lack of prospective data using GC in the neoadjuvant setting and the encouraging cumulative safety and outcome data with AMVAC

across several prospective phase II trials, we conclude that AMVAC should be considered a standard-of-care option for MIBC in the neoadjuvant setting. A randomized phase II study investigating neoadjuvant AMVAC and GC is currently planned through SWOG, and these results will help further define the role of AMVAC in MIBC. We are performing additional analyses of the molecular data generated from this study to ascertain whether alterations in baseline tumor samples can predict response to chemotherapy in the neoadjuvant setting, thus allowing for selection of sensitive patients and sparing others the toxicity of treatment and delay of definitive surgery.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

REFERENCES

- Grossman HB, Natale RB, Tangen CM, et al: Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *N Engl J Med* 349:859-866, 2003
- Neoadjuvant chemotherapy in invasive bladder cancer: Update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. *Eur Urol* 48:202-205, 2005; discussion 205-206
- Griffiths G, Hall R, Sylvester R, et al: International phase III trial assessing neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: Long-term results of the BA06 30894 trial. *J Clin Oncol* 29:2171-2177, 2011
- Neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: A randomised controlled trial—International collaboration of trialists. *Lancet* 354:533-540, 1999
- Montie JE, Clark PE, Eisenberger MA, et al: Bladder cancer. *J Natl Compr Canc Netw* 7:8-39, 2009
- Stenzl A, Cowan NC, De Santis M, et al: Treatment of muscle-invasive and metastatic bladder cancer: Update of the EAU guidelines. *Eur Urol* 59:1009-1018, 2011
- Fedeli U, Fedewa SA, Ward EM: Treatment of muscle invasive bladder cancer: Evidence from the National Cancer Database, 2003 to 2007. *The Journal of Urology* 185:72-78, 2011
- Donat SM: Integrating perioperative chemotherapy into the treatment of muscle-invasive bladder cancer: Strategy versus reality. *J Natl Compr Canc Netw* 7:40-47, 2009
- Bajorin DF, Herr HW: Kuhn's paradigms: Are those closest to treating bladder cancer the last to appreciate the paradigm shift? *J Clin Oncol* 29:2135-2137, 2011
- Sternberg CN, Yagoda A, Scher HI, et al: Methotrexate, vinblastine, doxorubicin, and cisplatin for advanced transitional cell carcinoma of the urothelium: Efficacy and patterns of response and relapse. *Cancer* 64:2448-2458, 1989
- 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* 18:3068-3077, 2000
- Dash A, Pettus JA 4th, Herr HW, et al: A role for neoadjuvant gemcitabine plus cisplatin in muscle-invasive urothelial carcinoma of the bladder: A retrospective experience. *Cancer* 113:2471-2477, 2008
- Sternberg CN, de Mulder P, Schornagel JH, et al: Seven year update of an EORTC phase III trial of high-dose intensity M-VAC chemotherapy and G-CSF versus classic M-VAC in advanced urothelial tract tumors. *Eur J Cancer* 42:50-54, 2006
- Falandry C, Horard B, Alexandre J, et al: Correlation of short telomeres (ST) with vulnerability, toxicity, and early death in elderly AOC patients receiving carboplatin: A multicenter GINECO trial. *J Clin Oncol* 30:569s, 2012 (suppl; abstr 9011)
- Garg MB, Lincz LF, Adler K, et al: Predicting 5-fluorouracil toxicity in colorectal cancer patients from peripheral blood cell telomere length: A multivariate analysis. *Br J Cancer* 107:1525-1533, 2012
- Edge S, Byrd DR, Compton CC, et al (eds): *AJCC Cancer Staging Manual* (ed 7). New York, NY, Springer, 2010
- Beahrs OH, Myers MH (eds): *AJCC Manual for Staging of Cancer* (ed 2). Philadelphia, PA, Lippincott, 1983
- Hoffman-Censits JH, Wong Y, Li T, et al: Dose intensity of cisplatin and gemcitabine (CG) for muscle invasive urothelial bladder cancer (MIUBC) in the neoadjuvant versus adjuvant settings: The Fox Chase Cancer Center (FCCC) experience. *J Clin Oncol* 27:182e, 2009 (suppl; abstr e16012)
- Gnrke A, Melnikov A, Maguire J, et al: Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nat Biotechnol* 27:182-189, 2009
- Cawthon RM: Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res* 37:e21, 2009
- Sternberg CN, de Mulder PH, Schornagel JH, et al: Randomized phase III trial of high-dose-intensity methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) chemotherapy and recombinant human granulocyte colony-stimulating factor versus classic MVAC in advanced urothelial tract tumors: European Organisation for Research and Treatment of Cancer Protocol No. 30924. *J Clin Oncol* 19:2638-2646, 2001
- Clavien PA, Barkun J, de Oliveira ML, et al: The Clavien-Dindo classification of surgical complications: Five-year experience. *Ann Surg* 250:187-196, 2009
- Shabsigh A, Korets R, Vora KC, et al: Defining early morbidity of radical cystectomy for patients with bladder cancer using a standardized reporting methodology. *Eur Urol* 55:164-174, 2009
- Novotny V, Hakenberg OW, Froehner M, et al: Systematic assessment of complications and outcome of radical cystectomy undertaken with curative intent in patients with comorbidity and over 75 years of age. *Urol Int* 90:195-201, 2013
- Kitamura H, Tsukamoto T, Masumori N, et al: Randomized phase III trial of neoadjuvant chemotherapy (NAC) with methotrexate, doxorubicin, vinblastine, and cisplatin (MVAC) followed by radical cystectomy (RC) compared with RC alone for muscle-invasive bladder cancer (MIBC): Japan Clinical Oncology Group study, JCOG0209. *J Clin Oncol* 31, 2013 (suppl 15; abstr 4526)
- Qu AQ, Jacobus SJ, Signoretti S, et al: Phase II study of neoadjuvant dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin (ddMVAC) chemotherapy in patients with muscle-invasive urothelial cancer (MI-UC): Pathologic and radiologic

AUTHOR CONTRIBUTIONS

Conception and design: Elizabeth R. Plimack, Jean H. Hoffman-Censits, Stephen A. Boorjian, Gary R. Hudes

Financial support: Elizabeth R. Plimack, Efrat Dotan

Provision of study materials or patients: Elizabeth R. Plimack, Jean H. Hoffman-Censits, Rosalia Viterbo, Edouard J. Trabulsi, Richard E. Greenberg, David Y.T. Chen, Costas D. Lallas, Yu-Ning Wong, Jianqing Lin, William Kevin Kelly

Collection and assembly of data: Elizabeth R. Plimack, Jean H. Hoffman-Censits, Rosalia Viterbo, Edouard J. Trabulsi, Richard E. Greenberg, David Y.T. Chen, Costas D. Lallas, Yu-Ning Wong, Jianqing Lin, Alexander Kutikov, Efrat Dotan, Timothy A. Brennan, Norma Palma, Essel Dulaimi, Reza Mehrazin, William Kevin Kelly, Robert G. Uzzo

Data analysis and interpretation: Elizabeth R. Plimack, Jean H. Hoffman-Censits, Edouard J. Trabulsi, Eric A. Ross, Reza Mehrazin

Manuscript writing: All authors

Final approval of manuscript: All authors

response, serum tumor markers, and DNA excision repair pathway biomarkers in relation to disease-free survival (DFS). *J Clin Oncol* 31:291s, 2013 (suppl 15; abstr 4530)

27. Siefker-Radtke AO, Kamat AM, Corn PG, et al: Neoadjuvant chemotherapy with DD-MVAC and bevacizumab in high-risk urothelial cancer: Results from a phase II trial at the University of Texas M. D. Anderson Cancer Center. *J Clin Oncol* 30:282s, 2012 (suppl; abstr 4523)

28. 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* 12:211-214, 2011

29. Raj GV, Iasonos A, Herr H, et al: Formulas calculating creatinine clearance are inadequate for determining eligibility for cisplatin-based chemotherapy in bladder cancer. *J Clin Oncol* 24:3095-3100, 2006

30. Blick C, Hall P, Pwint T, et al: Accelerated methotrexate, vinblastine, doxorubicin, and cisplatin

(MVAC) as neoadjuvant chemotherapy for patients with muscle-invasive transitional cell carcinoma of the bladder. *Cancer* 118:3920-3927, 2012

31. Iyer G, Hanrahan AJ, Milowsky MI, et al: Genome sequencing identifies a basis for everolimus sensitivity. *Science* 338:221, 2012

32. Chekaluk Y, Wu CL, Rosenberg J, et al: Identification of nine genomic regions of amplification in urothelial carcinoma, correlation with stage, and potential prognostic and therapeutic value. *PLoS One* 8:e60927, 2013

33. Gui Y, Guo G, Huang Y, et al: Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet* 43:875-878, 2011

34. Hurst CD, Platt FM, Taylor CF, et al: Novel tumor subgroups of urothelial carcinoma of the bladder defined by integrated genomic analysis. *Clin Cancer Res* 18:5865-5877, 2012

35. Kakehi Y, Ozdemir E, Habuchi T, et al: Absence of p53 overexpression and favorable response to cisplatin-based neoadjuvant chemotherapy in urothelial carcinomas. *Jpn J Cancer Res* 89:214-220, 1998

36. 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* 13:1384-1390, 1995

37. Stadler WM, Lerner SP, Groshen S, et al: Phase III study of molecularly targeted adjuvant therapy in locally advanced urothelial cancer of the bladder based on p53 status. *J Clin Oncol* 29:3443-3449, 2011

38. Canter D, Long C, Kutikov A, et al: Clinicopathological outcomes after radical cystectomy for clinical T2 urothelial carcinoma: Further evidence to support the use of neoadjuvant chemotherapy. *BJU Int* 107:58-62, 2011

GLOSSARY TERMS

null hypothesis: the statistical hypothesis that the observed difference results from chance alone.

telomeres: a tandem array of short DNA sequences that occur at the physical ends of chromosomes. Telomeres have addressed the issue of the inability of DNA polymerases to replicate the ends of DNA strands. As a result, during the course of replication, telomeres in somatic cells shorten with each cell division.

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